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Dengue Serotypes in Bangladesh: Whole Genome Sequencing and Comparative Genomics Facilitates Pathogenesis and Epidemiology Studies and Informs Improved Disease Control

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ABSTRACT

Dengue virus (DENV) is one of the most extensive arthropod-borne *(arbo)viruses worldwide, especially prevalent in tropical and subtropical countries, and which is responsible for causing an array of clinical disease manifestations in humans. The increasing frequency of dengue outbreaks and the expanding range over which both the virus and its vector of transmission, female* Aedes *mosquitoes, are endemic, are pressing global public health concerns. The ongoing lack of either an efficacious vaccine or antiviral drug has contributed to the escalating problem. Inadequate knowledge of DENV genomic architecture and pathogenesis has proven to be a major drawback when designing effective prevention and treatment options. Mounting case numbers of dengue infection are reported each year in Bangladesh, yet no full-length genome sequence data are available for DENV isolates from this densely populated, developing South Asian nation. Sequencing and characterization of the whole genome of Bangladeshi DENV isolates of different virus serotypes is therefore an important priority in order to identify therapeutic target determinants against which to develop effective measures to combat the disease.*

Keywords

Bangladesh, Dengue, Drug, Serotype, Vaccine, Virulence, Virus, Whole genome sequence.

Introduction

Dengue virus (DENV) is a ubiquitous, genetically diverse *ar*thropod-*bo*rne (arbo) virus with the potential to cause life-threatening infections in humans. It is transmitted via day-biting female mosquitoes of the genus *Aedes* (notably *Ae. aegypti, Ae. albopictus* and *Ae. polynesiensis*) that breed in stagnant, freshwater bodies [1,2]. The World Health Organization reports that over the last few decades the global incidence of dengue infection has increased markedly, with an estimated 390 million people now infected annually, of which around 25% (some 100 million patients) manifest clinically [3]. Currently, one half of the world's population lives in regions that place them at risk of infection. There are well over 100 countries endemic for dengue located mostly in tropical and subtropical zones across Africa, Asia, the Western Pacific, the Americas and the Caribbean, where *Aedes* mosquitoes are commonly found [4,5].

Most people infected with DENV are asymptomatic (75%) or show only mild symptoms. A significant minority (5%) suffer more severe illness and in a small proportion this is life-threatening. Clinical manifestations are broadly categorized into three classical types depending on severity of presentation, namely dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). While DF is an uncomplicated febrile illness, DHF and DSS may have fatal outcomes [6]. A spectrum of warning signs is attributed to DENV infection, which range from flu-like symptoms, lethargy and rashes for DF, to abdominal pain, accumulation of fluid, mucosal bleeding, plasma leakage and decreased platelet count for DHF, through to severe bleeding, typically from the gastrointestinal tract, and organ failure for DSS

[2]. While treatment of uncomplicated dengue cases (DF) is only supportive severe dengue cases (DHF and DSS) require hospital intensive care. Current annual global estimates of morbidity and mortality from severe dengue are 500,000 DHF cases and 22,000 deaths, mostly among children [3]. Reliable figures for DSS are not available.

DENV is a single-stranded positive sense RNA virus with a genome size of approximately 11 kilobases, belonging to the *Flavivirus* genus of the Flaviviridae family [7,8]. The genome of each virion encodes for three structural proteins, named envelope (E), membrane (M) and capsid (C), and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [9]. These genes are situated in the open reading frame of the genome which is flanked by 5' and 3' untranslated regions (UTRs) [10]. The UTRs and NS proteins are associated with viral replication and assembly, while E protein is involved in initial binding of the virion to the host cell membrane [11].

DENV isolates are categorized as being one of four distinct serotypes (DENV-1 to DENV-4) which share a genome sequence homology of between 60-70% and similarly cause recognized clinical symptoms [12]. The supposed presence of a fifth, more phylogenetically distinct, serotype (DENV-5) has been announced but this is yet to be confirmed [13,14]. The DENV genome is prone to increased rates of mutation, which leads to the presence of a number of discrete genotypes within each serotype [15,16]. Furthermore, recurrent infections pose a threat to patients as immunopathogenesis occurs [17,18]. This means that upon re-exposure to DENV of a heterologous serotype to that which caused primary infection seropositive patients exhibit enhanced disease manifestations [19], a phenomenon known as antibody-dependent enhancement of infection.

Despite continued progress in dengue research, it remains an ongoing challenge to identify definitive treatment and prevention options [20,21]. Therefore, it is of particular importance to reveal the genotypes of circulating DENV isolates distributed in different endemic countries, including Bangladesh, the focus of this article. This information may then be used to compare isolates in order to correlate the function of viral genomic determinants in the complex host-virus interaction and their possible role in pathogenesis.

Dengue control: Current schemes and their limitations

Since the second half of the twentieth century increasingly frequent human movement between countries, especially a growth in volume of intercontinental air travel, has facilitated dissemination of dengue across the globe [22]. Imported cases of dengue have become a major cause of public health concern in both developed and developing countries. Globalization of the economy and climatic changes may also play a role in the spread of the disease [23]. With the rising number of newly endemic areas and the high susceptibility of previously unexposed individuals resident in those areas to clinical infection, it is imperative to develop effective yet affordable strategies to reduce the impact of dengue worldwide. Important aspects to be taken into consideration when aiming to combat dengue include early virus detection and diagnosis of clinical infection, development of specific therapies and implementation of efficient vector control strategies [24].

A wide range of dengue detection and diagnostic tools are available at present, the use of each of which has advantages and drawbacks [25,26]. Different laboratory diagnostic techniques involving virus isolation, viral nucleic acid detection, serological methods, rapid detection tests and more are applicable to early and rapid detection of all dengue serotypes [2]. Several variable factors, such as sample collection time, storage and transportation, contribute to the differing sensitivity of tests performed under separate circumstances. Quite a few studies have highlighted the use of NS1 antigen and anti-NS1 antibody as a key dengue diagnostic device as it has the capacity to differentiate different serotypes and also helps to distinguish between primary and subsequent infections [27-29]. However, possibilities for improvement remain, with, for example, a reliable, cost-effective, highly specific and ultra-sensitive tool yet to be designed. The future development of suitable biosensors may eliminate such issues [30,31]. These employ nanobiotechnology hybrid materials to enhance a technique's sensitivity and selectivity while also reducing its detection time and high-throughput process time.

The absence of effective vaccines and antiviral drugs against dengue creates great hardships in clinical disease management [32]. Challenges to vaccine design include the noted phenomenon of enhanced pathology upon secondary infection by a serotype heterologous to that which caused primary infection, as well as genomic diversity between genotypes and serotypes of DENV. Hence, it is very difficult to prepare a vaccine that can immunize effectively against all DENV serotypes [16,33]. The first commercially approved dengue vaccine, the live-attenuated tetravalent Dengvaxia produced by Sanofi Pasteur, was released on a limited basis from December 2015 in 25 endemic countries but it is currently undergoing further review by the WHO before fresh recommendation for population level use [34]. This drastic action was taken due to the unfortunate occurrence of severe disease manifestations in immunized, previously seronegative individuals upon a subsequent dengue infection [35], a consequence of vaccine-induced antibody-dependent enhancement of infection. This has led to a profound loss of both government and public trust in this and other vaccines in some countries [36], notably the Philippines in which a vaccination campaign targeted at over one million 9-year-old students enrolled in public schools was abruptly halted [37]. Such a major drawback to Dengvaxia renews calls for additional tetravalent vaccine development [38]. Moreover, cheaper alternative vaccines to Dengvaxia, which requires three doses administered over the course of one year, are needed to encourage widespread immunization in dengue-affected developing countries such as Bangladesh.

In parallel to treatment options, in order to restrain DENV transmission and infection efficient vector control interventions need to be strategized. Destruction of breeding habitats of *Aedes* mosquitoes is a mandatory step to be carried out [10]. In addition,

community awareness programs must be initiated to educate people living in endemic locations as to the importance of vector control measures and dengue surveillance. Production of new generation insecticides is also crucial because of the increasingly reported resistance of *Aedes* to those leading insecticides currently in use [39,40].

Dengue in Bangladesh

Bangladesh is bordered by two countries that are major sources of dengue in South Asia. These are India to the west, north and east (a major centre of DENV transmission, with all four serotypes circulating) and, to the south east, Myanmar (where large-scale outbreaks occur typically every three to five years) [41]. Therefore, the nation is at constant risk of frequent incidence of dengue and is now considered hyper-endemic, with regular reporting of clinical cases from the start of this century. Sporadic transmission of DENV occurred in Bangladesh from 1964 to 1999 but a sudden and significant outbreak of DENV-3 in 2000 elevated the number of annually reported clinical cases to as high as 6,132 by 2002 [41,42]. Interestingly, 82% of hospitalized persons were adults and all deaths occurred in patients aged 5 years or older. In subsequent years dengue incidence declined slowly to as low as 375 confirmed cases in 2014. However, in 2016, a DENV-2 outbreak caused the highest number of clinical cases (around 6,100) in Bangladesh since 2002 [43]. In 2018, clinical incidence of dengue peaked at approximately 6,500 cases, apparently due to circulation of the DENV-3 serotype in the country, which had been notably absent during 2013-2017 [43]. A recently published study that examined dengue isolates over the three-year period 2015-2017 confirmed co-circulation of DENV-1 and DENV-2 serotypes in the nation's capital and largest city, Dhaka, suggesting the absence of DENV-3 and DENV-4 [29]. The epidemiology of dengue transmission in Bangladesh is therefore complex and fluid. Hence, close monitoring of circulating serotypes is essential in order to safeguard against sudden and unforeseen epidemics.

The frequency of reporting of dengue cases is greatest amid and just after the Bangladeshi monsoonal rains (June – October) as the conditions during this season (continuous drizzle, high temperature and humidity) are ideal for vector mosquitoes to breed [41]. In addition, developing countries in tropical regions like Bangladesh lack sufficient local community infrastructure and vector control programs to prevent breeding of mosquitoes and thereby to eliminate spread of the disease. Rather, the unsystematic rapid urbanization, increased population density, poor sanitation, slums with open water tanks, unprotected sewage drains and lack of waste disposal management are all human factors that promote the *Aedes* population to thrive [44]. In recent times, large groups of refugees from Myanmar have entered the country, an influx which places Bangladesh at risk of imported dengue cases becoming foci for subsequent autochthonous transmission [41].

Urgent action is required in order to reduce the escalating number of reported cases of dengue infection in Bangladesh [45]. In the most densely populated cities of Dhaka and Chittagong streets and houses are fogged with insecticides by the municipal authorities in an effort to eliminate the vectors. Unfortunately, however, this attempted control measure is relatively ineffective since adult mosquitoes but not larvae are susceptible. Continued use has led to resistance by mosquitoes developing to front-line insecticides. There is also a dearth of adequate healthcare provision, which results in inaccurate diagnosis, improper treatment and under-reporting [41]. Moreover, illiteracy, poverty and social inequalities are factors that have been associated with poor dengue management in prior studies [11].

Role of the viral genome in DENV virulence

Analysis of the DNA sequence of an organism's genome, in its entirety and determined at a single time, is known as whole genome sequencing (WGS). Its application to DENV reveals the complete sequence of the viral genome and facilitates molecular characterization of the virus and identification of significant genetic markers that may be associated with virulence properties [46,47]. An initial structural comparison between genomes of DENV isolates recovered from cases of DF and DHF revealed that charge differences among six encoded amino acids existed in structural (prM and E) and NS (NS4b and NS5) genes [48]. Determinants of DHF were found to reside in these regions and were assumed to modify virion-host interactions, regulate viral replication and code for transport proteins. Furthermore, alterations in 5' and 3' UTR sequences presumably change secondary structures of RNA. Previous investigations confirmed the significance of E protein amino acid substitutions (e.g. E71D, E126K and F4021) in neurovirulence enhancement [49,50]. Recently, several amino acid substitutions were observed in both structural and NS proteins that may contribute to the pathogenic capacity of DENV [51]. Further investigations are essential to understand more fully the influence and effect of such mutations on virus fitness and pathogenicity-conferring abilities.

Importance of WGS to combatting DENV

The novel application of WGS technology to different DENV serotypes will open new pathways by which to explore dengue research. This will help to overcome our current knowledge gap in regard to DENV genetics, virulence mechanisms and pathogenesis that underpin the spectrum of disease manifestations [52]. Moreover, the continuing absence of an effective vaccine and antiviral therapies may be attributed directly to this lack of underlying genomic information. At present, E protein sequences of globally disseminated DENV strains are readily accessible online but a very limited set of whole genome data is available for the purposes of research [51]. In addition, to date no WGS of DENV has been conducted on isolates collected from Bangladesh despite its status as a majorly affected dengue-endemic region. Thus, there is a pressing need to prioritize the use of this comprehensive method for analyzing the entire genome to characterize DENV serotypes isolated from clinical specimens collected from diverse locations and times throughout the country.

Several distinct lines of investigation can be devised to harness the value of such a genomic resource. These include evolutionary and epidemiological studies as well as comparative genomic analyses

between strains of the same serotype that cause varying degrees of disease severity [52]. In addition, the data can be examined to dissect the role of genetic polymorphisms in disease pathogenesis, to verify the stability of polymorphisms in the entire DENV genome and to determine how polymorphic loci can bring differences in disease severity. Furthermore, the application of WGS will help to understand virulence mechanisms and to identify the genes responsible. Also, such genomic data will contribute an invaluable data source to underpin the targeting of appropriate genes and gene products for dengue vaccine design and antiviral drug discovery.

Conclusion

In summary, the first WGS and genomic characterization of DENV isolates from Bangladesh will serve as an excellent reference and provide valuable genomic insights for future research investigations of dengue in the country. This will deepen our knowledge of dengue disease manifestations, virulence properties, epidemiology and phylogenetics, and thereby facilitate vaccine and therapeutics development.

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Authors' contributions

TT conceptualized the paper, which was developed further in discussion with AWTR. Both authors collated articles for review, wrote and critically reviewed various drafts, contributed to preparation of the final version and provided consent for submission.

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