

Genotypes of dengue virus circulate in dengue sentinel surveillance in Indonesia

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Abstrak

Latar Belakang: Demam berdarah dengue disebabkan oleh infeksi virus dengue (DENV) yang terdiri atas serotipe 1 sampai dengan serotipe 4. Masing-masing serotipe dibedakan lagi menjadi beberapa genotipe. Studi ini dilakukan untuk mendeteksi genotipe DENV yang bersirkulasi di lokasi surveilans sentinel dengue.

Metode: Untuk menentukan genotipe dilakukan analisis genetik menggunakan perangkat bioinformatik. Urutan nukleotida yang lengkap dari gen selubung DENV diperoleh melalui amplifikasi genetik menggunakan primer overlapping untuk masing-masing serotipe. Pohon filogenetik dibuat menggunakan perangkat lunak MEGA 6.0, metode Neighbor Joining dengan 1000 replikasi dan parameter Kimura-2. Sekuen nukleotida dari GenBank yang telah diketahui genotipe DENV digunakan sebagai referen.

Hasil: Jumlah sampel dengan gen selubung DENV yang dapat diamplifikasi adalah 64 dari 204 kasus yang terkonfirmasi. Empat belas sekuen dari DENV-1, 22 sekuen DENV-2, dan 28 sekuen DENV-3. Dari studi ini belum didapatkan sekuen untuk DENV-4. DENV-1 dan DENV-2 masing-masing masuk dalam kelompok genotipe I dan Cosmopolitan, sementara DENV-3 masuk dalam kelompok genotipe I.

Kesimpulan: Genotipe DENV di lokasi surveilans sentinel dengue sama dengan genotipe yang sebelumnya dilaporkan bersirkulasi di Indonesia. (*Health Science Journal of Indonesia 2016;7(2):69-74*)

Kata kunci: surveilans sentinel dengue, virus dengue, genotipe

Abstract

Background: Dengue hemorrhagic fever was caused by dengue virus (DENV). This virus has four serotypes, DENV-1 to DENV-4, in which each serotype consists of various genotypes. This study present the distribution of DENV genotype circulate in dengue sentinel surveillance sites.

Methods: We performed molecular analyses using bioinformatics tools to identify genotype of DENV. Sequencing targeting envelope gene was carried out on DENV-positive samples. Full-length sequence of envelope gene was obtained using overlapping primers for DENV 1, 2, 3 and 4. Phylogenetic tree was generated by Neighbor Joining using MEGA 6.0 with 1000 bootstrap replications and Kimura 2-parameter model. Known genotype of DENV sequences from Indonesia and other countries obtained from GenBank were included in the analyses as references.

Results: A total of 64 complete coding sequences of envelope gene from DENV-1, DENV-2 and DENV-3 were successfully sequenced from 204 confirmed DENV cases. There are fourteen sequences of DENV-1, 22 sequences of DENV-2 and 28 sequences of DENV-3. Unfortunately, there was no complete coding sequence of envelope gene obtained for DENV-4. DENV-1 and DENV-2 were grouped into genotype I and cosmopolitan genotype, respectively. The DENV-3 was grouped into genotype I. These viruses were belongs to various genotypes that have been circulating previously in Indonesia.

Conclusion: This finding suggests that the distribution of DENV genotype in sentinel sites remained stable. (*Health Science Journal of Indonesia 2016;7(2):69-74*)

Key words: dengue sentinel surveillance, dengue virus, genotype

Dengue virus (DENV) is the causative agent of dengue infection. DENV belongs to family *Flaviviridae* and genus *Flavivirus*. DENV serotypes have four genetically and antigenically different, known as DENV-1, DENV-2, DENV-3 dan DENV-4. Each serotype is divided into several genotypes based on the sequence variability on the envelope gene (E gene). DENV-1 consists of five genotypes (genotype I-V).¹ DENV-2 comprises of American genotype, Cosmopolitan, Asian I, Asian II, American Asian genotype and Sylvatic.² DENV-3 consists of 4 genotypes (genotype I-IV)³ and DENV-4 comprises of 4 genotypes (genotype I-III and sylvatic).⁴

Genotyping of DENV is essential as the identification of DENV genotype could provide information of genetic changes in DENV gene, and to detect the introduction of new genotype in certain area. In addition, DENV as RNA virus continues to evolve and the identification of DENV genotype could provide crucial information of the dynamic change of DENV.⁵

It was reported that genotype I and IV of DENV-1 circulated in Indonesia.⁶ So are cosmopolitan of DENV-2, genotype I of DENV-3 and genotype II of DENV-4.^{7,8} Therefore limited data was available regarding the distribution of DENV genotype in dengue sentinel surveillance sites, which serum samples were sent to National Institute of Health Research and Development (NIHRD). The surveillance was conducted to obtain information on epidemiology and virology of DENV and other arboviruses. In this study we present the distribution of DENV genotype circulate in dengue sentinel surveillance sites.

METHODS

Samples and DNA sequencing

DENV-confirmed samples were obtained from dengue cases of dengue sentinel surveillance systems sites in Deli Serdang (North Sumatra), Wonosari (Jogjakarta), Balikpapan (East Kalimantan), Bitung (North Sulawesi), Ambon (Maluku) and Mataram (West Nusa Tenggara) from September 2014 to December 2015. (Figure.1)

DENV E gene could be amplified from the sera of some samples, however for some other samples DENV should be isolated and propagated on cell culture. The archived clinical specimens that confirmed as DENV positive were cultured in Vero

76 cell line. The supernatant of Vero 76 cell line was collected from cell line showed cytopathic effect (CPE) followed by the viral RNA isolation using QiAmp Viral Mini Kit (Qiagen, Hilden, Germany) according manufacturer's instruction. The complete coding sequence of envelope gene was derived using overlapping primer of DENV 1, 2, 3, and 4⁹ with some modifications. Direct DNA sequencing was carried out using the Big Dye Terminator V.3.0 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) on ABI 3130 x 1 Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Genotype analysis

The overlapping nucleotide sequences were edited, assembled and aligned using BioEdit Sequence Alignment Editor Ver 7.0.5.2. Multiple alignment of DENV envelope gene sequences (1485 nt for DENV-1 and DENV-2, 1479 nt for DENV-3) was performed by ClustalW using MEGA 6.0.¹⁰ Then phylogenetic tree was generated by Neighbor Joining using the same software with 1000 bootstrap replicates and Kimura 2-parameter model. Existing genotype of DENV sequences from Indonesia and other countries obtained from GenBank were included in the analyses as references. Classifications scheme of genotype utilized as proposed by previous publications.¹⁻⁴

The study got ethical approval from National Institute of Health Research and development ethical committee No. LB.02.01/5.2/KE. 208/2015.

RESULTS

During study period, 204 samples were DENV positive out of 585 specimens sent to NIHRD. All DENV serotypes, DENV-1 to DENV-4 were found from all of sentinels in which DENV-3 was the dominant serotypes.

A total of sixty four complete coding sequences of envelope gene from DENV-1, DENV-2 and, DENV-3 were successfully sequenced from 204 confirmed DENV cases. There was no complete coding sequence of envelope gene of DENV-4 obtained in this study. Figure 2 illustrated the phylogenetic tree of envelope gene of DENV-1 in which consisted of fourteen sequences of DENV-1, grouped in genotype I. The samples were originated from all of sentinel

sites. The phylogenetic tree illustrated in Figure 2, shows that DENV-1 circulated in sentinel sites formed a cluster with those detected in Singapore 2007-2010. Nucleotide sequence identity between clades ranged from 97%-99%.

There were 22 samples that successfully sequenced for envelope gene of DENV-2. All sequences were grouped in cosmopolitan genotype. These sera

samples came from all of sentinel site except Ambon. The DENV-2 phylogenetic tree illustrated in Figure 3, showed that the Indonesian Cosmopolitan genotype were closely related to isolates that circulated previously in Indonesia^{7,11} also in neighboring country such as Brunei in 2006, Malaysia and Philippines in 2010, and Singapore in 2011. Homology sequences within clade were 97% -100%. This phylogenetic tree also showed that Indonesian Cosmopolitan belong to first sub-lineage. (Figure 3).



Figure 1. Map of Indonesia archipelago. The red bullet indicates the sentinel sites

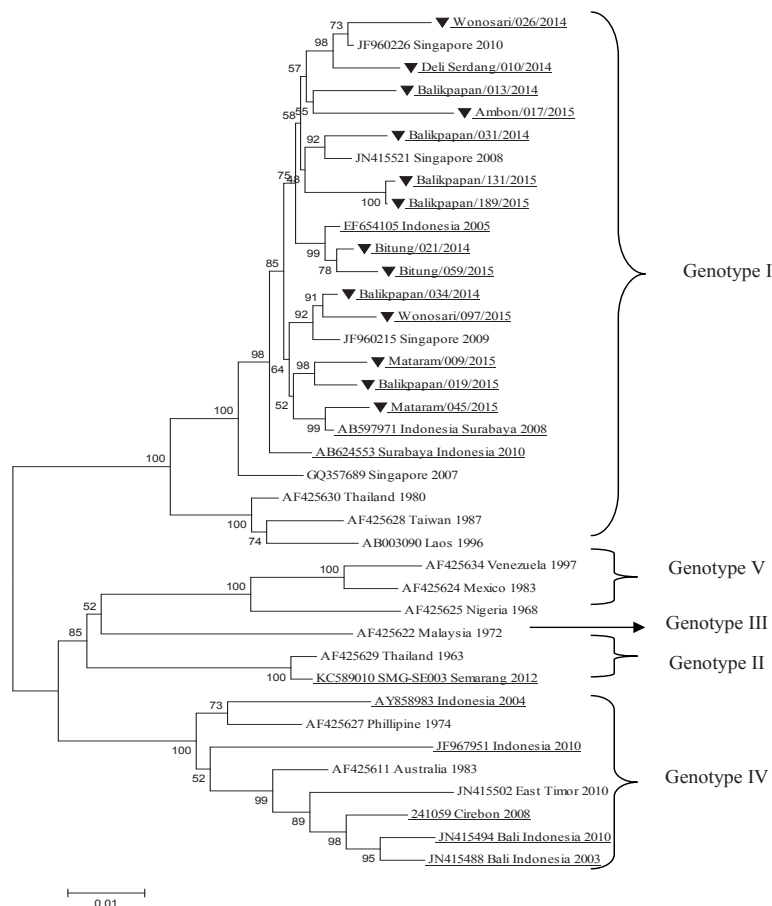


Figure 2. Phylogenetic Tree of DENV-1 constructed based on envelope gene (1485 nt). The Indonesian sequences were shown in underline, our sequence marked by red solid triangle (▼). Known strains as references from GenBank were noted by accession number, country and year of isolation. Genotypes are also indicated by reference sequence, classification scheme as proposed by Goncalvez, et al 2002.

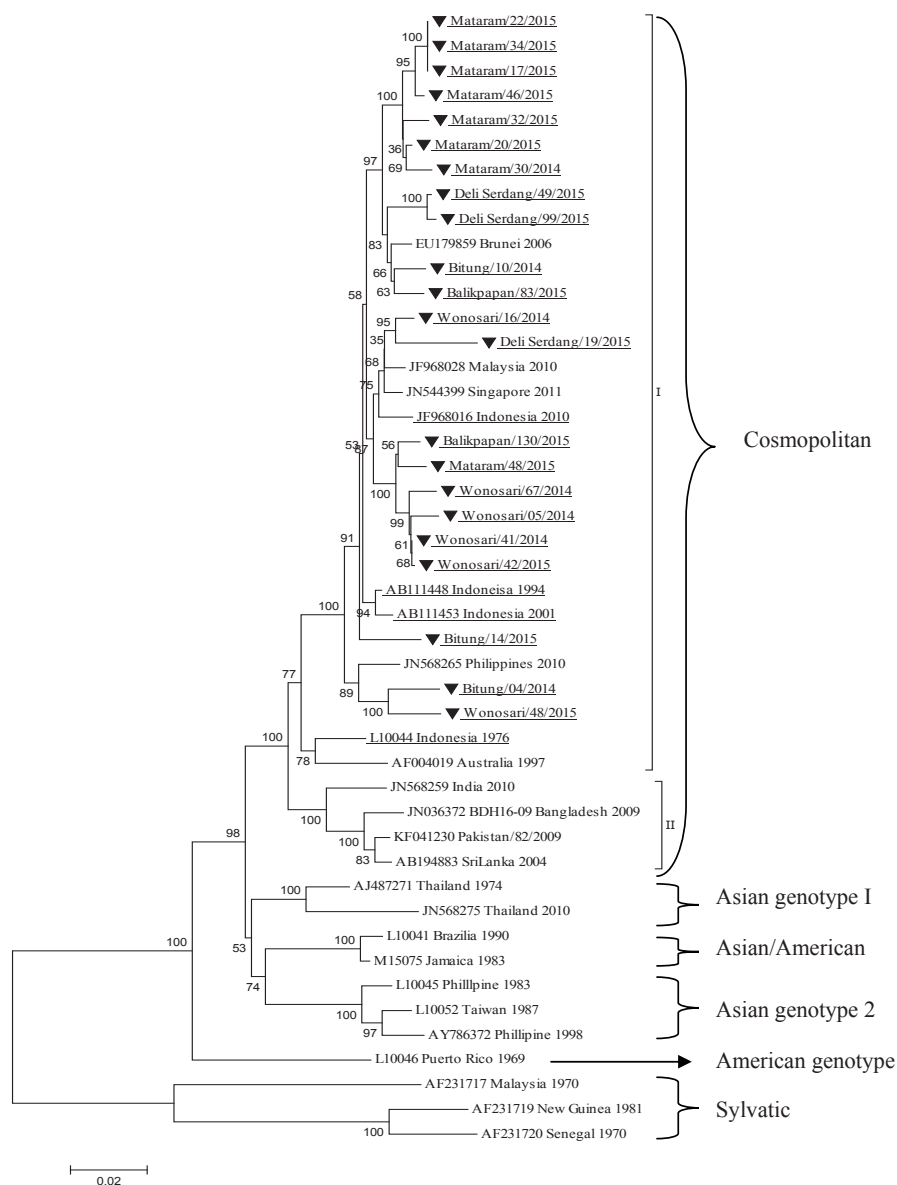


Figure 3. Phylogenetic tree of DENV-2 constructed based on envelope gene (1485 nt). The Indonesian sequences are designated with underline and sequences from dengue sentinel sites marked with solid triangle (▼). Strains from GenBank as references were denoted by accession number, country and year of isolation. Genotypes are also indicated by reference sequence, classification scheme as proposed by Twiddy et al 2002. Two Cosmopolitan sub-lineage indicated by I and II.

In addition, 28 sequences of DENV-3 were grouped in genotype I. (Figure. 4). The Origin of DENV-3 sequences found in this report were from Deli Serdang, Wonosari, Balikpapan and Bitung. Phylogenetic tree of DENV-3 showed that genotype I of DENV-3 circulated in sentinel sites were closely related to Malaysia 2006, Singapore 2009-2010 and Australia in 2008. These sequences showed more than 97% nucleotide homology. Phylogenetic tree analysis showed that DENV-3 circulated in sentinel sites had same ancestor.

DISCUSSIONS

This surveillance conducted in six district hospital located in six different islands which enable us to have representation of each region in Indonesian archipelago. It represented east (Maluku), central (East Kalimantan, North Sulawesi and West Nusa Tenggara) and west (Yogyakarta and North Sumatra) part of Indonesia. In this study the samples were collected from September 2014 to December 2015.

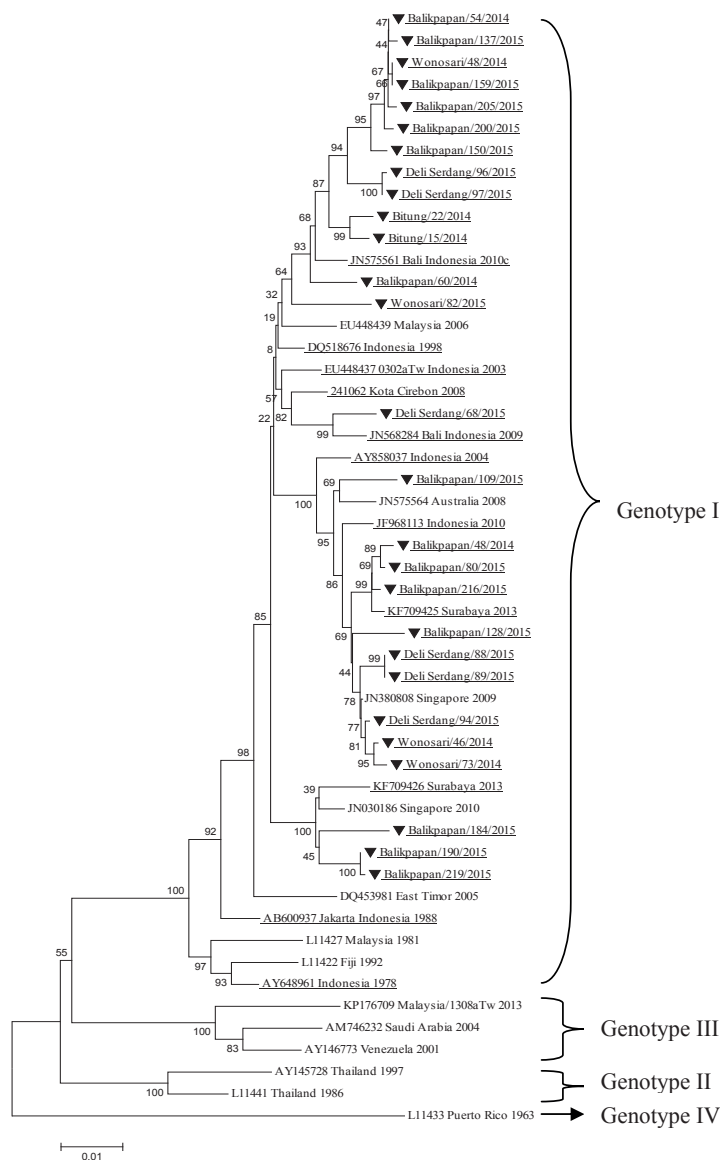


Figure 4. Phylogenetic Tree of DENV-3 constructed based on envelope gene (1479 nt). The Indonesian sequences were shown with underline. The sequences from dengue sentinel sites marked with solid triangle (▼). Sequences for reference from GenBank were denoted by accession number; country and year of isolation. Genotypes are also indicated by reference sequence, classification scheme as proposed by Lanciotti *et al* 1994.

It was reported by Sjatha *et al* (2012) that two genotypes of DENV-1 predominantly circulated in Indonesia, the genotype I and IV. The genotype IV of DENV-1 has been indigenous in Indonesia since 1948. It was likely to spread over the region and into the Indian Ocean countries and predicted diverged frequently around 1967.⁶ In Surabaya, another city in which endemic of dengue, genotype of the DENV-1 was reported to shift from genotype IV to I in 2010.¹² The genotype I circulated in the sentinel sites, and it have been circulated in Medan and Bandung in 2008 (data not shown), but another report suggesting that this genotype has been circulated in Indonesia since 2005.¹³ A recent study in Semarang has reported DENV-1 genotype II that was

the old genotype circulated in Indonesia,⁸ we did not find the genotype II in this study.

The Cosmopolitan genotype of DENV-2 has a wide geographical distribution including strains from Southeast Asia, Australia, India, China and the Middle East.¹⁴ Based on previous data, the Cosmopolitan genotype was separated into two geographically different sub-lineages. One sub-lineage observed in Southeast Asia, China and Oceania, other was observed in the India subcontinent.¹⁵ The Cosmopolitan genotype circulated in sentinel sites found in this study, was similar with the first sub-lineage.

DENV-3 is the most dominant serotypes found in this study and was grouped as genotype I. There were two sub-lineages of DENV-3 circulated in Indonesia that isolated before and after 1998, DENV-3 isolated after 1998 had gave greater impact on DHF epidemic.¹⁶ We found that all DENV-3 sequences from sentinel sites had close related to DENV-3 isolated after 1998. Other author have observed the existence of some intragenotypic groups that reveal amino acid changes in the envelope gene of DENV-3.¹⁷ Since DENV-3 had significantly higher nucleotide substitution rate and may potentially cause endemic outbreak,⁵ further study are needed to monitoring the spread of this virus in Indonesia.

Although this serotype was found in this study we are unable to obtain any DENV-4 sequences. To deal with this problem, in the future we could apply methods that utilize shorter fragments of E gene to determine the genotype of our DENV-4.

Laboratory-based dengue surveillance system in Indonesia has just initiated in late 2014 and still ongoing. Prior to that, samples from dengue cases were not collected continuously in sentinel system. Therefore we could not find comprehensive epidemiology and virology data from the related sentinel with consecutive years, the authors aware that many studies and incidental survey concerning dengue were carried out previously. Essential data was generated and dengue sequence could be obtained from public domain such as Genbank. However, continuous surveillance would be useful to understand the dynamic of dengue disease in Indonesia. Even though, some of the information about DENV genotype in Indonesia still can be found in some publication and also in Gene Bank.

In conclusion, we have presented the distribution of DENV genotype in dengue sentinel surveillance sites. These viruses were belongs to various genotypes that have been circulating previously in Indonesia.

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