BJMS
Borneo Journal of Medical Sciences

LETTER TO THE EDITOR

Detection of Four Mismatched Nucleotides between DENV4 Specific C-prM Primer (rTS4) and Current DENV 4 Sequences in Sabah

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Received: 13 February 2019

Accepted: 30 April 2019

How to Cite:
Aung, T., Emran, A., Thein, T., Gintarong, T., & Kobayashi, N. (2019). Detection of four mismatched nucleotides between DENV4 specific C-prM primer (rTS4) and current DENV 4 sequences in Sabah. *Borneo Journal of Medical Sciences (BJMS)*, *13* (2), 61–63. Retrieved from https://jurcon.ums.edu.my/ojums/index.php/bjms/article/view/1696

Keywords: dengue 4 C-prM primers

Dear Sir/ Madam,

Dengue is a mosquito-borne viral disease caused by dengue virus serotype 1 to 4 (DENV 1 to 4). Dengue outbreaks are common in both east and west Malaysia. Between July 2016 and December 2017, 200 serum samples were found to be positive for dengue by rapid test (Panbio Dengue Duo Cassette and Panbio Dengue Early rapid test kits; Abbott, Australia) of samples collected from patients in Sandakan and Kudat of Sabah state during dengue outbreaks. Serotypes of the dengue viruses were determined by reverse transcriptase polymerase chain reaction (RT-PCR) followed by nested PCR. C-prM primers designed by Lanciotti et al.[1] and later redesigned by Chien et al.[2] were used for the purpose. All the four dengue serotypes were detected with PCR products with specific sizes in gel electrophoresis. However, in four samples, no serotype-specific band was amplified by the nested PCR, while they were dengue-positive in RT-PCR showing 511 base pair (bp) amplicon. It was first presumed that these DENV might belong to a new DENV serotype, accordingly nucleotide sequences of the 511 bp amplicons of the four samples were determined. As a result, all the four samples (K35, K81, S23, and K75) (K and S stand for Kudat and Sandakan respectively) were found to belong to DENV4. Then the sequences of these samples were aligned with that of DENV 4 reverse primer (rTS4) (Figure 1).

The DENV4 specific primer rTS4 was found to have four mismatched nucleotides to the current DENV4 sequences in Sabah leading to the absence of DENV4 specific bands (260 bp) in nested PCR.

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                    TCAATATGCTGAAACGCGAGAGAAACCG Primer mD1(Dengue forward primer)
K35                 TTCAATATGCTGAAACGCGAGAGAAACCGCGTATCAACCCCTCAAGGGTTGGTGAAGAGA
K81                 ------------AAACGCGAGAGAAACCGCGTATCAACCCCTCAAGGGTTGGTGAAGAGA
S23                 -TCAATATGCTGAAACGCGAGAGAAACCGCGTATCAACCCCTCAAGGGTTGGTGAAGAGA
K75                 ------------AAACGCGAGAGAAACCGCGTATCAACCCCTCAAGGGTTGGTGAAGAGA
rTS4-reverse        ------------------------------------------------------------

K35                 TTCTCAACCGGACTTTTCTCCGGGAAAGGACCCTTACGGATGGTGCTAGCATTCATCACG
K81                 TTCTCAACCGGACTTTTCTCCGGGAAAGGACCCTTACGGATGGTGCTAGCATTCATCACG
S23                 TTCTCAACCGGACTTTTCTCCGGGAAAGGACCCTTACGGATGGTGCTAGCATTCATCACG
K75                 TTCTCAACCGGACTTTTCTCCGGGAAAGGACCCTTACGGATGGTGCTAGCATTCATCACG
rTS4-reverse        ------------------------------------------------------------

K35                 TTTTTGCGGGTTCTTTCCATCCCACCAACAGCAGGGATTCTGAAAAGATGGGGACAGTTG
K81                 TTTTTGCGGGTTCTTTCCATCCCACCAACAGCAGGGATTCTGAAAAGATGGGGACAGTTG
S23                 TTTTTGCGGGTTCTTTCCATCCCACCAACAGCAGGGATTCTGAAAAGATGGGGACAGTTG
K75                 TTTTTGCGGGTTCTTTCCATCCCACCAACAGCAGGGATTCTGAAAAGATGGGGACAGTTG
rTS4-reverse        ------------------------------------------------------------

K35                 AAAAAGAATAAGGCCATCAAGATATTGATTGGATTCAGGAAGGAGATAGGCCGCATGTTA
K81                 AAAAAGAATAAGGCCATCAAGATATTGATTGGATTCAGGAAGGAGATAGGCCGCATGTTA
S23                 AAAAAGAATAAGGCCATCAAGATATTGATTGGATTCAGGAAGGAGATAGGCCGCATGTTA
K75                 AAAAAGAATAAGGCCATCAAGATATTGATTGGATTCAGGAAGGAGATAGGCCGTATGTTA
rTS4-reverse        ------------------------------------------------------------G

K35                 AACATCTTGAATAGGAGAAGAAGGTCAACAATGACATTGCTGTGTTTGATTCCCACCGTA
K81                 AACATCTTGAATAGGAGAAGAAGGTCAACAATGACATTGCTGTGTTTGATTCCCACCGTA
S23                 AACATCTTGAATAGGAGAAGAAGGTCAACAATGACATTGCTGTGTTTGATTCCCACCGTA
K75                 AACATCTTGAATAGGAGAAGAAGGTCAACAATGACATTGCTGTGTTTGATTCCCACCGTA
rTS4-reverse        AACATCCTGAACGGGAGAA------------------------------------------
                    ****** ****  ******

K35                 ATGGCGTTTCACCTGTCAACAAGAGATGGCGAACCCCTCATGATAGTGGCAAAACACGAA
K81                 ATGGCGTTTCACCTGTCAACAAGAGATGGCGAACCCCTCATGATAGTGGCAAAACACGAA
S23                 ATGGCGTTTCACCTGTCAACAAGAGATGGCGAACCCCTCATGATAGTGGCAAAACACGAA
K75                 ATGGCGTTTCACCTGTCAACAAGAGATGGCGAACCCCTCATGATAGTGGCAAAACACGAA
rTS4-reverse        ------------------------------------------------------------

K35                 AGGGGGAGACCTCTCTTGTTCAAGACAACAGAAGGAATCAACAAATGCACCCTCATTGCC
K81                 AGGGGGAGACCTCTCTTGTTCAAGACAACAGAAGGAATCAACAAATGCACCCTCATTGCC
S23                 AGGGGGAGGCCTCTCTTGTTCAAGACAACAGAAGGAATCAACAAATGCACCCTCATTGCC
K75                 AGGGGGAGACCTCTCTTGTTCAAGACAACAGAAGGAATCAACAAATGCACCCTCATTGCC
rTS4-reverse        ------------------------------------------------------------

K35                 ATGGACCTGGGTGAAATGTGTGAAGACACTGTCACGTATAAATGTCCTCTACTGGTTAAC
K81                 ATGGACCTGGGTGAAATGTGTGAAGACACTGTCACGTATAAATGTCCTCTACTGGTTAAC
S23                 ATGGACCTGGGTGAAATGTGTGAAGACACTGTCACGTATAAATGTCCTCTACTGGTTAAC
K75                 ATGGACCTGGGTGAAATGTGTGAAGACACTGTCACGTATAAATGTCCTCTACTGGTTAAC
rTS4-reverse        ------------------------------------------------------------

K35                 ACCGAACCTGAAGACATTGACTGTTGGTG
K81                 ACCGAACCTGAAGACATTGA---------
S23                 ACCGAACCTGAAGACATTGA---------
K75                 ACCGAACCTGAAGACATTGA---------
rTS4-reverse        ----------------------------
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Figure 1 Alignment of DENV4 sequences of samples (K35, K81, S23, and K75) and rTS4 primer sequence. Primer mD1 sequence is shaded and reverse complement sequence of rTS4 primer is bolded. Asterisk indicates identical nucleotide.

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