LETTER TO EDITOR

Re-Emergence of Dengue 4 Virus

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Sir,

Dengue is a mosquito borne disease caused by any of the four serotypes of dengue virus. Dengue 4 virus (DEN-4) was once the predominant serotype found in Malaysia causing up to 60% of dengue fever (DF) cases in 1960's[1]. Since then, however, other serotypes notably DEN-2 and DEN-3 have become the predominant serotypes isolated among DF and dengue hemorrhagic fever (DHF) patients. DEN-4 made up only ~ 5% of the isolates and in the last five years (1995- 2000), no DEN-4 was isolated from patients attending the University Malaya Medical Center (UMMC) even during the peak of DF and DHF cases yet in Malaysia in 1998[2]. We report here isolation of five DEN-4 isolates from patients attending UMMC during the months of Jan - June, 2001 (Table I). DEN-4 virus was detected from infected cell culture using immunofluorescence staining with DEN-4 specific monoclonal antibodies. The presence of DEN-4 was confirmed here by multiplex reverse transcriptase polymerase chain reaction (RT-PCR) performed using a forward primer, DV1 and sets of four serotype specific reverse primers DSP1, DSP2, DSP3 and DSP4 to amplify a portion of NS3 region of the different dengue virus serotypes[3]. The following amplification parameters were used: reverse transcription at 48°C for 45 min; denaturation at 95°C for 2 min; and 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 30 sec and final extension at 72°C for 5 min. Amplified DNA fragments were electrophoresed in 1.2% agarose gel. DEN-2 and DEN-4 genomic RNAs and water was included as controls. DEN-2 generated an expected DNA band size of approximately 362 bp (Fig.1, lane 2) while DEN-4 generated a band of about 426 bp (Fig.1, lane 3). RT-PCR of all five DEN-4 isolates resulted in bands of 426 bp in size (Fig.1, lane 4-8). The presence of the predicted DNA band size indicated that all five isolates were DEN-4. This was later confirmed by partial sequencing of the amplified genome (data not shown). Successive isolation of DEN-4 within the first six months of 2001 after five years of no isolation could reflect re-emergence of DEN-4 in Malaysia. This could pose a public health threat since the virus has not been seen in the population for sometime, thus, it is not expected that the general population would have developed specific immunity against the virus. Further monitoring of the virus is therefore necessary and effective molecular techniques such as the multiplex RT-PCR and RNA/DNA sequencing could help in identification of the origin of the virus.

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Table I: Dengue 4 virus isolated in 2001 from dengue fever patients attending the University Malaya Medical Center, Kuala Lumpur

Clinical Isolates No.	Isolation Date	Patients		
		Age	Sex	Race
22713/01	January	42	Male	Indian
23096/01	April	23	Male	Indian
23264/01	May	41	Male	Malay
23298/01	June	35	Male	Malay
23314/01	June	17	Male	Malay

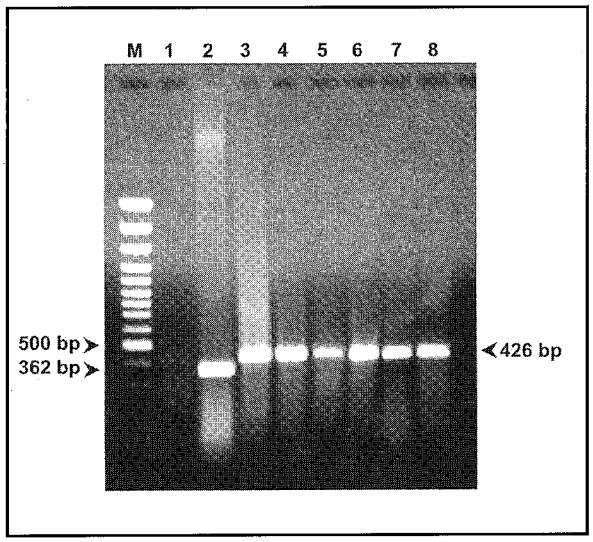

Fig. 1: Detection of dengue 4 virus using multiplex RT-PCR. Partial NS3 gene was amplified using specific oligonucleotide primers as described by Seah et al., (1995)[3]. Total RNA was extracted using Trizol (Life Technologies, USA) and RT-PCR was performed using Access RT-PCR Kit (Promega, USA). 100 bp marker (M); negative control (Lane 1); dengue 2 virus positive control (Lane 2); dengue 4 virus positive control (Lane 3); clinical isolates 22713 (Lane 4); 23314 (Lane 5); 23264 (Lane 6); 23096 (Lane 7); 23298 (Lane 8).

References

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