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A generic assay for whole-genome amplification and deep sequencing of enterovirus A71

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A B S T R A C T

Enterovirus A71 (EV-A71) has emerged as the most important cause of large outbreaks of severe and sometimes fatal hand, foot and mouth disease (HFMD) across the Asia-Pacific region. EV-A71 outbreaks have been associated with (sub)genogroup switches, sometimes accompanied by recombination events. Understanding EV-A71 population dynamics is therefore essential for understanding this emerging infection, and may provide pivotal information for vaccine development. Despite the public health burden of EV-A71, relatively few EV-A71 complete-genome sequences are available for analysis and from limited geographical localities. The availability of an efficient procedure for whole-genome sequencing would stimulate effort to generate more viral sequence data. Herein, we report for the first time the development of a next-generation sequencing based protocol for whole-genome sequencing of EV-A71 directly from clinical specimens. We were able to sequence viruses of subgenogroup C4 and B5, while RNA from culture materials of diverse EV-A71 subgenogroups belonging to both genogroup B and C was successfully amplified. The nature of intra-host genetic diversity was explored in 22 clinical samples, revealing 107 positions carrying minor variants (ranging from 0 to 15 variants per sample). Our analysis of EV-A71 strains sampled in 2013 showed that they all belonged to subgenogroup B5, representing the first report of this subgenogroup in Vietnam. In conclusion, we have successfully developed a high-throughput next-generation sequencing-based assay for whole-genome sequencing of EV-A71 from clinical samples.

1. Introduction

Enterovirus A71 (EV-A71) belongs to the Enterovirus A species of the family *Picornaviridae*, and is genetically divided into three genogroups (A, B, and C). The latter two are further divided into subgenogroups, denoted B0 – 5 and C1 – 5, respectively. Since 1997, EV-A71 has emerged as the most important cause of large outbreaks of severe and sometimes fatal hand, foot and mouth disease (HFMD) across the Asia-Pacific region (Solomon et al., 2010; Xing et al., 2014). In Vietnam, EV-A71-related HFMD was first described in 2003, and became a notifiable illness in 2008. Between 2011 and 2012, more than 200,000 hospitalized cases due to HFMD were reported in Vietnam, of which 207 died as a consequence of clinical complications (including cardio-pulmonary compromise with

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