Abstract

Introduction: Oseltamivir and zanamivir are neuraminidase inhibitors (NAIs) with important roles as drugs for prophylaxis and treatment of influenza. Whereas there have been reports of in vivo resistance of influenza B viruses to NAIs, currently there is lack of information regarding sensitivity or resistance to these drugs in influenza B viruses circulating in Kenya. Here, we report the isolation of influenza B viruses, phenotypic assessment to NAI activities and molecular characterization of NAI-relevant mutations in viruses that circulated in Kenya in the period 2011-2012. Materials and Methods: Influenza B viruses were isolated from patient nasopharyngeal specimens by inoculation onto MDCK monolayers. For phenotypic determination, enzyme inhibition assay using fluorescent MUNANA substrate was used. Known NA inhibitor-resistant and inhibitor-sensitive viruses were included in the assays as controls. IC50 values were determined using curve fitting implemented in Grafit version 7.0 software which is based on 50% of fitted upper asymptote. For molecular characterization of the mutations relevant in NAI resistance, RNA was extracted from the isolates followed by PCR amplification of NA gene segments using gene-specific primers. Nucleotide sequencing of the amplicons were performed using the Sanger dideoxy termination chemistry implemented using the BigDye technology prior to analyses using a suite of bioinformatics tools. Results and Discussion: Twenty four influenza B viruses were isolated and assed in this study. The mean IC50s of the isolates ranged from 17.1nM - 70.1nM for Oseltamivir and 0.0nM - 12.6nM for Zanamivir which were all within the 2011 WHO sensitive limits of 8-128nM for oseltamivir carboxylate and 0.5-12nM for Zanamivir. None of the isolates analyzed depicted oseltamivir or Zanamivir resistance at the eight amino acid positions E119, R152, D198, I222, S250, H274, R371, and G402 in the neuraminidase protein previously found to be associated with resistance or reduced susceptibility to oseltamivir and/or zanamivir. In conclusion, NAIs drugs were effective in treating influenza cause type B viruses during the 2011-2012 Kenyan seasons