Characterization of neuraminidase inhibitor susceptibility of influenza A isolates obtained in Kenya, 2008-2011

Abstract:

Background: Vaccines and antivirals are the mainstay for mitigation and clinical management of influenza infections. However, due to the ever-changing antigenic profile, vaccine formulations are revised every year to make them efficacious. Neuraminidase (NA) inhibitors, mainly oseltamivir and zanamivir, function both as prophylactic and as treatment agents. In NA inhibition by antivirals, inhibitor molecules mimic NA's natural substrate and bind to the active site, preventing NA from cleaving host cell receptors and releasing new virus. Currently, there exist no data on antiviral susceptibility profiles of influenza A isolates circulating within the Eastern African region. Here we characterised the antiviral susceptibility of the 2008-2011 influenza A viruses circulating in Kenya by combining both the genotypic data involving known molecular markers in NA protein responsible for drug resistance and IC50 data generated from NA inhibition assays. Materials and Methods: Nasopharyngeal swab specimens from consenting outpatients aged ≥ 2 months were obtained and transported to the National Influenza Centre and screened by real-time RT-PCR using primers targeted at the matrix and haemagglutinin genes of influenza A subtypes. Positive specimens were inoculated onto MDCK monolayers to isolate virus. RNA was extracted from virus isolates followed by PCR amplification of NA gene segments. Nucleotide sequencing was performed using the BigDye chemistry prior to analyses using a suite of bioinformatics tools. Drug susceptibility was determined by enzyme inhibition assay using fluorescent substrate with known NA inhibitor–resistant and –sensitive viruses as controls. IC50 values were determined using curve-fitting software (Grafit 7.0), which is based on 50% of fitted upper asymptote. Results: Of 836 influenza A virus isolates obtained (2008-2011), 108 (13%) were analysed for markers of resistance to NA inhibitors: 64% (7/11) of the 2008 seasonal influenza A/H1N1 isolates analysed showed oseltamivir-resistant marker H275Y, while all 33 (100%) influenza A/H3N2 isolates obtained showed sensitivity to oseltamivir. Genetic analyses of the A (H1N1) pdm09 isolates obtained in 2009-2010 showed that all were sensitive to oseltamivir. All 14 influenza A/H3N2 isolates obtained in 2011 were also shown to be sensitive to oseltamivir. A total of 28 isolates were further subjected to phenotypic susceptibility assay. The mean zanamivir IC50 values were 1.75, 2.53 and 1.84 nM for the subtypes H1N1, pH1N1 and H3N2, respectively. Two of the 2008 sH1N1 and one of the sH1N1 obtained in 2009 showed normal sensitivity to oseltamivir in the NA inhibitor susceptibility assay (mean IC50 of 1.28 nM). The rest of the 2008-2009 sH1N1 analysed (n = 8) showed highly reduced sensitivity to oseltamivir. The IC50 values in the fluorescent assay ranged from 73 to 984 nM. Pandemic A/H1N1 strains obtained between 2009 and 2011 indicated oseltamivir IC50 values of 1.60 to 6.32 nM—categorised as normal sensitivity. All 8 candidate influenza A/H3N2 isolates obtained between 2008 and 2011 were sensitive to oseltamivir, with IC50 values ranging from 0.16 to 0.94 nM. The 2011 WHO ranges and median IC50 values for oseltamivir carboxylate were 0.4 to 10 nM and 0.5 nM, 0.1 to 5 nM and 0.2 nM, and 0.2 to 10 nM and 0.6 nM for wild-type sH1N1, sH3N2 and pH1N1, respectively. The 2011 WHO ranges and median IC50 values for oseltamivir carboxylate were 257 to 3455 nM and 458.2 nM and 132 to 2179 nM and 191.3 nM for mutant types sH1N1 and pH1N1, respectively. The WHO IC50 values for zanamivir, both for mutant and wild-type strains, ranged from 0.2 to 3 nM for all

subtypes, with no significant differences between the mutant and wild-type strains for each subtype. Conclusion: Overall, our genotypic data demonstrate that there was oseltamivir resistance in seasonal influenza A (H1N1) viruses isolated in Kenya in 2008-2009. Most of the 2008-2009 sH1N1 isolates depicted highly reduced sensitivity to oseltamivir. This was due to the presence of the H275Y mutation in the NA protein sequence. H275Y mutation increased the IC50 value by 50- to 100-fold. Resistance to NA inhibitors was found to be specific to both drug and virus subtype. The drug susceptibility profile will be best informed using both elevated IC50 vales and known molecular markers of resistance.