Abstract

Background: The genome of a human coronavirus (HCoV) is composed of a linear, single-stranded, nonsegmented, positive-sense RNA of 27-32 kb. The pol gene of HCoV found in ORF1ab, is a good molecular chronometer for molecular characterization of HCoV types because in a region of ~900bp towards the 5', it contains two conserved flanks with a hypervariable middle. Thus, this region of the pol gene has been used to type all known HCoVs. Thus, molecular typing using this gene segment corresponds well to the classical typing based on serological cross-reactivity which groups CoVs into four groups. HCoVs cause a variety of mild and severe respiratory tract diseases including SARS and MERS. To date there are six known types of HCoVs. Although studies have shown evidence of global distribution of HCoVs and the diseases they cause, there is limited information on their presence, distribution and genetic characteristics in Kenya. Objective: To isolate, type and infer the genetic diversity of HCoVs that circulated in Kenya from 2009-2012 using the pol gene. Methods: Archived nasopharyngeal (NP) swab specimens from consenting outpatients aged ≥2 months were screened by real-time RT-PCR using HCoVspecific primers. Positive specimens were inoculated onto LLCMK2 monolayers to isolate the virus. RNA was extracted from virus isolates followed by PCR amplification of the HCoV pol gene using gene-specific primers. Nucleotide sequencing of amplicons was carried out using the BigDye chemistry prior to analyses using a suite of bioinformatics tools. Results and discussion: 29 of the 417 (7%) NP samples tested were positive for HCoV. The highest proportion (33%) were HCoV-NL63 followed by HCoV-HKU1 (30%), HCoV-OC43 (27%) and HCoV-229E (10%) respectively. SARS-CoV and MERS-CoV were not detected. Of the 29 positive samples, 14 (47%) yielded viral isolates for nucleotide sequencing. Sequence and Phylogenetic analyses identified 4 HCoV-HKU1, 5 HCoV-NL63, 4 HCoV-OC43 and 3 HCoV-229E. Mutation analyses revealed that 2/3 of the Kenyan HCoV-229E had Y4682L and one had F4821T amino acid substitutions relative to the prototype (GenBank Acc. No. NC_002645.1) The other human coronavirus types (HKU1, NL63 & OC43) had a few disparate silent mutations and were phenotypically identical to their respective prototypes. Conclusion: Four types of HCoVs circulated in Kenya during the study period. Genetic diversity in the hypervariable region of the pol gene was only observed in the HCoV-229E