Virus Genes
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SHORT REPORT

Genotypes of rhinovirus detected among children in two communities of South-West Nigeria

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Abstract

Rhinoviruses (RVs) are the most common etiological agent implicated in respiratory infections among infants and children. There are currently no approved antivirals and vaccine for use against the virus; hence, the need for information on the genotypes of rhinovirus from developing countries of the world with high burden of the infection. This study determined the genotypes of rhinovirus circulating among children in selected cities in Nigeria. Nasopharyngeal and oropharyngeal samples were carefully collected from children showing signs of respiratory infection in two communities in South-west Nigeria. Polymerase Chain Reaction was used to amplify the hypervariable part of the 5'- non-coding region, the entire viral protein gene 4 and the 5' terminus of the VP2 gene of RV. Nucleotide BLAST and phylogenetic analyses were used to genotype the isolates. Of the samples analysed, 12.7% showed rhinovirus positivity. All the three genotypes of rhinovirus were detected with genotype C (71.4%), being the predominant. Multiple strains of rhinovirus were found circulating. We showed for the first time the genotypes and strains of rhinovirus circulating in Nigeria. Further studies are required to highlight transmission patterns and disease severity among rhinovirus species in Nigeria.

Keywords Rhinovirus · Genotypes · Molecular · Phylogenetics · Nigeria

Introduction

Rhinoviruses (RVs) are the most common etiological agent implicated in respiratory infections among infants and children [1]. The RVs, which were previously thought to be associated with only mild infections of the upper respiratory tracts, have now been shown to cause severe infections of both the upper and lower respiratory tracts including pneumonia, bronchiolitis, wheezing, asthma exacerbation as well as chronic obstructive pulmonary disease (COPD) [2, 3].

The RVs are members of the enterovirus genus in the *Picornaviridae* family [1]. They are small, non-enveloped, single-stranded, positive-sense RNA viruses [4] with a genome of approximately 7.2 kb [2]. The viral genome consists of a single open reading frame (ORF) that encodes eleven proteins, including four capsid proteins (VP1, VP2, VP3, and VP4) [2, 5]. The non-coding region (NCR), together with the capsid region has been used extensively in the detection and genotyping of RVs [2, 5–7].

Over 160 genotypes, classified into three species (A, B, and C) have been identified [1, 2, 6]. Although there are currently no approved antivirals and vaccine for use against this pathogen, efforts are ongoing in these areas to produce effective agents [8].

Information on the genotypes of RV from developing countries of the world with high burden of respiratory infections is vital to the ongoing global efforts towards effective vaccine and antiviral development. This study was therefore designed to determine the genotypes of rhinovirus

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circulating among children in selected cities (Iwo and Ibadan) in Southwestern Nigeria.

Samples for the study were collected between January and May 2018, from children in a Staff School in Iwo as well as children attending routine immunization clinics in two primary health centres in Ibadan South-East Local Government.

Samples were collected from children showing symptoms of respiratory infection [9]. Informed assent was obtained from parents/guardian, and only children whose day of onset of symptoms were within seven days were included in the study. Both nasopharyngeal and oropharyngeal samples were carefully collected from each of the study participants and transported on ice to the Department of Virology, College of Medicine, University of Ibadan for laboratory analysis. The study protocol was approved by the University of Ibadan/University College Hospital ethics committee with approval number UI/EC/14/0284.

Viral RNA was extracted from the clinical samples using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A pair of primer (forward—5'-GCA TCI GGY ARY TTC CAC CAC CAN CC 3'; reverse—5'- GGG ACC AAC TAC TTT GGG TGT CCG TGT 3') was used to amplify a 549 bp region spanning the hypervariable part of the 5'- non-coding region (NCR), the entire viral protein (VP) gene 4 and the 5' terminus of the VP2 gene of RV [7]. The PCR amplification was initiated at 94 °C for 1 min, followed by 35 cycles of 94 °C for 1 min; 42 °C for 1 min; 72 °C for 2.5 min, and a final extension temperature of 72 °C for 7 min [10]. The samples were also screened for other respiratory viruses including human bocavirus and adenovirus following previously published protocols [11, 12].

PCR amplicons with expected RV products were selected for bi-directional sequencing. Sequencing was performed with the same pair of primers above using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and analysed on an ABI prism 3130xl genetic analyzer (Applied Biosystems).

Electropherograms were assembled, and consensus sequences were generated with CLC Main Workbench 7.6.2 software (CLC bio, Cambridge, MA, USA). The sequences were used in nucleotide BLAST analysis. The Sequences obtained in the study (MH746715-MH746727) as well as reference sequences of human rhinoviruses [5] retrieved from GenBank (http://www.ncbi.im.nih.gov) were used for phylogenetic analysis. Phylogenetic tree was constructed using the Neighbour-Joining algorithm on the MEGA 5.05 software. The test of significance of the tree topology was done by bootstrapping with 1000 replicates. Isolates were assigned a serotype identity based on the clustering pattern on the phylogenetic tree.

Results and discussion

A total of 118 samples (one from each participant) were analysed. The number of male participants exceed the females; however, there is no significant difference in rhinovirus positivity between both genders (Table 1). Forty-three of the participants (≈36.5%) were aged 0–6 months (Table 1). At least one of the respiratory viruses was detected in sixty-two of the samples (Fig. 1). Comprehensive data on the other viruses detected as well as co-infectivity is given in another report. Fifteen (12.7%) of the total samples analysed had detectable rhinovirus RNA. The highest percentage positivity of RV was detected in the month of January (Supplementary Table S1). Cough, rhinorrhea and fever are the symptoms found among participants that screened positive for rhinovirus.

Thirteen of the 15 rhinovirus-positive samples were successfully sequenced and genotyped. The genotypes C, A, and B (71.4%, 21.5%, and 7.1%, respectively) were detected. Phylogenetic analysis showed that multiple strains of rhinovirus, including: A10, A12, C12, C17, C22, C36, C37, and C51 were circulating among the study population (Supplementary Fig. S1).

The current study analysed the genetic epidemiology of human rhinovirus (HRV) among children in 2 south western Nigerian communities. Majority of the children sampled were symptomatic for respiratory illness, with 88(74.6%) having a cough and 77(65.3%) having rhinorrhea. Our detection rate of 15(12.7%) is relatively lower compared to that of similar studies done in Africa [5, 6,

Table 1 Rhinovirus detection among study participants. $P = 0.313$

Age group	No tested			No Positive		
	Male	Female	Total	Male	Female	Total (%)
0–6	26	17	43	2	2	4 (9.3)
7–12	13	13	26	3	1	4 (15.4)
13–18	2	9	11	1	2	3 (27.3)
19–24	11	1	12	3	1	4 (33.3)
> 24	17	9	26	–	–	–
Total (%)	69	49	118	9 (13.0)	6 (12.3)	15 (12.7)

Fig. 1 Venn Diagrammatic presentation of co-infectivity of Rhinovirus with other respiratory viruses screened. The values within regions of overlap depict multiple infections, while the values outside the overlap regions are indicative of mono infection. Twenty, 14 and 7 people had dingle infection with bocavirus, adenovirus and rhinovirus, respectively

13]. Monthly distribution showed that majority of infections occurred in the early months of January and February, this observation is consistent with that of a study in Washington which also reported high detection rates of HRV in the months of January and February [14]. This spike in level of HRV cases detected in the winter months might be attributed to the harsh weather conditions, coupled with other factors like increased time spent indoors contributing to increased risk of person to person spread of respiratory viruses such as HRV.

Molecular analysis identified the circulation of HRV species A, B, and C among our study population, with genotype C being the most abundant (71.4%). Recent studies from Kenya and South Africa [5, 13] have also shown high levels of species A and C circulation. Species B seems not to be efficiently transmitted around Africa with most studies including our study showing low species B detections [5, 13]. However, reports from outside Africa show contrasting species distribution, for instance high level species B detections have been reported between 2009 and 2012 in Spain and Thailand [15, 16], whereas HRV A was reported to be the main circulating species in USA, UK, Netherlands, and Japan within the same period of 2009–2012 [17, 18]. We identified six clades among species C (C12, C17, C22, C36, C37, and C51) and two clades for species A (A10 and A12). We were unable to associate disease severity with species; however, studies have associated species C with severe lower respiratory tract illness compared to HRV A [19, 20], though there have been some conflicting reports.

The main limitations of our study include the short duration of sampling which did not cover the two major seasons in Nigeria, our inability to screen for more respiratory viruses such as respiratory syncytial virus (RSV) and Influenza, and finally insufficient clinical information collected during the study period which did not allow for association of clinical severity of HRV infection with species clades identified. Regardless of these limitations we were able to characterize HRV from clinical samples among children showing signs of respiratory tract infections in Nigeria.

This is the first report of molecular characterization of HRV among children in Nigeria. We have shown the presence of HRV species A, B, and C with species C being the most diverse. This might not represent the actual picture of the distribution of HRV species circulating in Nigeria, but it gives insight into what the pattern of HRV transmission might be. Further large-scale studies would, however, be needed to highlight transmission patterns and disease severity among HRV species in Nigeria.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-021-01841-0.

Authors' contributions Conceptualization, OOO and YTN; Methodology, OOO and YTN; Formal Analysis, OOO; Investigation, OOO and YTN; Resources, OOO, YTN, JOA and ML; Data Curation, OOO, YTN, JOA and ML; Writing—Original Draft Preparation, OOO;

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Data availability All data generated or analysed during this study are included in this published article and the nucleotide sequence of the study isolates (Accession numbers MH746715-MH746727) can be obtained in Genbank.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval The study was approved by the University of Ibadan/University College Hospital ethics committee with approval number UI/EC/14/0284.

Consent to participate Informed assent was obtained from parents/guardian of the participants.

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