DRUG DISCOVERY

Population genetics and diversity of human adenovirus in two tertiary hospitals in Southern Nigeria

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ABSTRACT

The study of dynamic changes in genetic heterogeneity can shed light on the evolution of viruses because it is known that the genetic variety of viral populations within their hosts affects disease progression, treatment outcome, medication resistance, cell tropism and transmission risk. Our study's primary goal is to identify trends in the genetic makeup and variety of human adenoviruses using samples taken from children who attend two distinct tertiary hospitals in Southern Nigeria's Benin City and Nnewi. In Benin City and Nnewi, 330 pediatric patients' samples from tertiary hospitals were gathered and after DNA extraction and Sanger sequencing, the samples were examined. The obtained genetic information matrix was used to calculate the Jaccard similarity index, genetic distance and genetic diversity studies. According to the Analysis of Molecular Variance (AMOVA), this variation was more evenly distributed within each site's population (97%) than it was between them (3%). The effective number of alleles Ne, which represents genetic diversity at the population level, was 2.358 for Benin City and 2.255 for Nnewi, respectively. This shows that each of the studied loci had an average of 2.358 and 2.255 alleles per locus in these two populations. As a result, Nnewi has less genetic diversity of human adenovirus than Benin.

Keywords: Genetic diversity, human adenovirus, AMOVA, loci, children, population

1. INTRODUCTION

Viral gastroenteritis is a very widespread viral virus that affects many people all over the world. It presents a significant risk, particularly to those who are more vulnerable, such as children, the elderly and those with weakened immune systems (Lion, 2014). Gastroenteritis may be brought on by a variety of enteric viruses. According to epidemiological research, rotaviruses, astroviruses, enteric adenovirus serotypes 40 and 41 and the calicivirus family (Norovirus and Sapovirus) are the primary causes of acute gastroenteritis in infants and young children. Enteric human adenoviruses, which can cause outbreaks and rare

episodes of acute diarrhea, are estimated to be the third most prevalent non-bacterial cause of acute diarrhea in children.

The genus Mastadenovirus, which includes seven identified species and ranges in size from A to G, belongs to the family Adenoviridae, which has seveis where human adenoviruses (HAdVs) are categorized. Hemagglutination and serum neutralization reactions have been used to classify 51 serotypes to date, however genomic data has lately led to the identification of several novel and recombinant adenovirus types. Seven species of adenoviruses have been identified and over 60 distinct kinds have also been discovered. HAdVs are double-stranded, linear DNA viruses with genomes that are between 34 and more than 37 kb in size and 40 genes (Robinson et al., 2013). The disease spectrum not only includes gastroenteritis but also respiratory, ocular and urinary tract infections (Lion, 2014). Despite the clinical course typically being moderate and self-limiting, infections can occasionally lead to outbreaks with severe progression and a fatal conclusion, even in immunocompetent individuals.

People with normal immune systems and immune deficiencies have both experienced chronic illnesses related to adenovirus. These ailments have the potential to transmit deadly diseases. Adenovirus infections cause various disease patterns that vary depending on the viral species. Adenovirus species F types 40 and 41 are referred to as enteric adenoviruses since illness has been associated with them. Additionally, other species like A, C and D have been connected to diarrhea. In Nigeria, viral gastroenteritis has not been sufficiently studied and the particular contribution of adenovirus to childhood diarrhea is still unknown. Our study's major objective is to identify patterns in the genetic makeup and diversity of human adenoviruses from samples taken from kids who attended two separate tertiary hospitals in Southern Nigeria.

2. MATERIALS AND METHODS

Study area description

This cross-sectional study was conducted in two tertiary teaching hospitals, one in Nnewi, Anambra State and the other in Benin City, Edo State. The University of Benin Teaching Hospital in Benin City, Edo State and Nnamdi Azikiwe University in Nnewi, Anambra State, both participated in this study. After the samples had been prepared and the genetic materials had been extracted, the samples were examined at Iykeson Molecular and Diagnostic Laboratories in Awka.

DNA Extraction

Using the Quick-DNATM Viral Miniprep Kit (Zymo Research) in accordance with the manufacturer's instructions, DNA was extracted from all antigen positive adenovirus diarrhoeic feces. The collected RNA was then stored directly at -70°C until polymerase chain reaction.

PCR Amplification

Adenovirus PCR was performed using a 25-l final reaction volume, 1l of DNA, adenovirus forward and reverse primers (final concentrations of 100 nM each), PCR master mix (Taq Polymerase, dNTP, MgCl2 and reaction buffer) and PCR-grade water are the ingredients (as shown below) Menasra and Bouzaher, (2021). All amplifications were done on a Thermal Cycler (Applied Bio systems 2400 and Gene Amp PCR system 2400). 40 cycles at 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds and one cycle at 72°C for 5 minutes were utilized as the amplification conditions.

AdV F: GCC ACG GTG GGG TTT CTA AAC TT
AdV R: GCCCCAGTGGTCTTACATGCACATC

Bioinformatics analysis

Data were captured using ABI 3730 software, and analyzed using R packages in Rstudio v3 software. Measures of genetic variability were determined using GenAlEx 6.5, genetic diversity within and among accessions using GenAlEx 6.5. Phylogenetic analysis was constructed using the Ugene Unipro v44.

3. RESULTS

Genetic Diversity

In the tertiary hospitals in Benin and Nnewi, respectively, 97.22 percent and 96.6 percent of 324 loci were polymorphic with substantial allelic diversity and heterozygosity (Figure 1). We discovered a total of 1091 alleles across all 324 loci, with three or four alleles present at the majority of them (the most polymorphic loci) and two alleles present at only a few loci (Figure 2). There was an average of 2.358 and 2.255 alleles per locus among the examined loci in Benin and Nnewi, respectively, demonstrating that genetic

diversity at the population level consisted of these two populations (Figure 3). The number of private alleles for the two populations are 0.747 (Benin) and 0.620 (Nnewi). For Na, Benin was the highest population with Na=2.747, while Nnewi was the lesser variable one with Na=2.620 (Figure 3, Table 1).

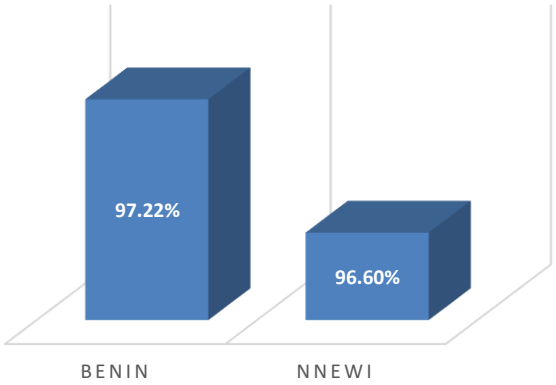

Figure 1 Identified polymorphic sites

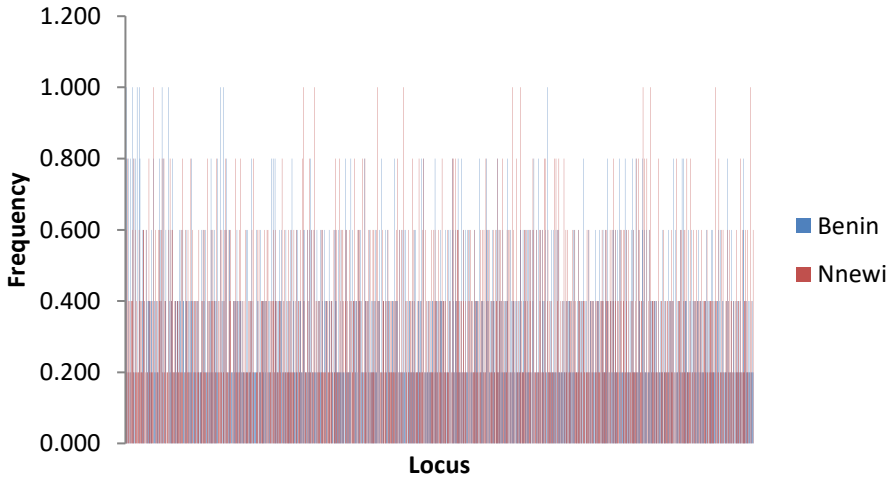

Figure 2 Allele frequency**:** Histogram depicting the allele frequency per locus

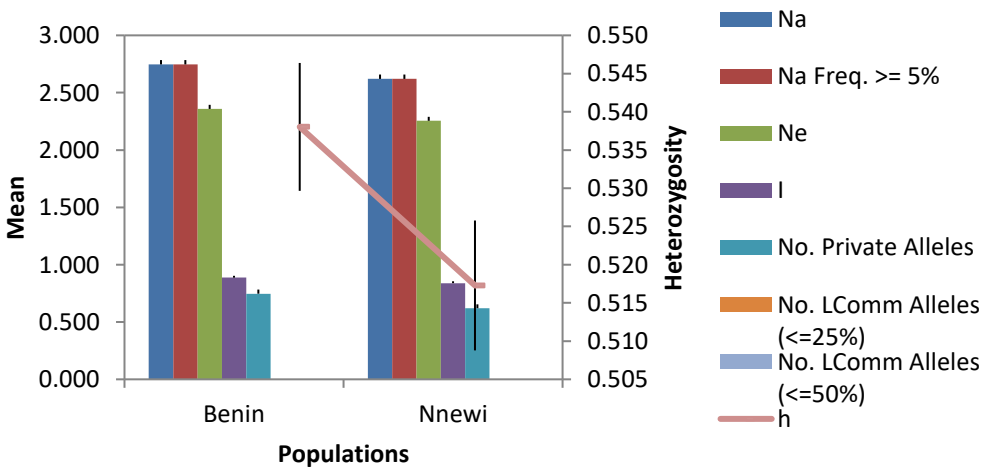

Figure 3 Allelic patterns across population: Histogram depicting the mean and standard errors across loci by location for the following statistics: Mean numbers of alleles (Na), alleles with a frequency higher than 5% (Na Freq. ≥ 5%) effective alleles (Ne), information index (I), number of private alleles (No. Pr. Al) and haploid diversity (h)

In addition, the genetic diversity parameters at the population level for the 10 sequences between the two sites were calculated to evaluate their overall informativeness (Table 1). The results showed that for Nnewi and Benin, respectively, the number of unique alleles (Na) ranged from 2.620 to 2.747, the number of effective alleles (Ne) from 2.255 to 2.358, the information index (I) between 0.839 and 0.887, diversity (h) from 0.517 to 0.538 In general, polymorphism and less discriminative sequences were found at all sites.

Table 1 Allelic patterns across population

Statistics	Mean values		Standard Error (SE) values	
Population	Benin	Nnewi	Benin	Nnewi
Na	2.747	2.620	0.038	0.038
Na Freq. >= 5%	2.747	2.620	0.038	0.038
Ne	2.358	2.255	0.036	0.035
I	0.887	0.839	0.016	0.016
No. Private Alleles	0.747	0.620	0.036	0.034
No. LComm Alleles (<=25%)	0.000	0.000	0.000	0.000
No. LComm Alleles (<=50%)	0.000	0.000	0.000	0.000
h	0.538	0.517	0.008	0.008
uh	0.673	0.647	0.010	0.011

Genetic distance

The genetic separation between the adenovirus in Benin and Nnewi, as calculated by Nei, (1978), is 0.380 (Table 2).

Table 2 General genetic diversity for the two locations

Benin	Nnewi	
0.000		Benin
0.380	0.000	Nnewi

In table 3, the Human adenovirus species with the lowest percentage similarity to its close relatives in Genbank was 938%, indicating related species. According to a review by Janda and Abbott from 2007, sequencing generally (>90%) but less frequently (65–83%) enables genus identification. To improve the accuracy of identification, a stricter boundary for species delineation was suggested (Meier-Kolthoff et al., 2013). The species included in this investigation had pairwise nucleotide similarity values that fell within the range required for accurate species identification.

Table 3 The human adenovirus identification, accession number and its close relatives in NCBI that isolated from children Attending tertiary hospitals in Benin and Nnewi

No.	Sample codes	Isolates	Accession numbers	Similarity to close relatives in NCBI (%)	Accession no of close relatives in NCBI
1	1_Alfred-Adeno_B05_05	*Human adenovirus sp.*	ON128719	100	MK995001.1
2	2_Alfred-Adeno_C12_09	*Human adenovirus sp.*	ON128720	98	MF962525.1
3	3_Alfred-Adeno_D05_11	*Human adenovirus sp.*	ON128721	98	MF962521.1
4	4_Alfred-Adeno_E05_14	*Human adenovirus sp.*	ON128722	98	MF962520.1
5	5_Alfred-Adeno_F05_17	*Human adenovirus sp.*	ON128723	98	MF962511.1
6	6_Alfred-Adeno_D12_12	*Human adenovirus sp.*	ON128724	98	MF962509.1
7	7_Alfred-Adeno_H05_23	*Human adenovirus sp.*	ON128725	98	MF962499.1
8	8_Alfred-Adeno_A06_03	*Human adenovirus sp.*	ON128726	98	MF962498.1
9	9_Alfred-Adeno_B06_06	*Human adenovirus sp.*	ON128727	98	MF962494.1
10	10_Alfred-Adeno_C06_09	*Human adenovirus sp.*	ON128728	98	MF962492.1

The phylogenetic analysis revealed that isolates from Benin and Nnewi were spread throughout the same cluster. Between isolates acquired from Nnewi and Benin and close associates from the Genbank, there is no significant phylogenetic connection (Figure 4). Therefore, it appears that distance affects evolutionary relationships. To solve this conundrum, more studies with a larger population are necessary. The evolutionary and functional links between the isolates can also be shown by examining a set of conserved genes, which is outside the purview of this work.

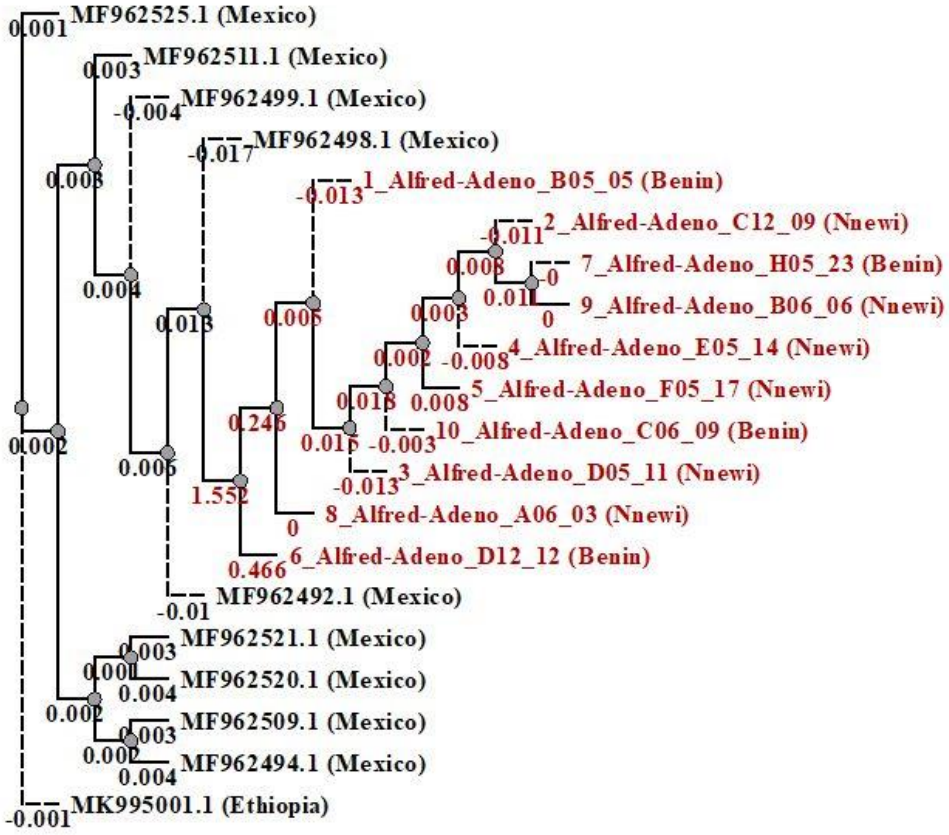

Figure 4 Phylogenetic tree illustrating the relationship among the human adenovirus identified and their close relatives in NCBI

Analysis of molecular variance (AMOVA) (Figure 5, Table 4) revealed that population diversity was significantly higher inside each community (97%) than between them (3%). Benin has the largest proportion of polymorphic loci (97.22%), followed by Nnewi with 96.60%.

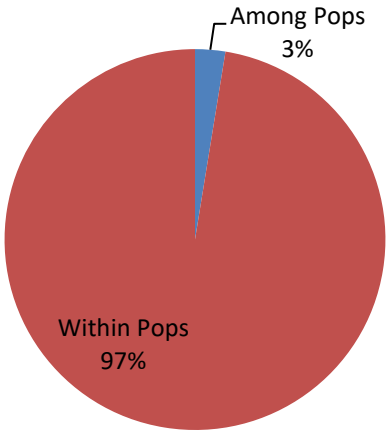

Figure 5 Percentages of molecular

Based on the sites' distance matrices, the major patterns between them were discovered and plotted using coordinate analysis (PCoA) (Figure 6). The overall variation within Human adenovirus was described by the first two PCoA axes in 67.48% of cases (Figure 6).

Table 4 Analysis of Molecular Variance (AMOVA) within and between sites

Source	df	SS	MS	Est. Var.	%
Among Pops	1	120.900	120.900	2.810	3%
Within Pops	8	854.800	106.850	106.850	97%
Total	9	975.700		109.660	100%

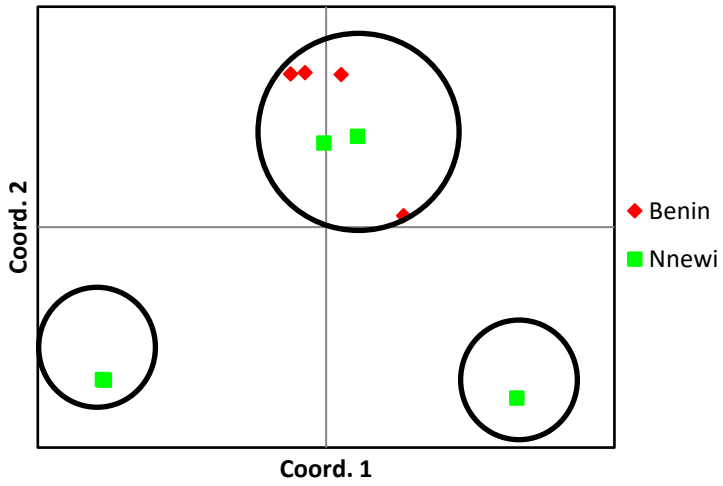

Figure 6 Principal Coordinate Analysis (PCoA) for human adenovirus in the both sites using a dissimilarity distance matrix.

4. DISCUSSION

Human adenovirus (HAdV) and human astrovirus frequently cause gastroenteritis (HAstV). Information on the prevalence and diversity of enteric viruses is essential for management and prevention methods (Gelaw et al., 2019). The levels of genetic diversity are essential for a species' ability to adapt to environmental change and the emergence of disease resistance. They define the viability and evolutionary potential of populations and are widely used to estimate species trends and future hazards (Booy, 2000). The genetic diversity of human adenovirus populations can be examined with accuracy and reliability by using molecular markers, as has been shown (Haider, 2011).

In this study, we examined the genetic diversity of human adenoviruses collected from 2 geographically distinct places, Benin and Nnewi, using 10 DNA sequences based on sanger sequencing. The results showed a slight difference in the number of alleles of each locus, ranging from 2.620 to 2.747. Additionally, the variances in each locus' values show that there is significant genetic variation among the populations at these 324 loci, emphasizing their significance in relation to the human adenovirus.

Benin's Ne value is 2.358 and Nnewi's is 2.255, according to analysis of human adenovirus genetic diversity at the population level, whereas their respective I value range from 0.887 to 0.839. The lower results show that Nnewi has a lesser genetic diversity of human adenovirus than Benin. The genetic diversity of viruses' hosts is increasing due to their rapid rates of mutation, short generation durations, and enormous population sizes (Duffy et al., 2008). It is known that the genetic diversity of viral populations within a host affects how well a treatment works. It influences cell and tissue tropism, transmission risk, illness progression and the emergence of medication resistance (Quer et al., 2017; Tsibris et al., 2009; Vignuzzi et al., 2006; Webber et al., 2017; Puller et al., 2017). Given that the human adenoviruses in Benin and Nnewi have a closer phylogenetic link than the more distantly related adenoviruses in Mexico and Ethiopia, it is possible that the lower genetic diversity of the human adenovirus in Nnewi is due to mutations. Additionally, the PCA analysis revealed that the human adenovirus populations from Benin and Nnewi share a genetic similarity (Figure 5).

5. CONCLUSION

In the current study, 10 DNA sequences based on sanger sequencing were gathered from 2 geographically distinct places, Benin and Nnewi, to explore the genetic diversity of human adenoviruses. According to the study's findings, there was little to no gene flow among the human adenovirus populations, and people from Benin had the most genetic variety. Geographic isolation may be a key factor in the genetic diversity of human adenovirus populations.

Ethical approval

Using the Quick-DNATM Viral Miniprep Kit (Zymo Research) based on the manufacturer's instructions. DNA Extraction & PCR amplification was performed by ethical guidelines.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Booy G, Hendriks R, Smulders JJ, Van Groenendael MJM, Vosman B. Genetic diversity and the survival of populations. Plant Biol 2000; 2(04):379-395.
2. Duffy S, Shackelton LA, Holmes EC. Rates of evolutionary change in viruses: Patterns and determinants. Nat Rev Genet 2008; 9(4):267-276.
3. Gelaw A, Pietsch C, Liebert UG. Genetic diversity of human adenovirus and human astrovirus in children with acute gastroenteritis in Northwest Ethiopia. Arch Virol 2019; 164 (12):2985-2993.
4. Haider N. Chloroplast-specific universal primers and their uses in plant studies. Biol Plant 2011; 55(2):225-236.
5. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils and pitfalls. J Clin Microbiol 2007; 45(9):2761-2764.
6. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev 2014; 27 (3):441-462.
7. Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP. When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 2013; 195(6):413-418.
8. Menasra A, Bouzaher S. GIS tools for landscape character assessment: Case of Ziban region in Algeria. Geomatics, Landmanagement and Landscape 2021.
9. Nei M. The theory of genetic distance and evolution of human races. Jpn J Hum Genet 1978; 23(4):341-369.
10. Puller V, Neher R, Albert J. Estimating time of HIV-1 infection from next-generation sequence diversity. PLoS Comput Biol 2017; 13(10):e1005775.
11. Quer J, Rodríguez-Frias F, Gregori J, Tabernero D, Soria ME, García-Cehic D, Perales C. Deep sequencing in the management of hepatitis virus infections. Virus Res 2017; 239: 115-125.
12. Robinson CM, Singh G, Lee JY, Dehghan S, Rajaiya J, Liu EB, Chodosh J. Molecular evolution of human adenoviruses. Sci Rep 2013; 3(1):1-7.
13. Tsibris AM, Korber B, Arnaout R, Russ C, Lo CC, Leitner T, Kuritzkes DR. Quantitative deep sequencing reveals dynamic HIV-1 escape and large population shifts during CCR5 antagonist therapy in vivo. PloS one 2009; 4(5):e5683.
14. Vignuzzi M, Stone JK, Arnold JJ, Cameron CE, Andino R. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. Nature 2006; 439(7074):344-348.
15. Webber QM, Fletcher QE, Willis CK. Viral richness is positively related to group size, but not mating system, in bats. Ecohealth 2017; 14(4):652-661.