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Complete genome sequences of nine double recombinant vaccine-derived novel oral poliovirus type 2 genomes from Nigeria 2023–2024

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ABSTRACT We report the complete genome sequences of nine double recombinant vaccine-derived novel oral poliovirus type 2 genomes from acute flaccid paralysis (AFP) cases (n = 3), AFP case contacts (n = 4), and environmental surveillance sampling (n = 2) in Nigeria.

KEYWORDS poliovirus, nOPV2, next generation sequencing

Poliovirus, from the family *Picornaviridae*, is a single-stranded, positive-sense RNA (+ssRNA) virus with a genome of approximately 7,500 nucleotides and a causative agent of poliomyelitis. To better address the global evolving risk of type 2 circulating vaccine-derived poliovirus (cVDPV2), a novel oral poliovirus type 2 (nOPV2) vaccine was genetically engineered and first distributed to countries in March 2021 (1, 2). The nOPV2 vaccine is a modified version of the preexisting type 2 monovalent OPV (mOPV2) vaccine and provides comparable protection against poliovirus while being more genetically stable and less likely to revert to a form that can cause paralysis (1). The nOPV2 vaccine's increased genetic stability should result in a reduced risk of seeding new cVDPV2 emergences compared to the mOPV2 (Sabin 2) vaccine.

The nine samples were received at the Centers for Disease Control and Prevention via FTA cards from the WHO National Polio Laboratory (polioeradication.org), University of Maiduguri Teaching Hospital, Maiduguri, Nigeria (see Table 1). Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Cat. No. 52906; Qiagen), and cDNA was generated by using SuperScript IV First-Strand Sequencing System (Cat. No. 18091200; Invitrogen) using random primers. Klenow Fragment (3′–5′ exo-) (Cat. No. M0212S, NEB) was used for cDNA second-strand synthesis. For Illumina sequencing, the library was prepared using the Nextera XT Library Kit (Cat. No. FC-131-1096; Illumina) and sequenced on the Illumina MiSeq platform (2 × 250 bp).

Raw read data were processed using VPipe version 1.0, our in-house pipeline for processing viral specimens (3), using default parameters. Additionally, a reference-based assembly was performed in Geneious Prime version 2023.1.1 using default parameters and a medium sensitivity. Genome alignments were performed using MAFFT version 7.490 to compare with the reference genome (GenBank accession AY184220) and verify results and identify recombination events (see Table 1). The MrBayes version 3.2.6 plugin in Geneious Prime using an HKY substitution model and a gamma rate variation was used to make the capsid tree (see Fig. 1), and regions of recombination events were identified as Enterovirus species C.

We isolated the full nOPV2 genomes from patients, contacts, and environmental sampling in Nigeria that have undergone double recombination events and were

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TABLE 1 Sequencing summary of nine double recombinant circulating vaccine-derived nOPV2 genomes from Nigeria

GenBank accession	SRA accession	Sample name	Collection year	Type	Country	State	Surveillance type	Genome length (nt)	Average coverage	SNP count when compared to VP1 reference (AY184220)	Total no. of pre-processed reads	Total no. of post-processed reads	Total no. of mapped reads	GC content (%)
PQ059262	SRR30089174	NIE23-001	2023	nOPV2	Nigeria	Katsina State	AFP case contact	7,442	1,262.7	12	2,224,238	439,508	68,963	46.2
PQ059263	SRR30089173	NIE23-002	2023	nOPV2	Nigeria	Kano State	AFP case	7,442	366.0	13	1,600,498	302,501	20,323	46.0
PQ059264	SRR30089172	NIE23-003	2023	nOPV2	Nigeria	Kano State	Environmental	7,442	867.8	16	1,881,262	372,247	44,987	46.0
PQ059265	SRR30089171	NIE23-004	2023	nOPV2	Nigeria	Kano State	Environmental	7,442	78.3	16	713,470	32,591	4,058	46.0
PQ059266	SRR30089170	NIE24-005	2024	nOPV2	Nigeria	Kano State	AFP case	7,442	403.5	13	2,029,418	757,739	25,357	46.2
PQ059267	SRR30089169	NIE24-006	2024	nOPV2	Nigeria	Kano State	AFP case contact	7,442	1,031.2	16	2,159,638	407,361	59,321	46.2
PQ059268	SRR30089168	NIE24-007	2024	nOPV2	Nigeria	Kano State	AFP case contact	7,442	503.1	15	1,406,028	494,068	30,176	46.1
PQ059269	SRR30089167	NIE24-008	2024	nOPV2	Nigeria	Kano State	AFP case	7,442	104.7	17	208,442	100,142	6,089	46.0
PQ059270	SRR30089166	NIE24-009	2024	nOPV2	Nigeria	Kano State	AFP case contact	7,442	139.5	12	862,648	197,435	8,816	46.0

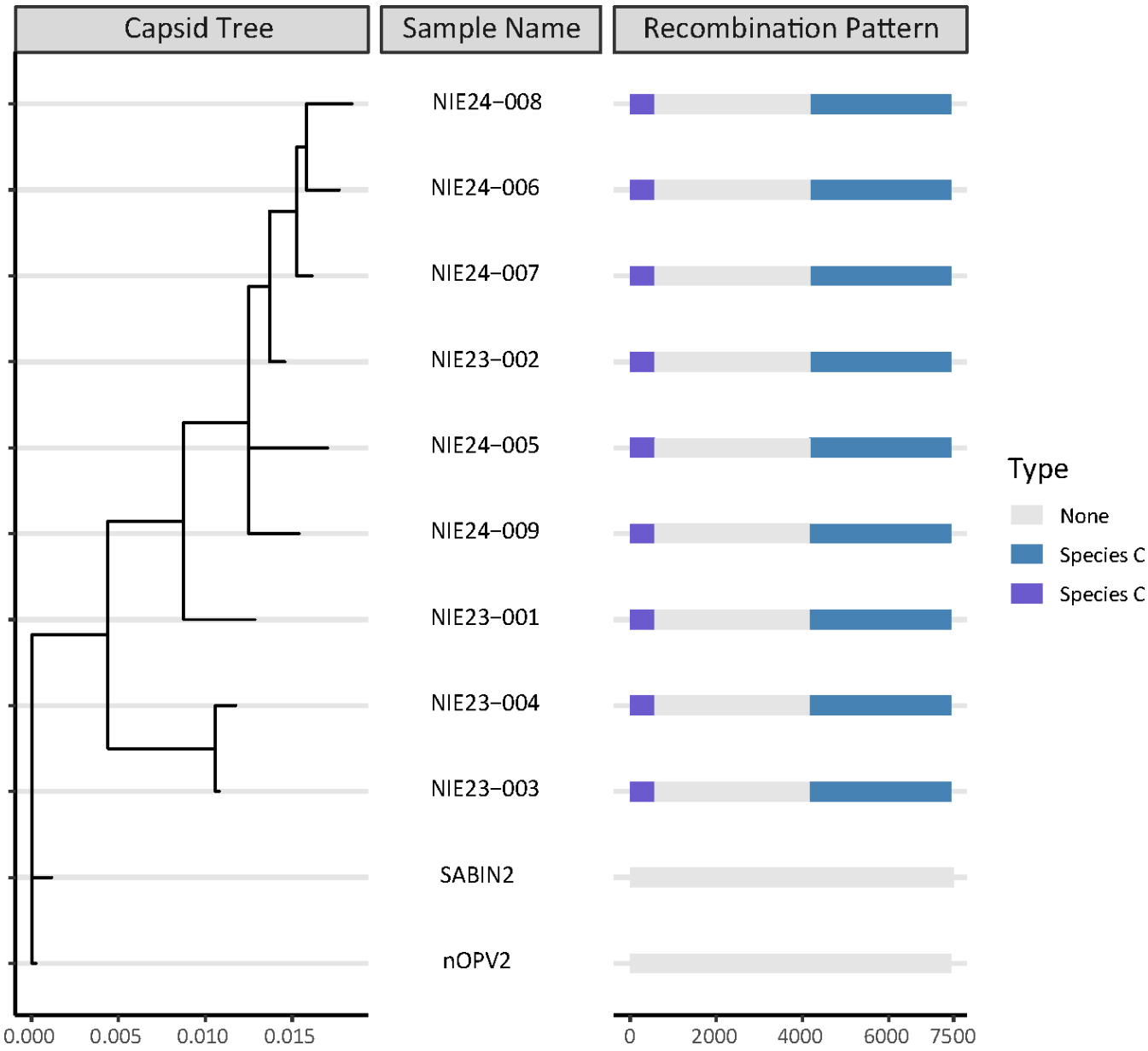

FIG 1 Double recombinant cVDPV nOPV2 Nigeria samples showing a capsid tree and the recombination pattern of their full-length genomes. All samples are in the NIE-KTS-1 emergence group and show similar double recombination events. Sabin2 (AY184220) and nOPV2 (MZ245455) sequences are shown for reference. "None" delineates areas where no recombination was observed. Purple shows the 5′ untranslated region recombinant area matching an Enterovirus species C. Blue shows the P2/P3 recombinant area of the genome matching a different Enterovirus species C. All nine full-length genomes show a similar recombinant pattern, and all genomes show a 98%–99.8% sequence pairwise percent identity to each other.

identified as VDPV2s (4). The entire 5′ untranslated region and the non-structural region (P2/P3) of the genome showed evidence of recombination (see Fig. 1), essentially removing the nOPV2 modifications except three nucleotide markers within the capsid (P1) region. The identification of these double recombinant genomes is responsible for classifying a new cVDPV2 emergence group from nOPV2 origin in Nigeria called NIE-KTS-1, first detected in an AFP contact in 2023. This emergence group in Nigeria is in addition to the previously reported cVDPV nOPV2 emergence group, NIE-KBS-1 previously detected in 2023 as well (5), yet distinctly different cVDPV nOPV2 strains. Identifying these nine double recombinant genomes from AFP case patients is noteworthy for

tracking the genetic characterization of all circulating nOPV2 strains at the current stage of nOPV2 vaccine use.

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DATA AVAILABILITY

The genome sequences have been deposited in GenBank with the accession numbers PQ059262-PQ059270. The postprocessed FASTQ reads have been deposited in the Sequence Read Archive with the run accession numbers SRR30089166-SRR30089174.

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