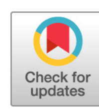

Nearly Complete Genome Sequences of Type 2 Sabin-Like Polioviruses from Northern Nigerian Poliovirus Surveillance, 2016 to 2018

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ABSTRACT We sequenced 109 type 2 Sabin-like poliovirus isolates that had been collected from acute flaccid paralysis patients or healthy children in Nigeria. Understanding the genetic makeup of these viruses may contribute to polio eradication efforts.

The Global Polio Eradication Program has reduced the number of annually diagnosed wild polio cases worldwide by >99.9% (1), from an estimated 350,000 cases in 1988 to 5 wild poliovirus cases in Afghanistan and Pakistan in 2021. To support polio eradication efforts, we sequenced and analyzed 109 type 2 Sabin-like poliovirus isolates that had been collected from surveillance in northern Nigerian from 2016 to 2018. These sequences were divided into two groups, i.e., samples collected from routine acute flaccid paralysis (AFP) surveillance (AFP group, $n = 84$) and samples from a stool survey of healthy children (HC) (HC group, $n = 25$). AFP surveillance is the primary case-based syndromic surveillance system for detecting poliovirus worldwide (2, 3). Targeted HC stool surveys collect and test stool samples from high-risk HC when there is a high degree of suspicion of circulating poliovirus (4).

Polioviruses, the causative agent of polio, belong to the *Enterovirus C* species, in the family *Picornaviridae*. Viruses were isolated using the recommended WHO isolation protocol described previously (5–7). A stool suspension was inoculated in cell culture with two cell lines that contain the poliovirus receptor. Cytopathic effect (CPE)-positive cell cultures were screened by real-time PCR to determine the poliovirus serotype. Whole-genome sequencing was performed on all isolates. The Qiagen QIAamp viral RNA Mini kit was used to extract RNA. After RNA was extracted from the poliovirus tissue culture samples, nonviral DNA was digested using the Ambion DNA-free DNA removal kit. First-strand cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen) and primers containing 8-nucleotide random sequences with an additional 20-nucleotide PCR tag (8). Second-strand synthesis was completed using the Klenow fragment of DNA polymerase I, primed with the same tagged random primers. The resulting double-stranded cDNA was amplified using Applied Biosystems AmpliTaq Gold polymerase, primed with the same 20-nucleotide PCR tag. The random amplicon was cleaned using 1.8× AMPure XP solid-phase reversible immobilization (SPRI) bead cleanup. Illumina

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TABLE 1 Recombination and reversion sites

Group	No. (%)				
	Recombination[a]	Reversion at 5′ UTR position 481[b]	Reversion at VP1 position 143[c]	Reversion at 5′ UTR position 481 and VP1 position 143	Total[d]
AFP	2 (2)	30 (36)	23 (27)	17 (20)	84
HC	0 (0)	9 (36)	5 (20)	1 (4)	25

[a] Intertypic recombination between Sabin strains.
[b] Reversion site in 5′ UTR.
[c] Reversion site in capsid VP1.
[d] Total number of nearly complete genomes.

libraries were generated using the Nextera XT library preparation kit and sequenced on an Illumina MiSeq instrument using a 500-cycle paired-end run with multiplexing. The read length was 250 bp. Raw read counts ranged from 100,732 to 870,130 reads for all samples. Average read coverages across the assembled genomes ranged from 107.24× to 2,124.79×.

Next-generation sequencing data were analyzed using VPipe (9). The preprocessing steps for the reads included trimming, host removal, and read deduplication. Human reads were removed using Bowtie 2 v2.3.3.1 (10). Primer trimming, adapter trimming, and sequence quality score filtering were performed using Cutadapt v1.8.3 (11). The Python script Dedup.py was used to deduplicate reads (8), which were assembled *de novo* using SPAdes v3.7.0 (12) with default k-mer settings. In addition, reference mapping using Geneious v2020.0.5 was performed using the clean reads, the contigs from SPAdes, and the Sabin reference genome (GenBank accession number AY184220.1). Recombination and reversion sites were analyzed using Geneious.

We compared recombination and reversion sites within samples in the two groups. Recombination was observed in 2 isolates from the AFP group but not in isolates from the HC group, and some reversions were observed in both groups (Table 1). Only intertypic recombination between Sabin poliovirus strains (e.g., Sabin 2/Sabin 1 or Sabin 2/Sabin 3 recombinants) was observed. Reversion at nucleotide position 481 in the 5′ untranslated region (UTR) (13) was observed in both groups; 10 of 25 HC sequences had this position reverted from A to G, whereas 47 of 84 AFP sequences had this reversion (Table 1). These observations are comparable to those of an early type 2 Sabin-like poliovirus study (14).

Data availability. The sequencing data have been deposited in the NCBI SRA under BioProject accession number PRJNA779084 and SRA accession numbers SRR16902533 and SRR16902641 and in GenBank under accession numbers ON596331 to ON596439.

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