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**Isolation and Molecular Characterization of Polio Virus Isolates from Sewage Samples in Some Selected Settlements and Internally Displace Person (IDPS) Camps in Maiduguri Metropolis**Muhammad Talle<sup>1</sup>, Auwalu Uba<sup>2</sup>, Fatima Tahir<sup>2</sup>, Modu Mallam Ali<sup>3</sup> and Kaltum T. Babagana<sup>3</sup>

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**Author for Correspondence:** muhammادت6@gmail.com/+234-803-472-2684**Abstract**

Acute Flaccid Paralysis (AFP) surveillance is the gold standard for polio virus surveillance in polio eradication initiative globally but environmental surveillance complements AFP surveillance, help monitor the genetic diversity of isolated poliovirus and can help to verify that circulation of wild polio virus has stopped in an area. The study was carried out to isolate and characterize polio virus isolates from sewage in an effort to complement the acute flaccid paralysis surveillance for wild poliovirus in Maiduguri metropolis. The samples were concentrated using the two-phase separation method, and treated following the WHO guidelines on environmental surveillance for the detection of poliovirus. Isolation of Polio virus was done by tissue culture technique using RD cell line (Rhabdomyosarcoma) and L20B cell line (A genetically engineered mouse cell line) obtained from WHO National Polio Lab (UMTH Maiduguri), adopting the WHO standard procedures. All positive culture (CPE, detached, round and refractile cells) were harvested for ITD (Intratypic differentiation) using Realtime PCR. A total of 36 litres of Sewage samples from 12 settlements were analyzed (3litres from each settlement) out of which 10 samples were found to be positive for serotypes P2 and P3 with isolation rate of 10 (27.8%). Type 3 serotype has the highest isolation rate 6(60%) followed by type 2 serotype 4(40%). Distribution by settlements revealed highest isolation rate of 4(11.1%) from Abbaganaram site followed by Bulabulun Gidan Zana 3(8.3%) while budum round about, Gwange sabon layi and Zaman clinic sites reported one serotype each 1(2.8%). Isolation from IDPs camps out of the 30 liters of sewage analysed, revealed only Non-Polio Enteroviruses (NPENT) with isolation rate of 9(30%) with highest distribution from Dalori camp1 and teachers village each with 2(6.7%) followed by Dalori camp2, Gubio camp, Muna custom, Muna Haruna and Farm centre

camp each with 1(3.3%). No isolation was reported from NYSC, Bakassi and Goni-kachalla camps. The results confirm the prevailing immunity gap among children in IDPs camps despite immunization campaigns using bivalent oral polio Vaccine (bOPV). It is expected that children will shed the virus and therefore could be isolated from sewage but only NPENT were isolated (No Sabin strains) in all the camps visited indicating non-response of the IDP's children to the vaccines. This could be attributed to malnutrition which has been reported in IDPs camps as malnourished children may have different immunological response to vaccines. In comparison to some selected settlements a number of sabin-like strains were isolated indicating a good vaccine response and shedding by the immunized children which can as well help to boost herd immunity.

**Key words:** Polio virus, Environmental surveillance, Maiduguri.**Introduction:**

Effective environmental surveillance relies upon the Polio virus (PV) excretion pattern characteristically exhibited by infected individuals. PV transmission is via person-to-person, both via the faecal-oral and the oral routes (the latter being most probable in developed countries with high hygiene standards). Irrespective of the presence or absence of clinical symptoms, PV replication in infected individuals initially occurs in the pharynx and usually continues in the para-intestinal submucosal lymphatic tissue for several weeks to a few months after initial infection. Throughout this period virus is excreted into the faeces and shed into the environment. Shedding may be intermittent and is affected by the immune status and immune competence of the individual. Past natural infection with wild polio virus (WPV) and vaccination with OPV serve to significantly reduce the extent and duration of PV shedding. The amount

of virus excreted into stools is known to be variable, with maximal amounts reaching 10<sup>7</sup> infectious doses/day per person. Poliovirus remains infectious in the environment for varying lengths of time depending on the immediate conditions. PVs are relatively stable in aqueous environments at ambient temperatures, and adsorption to various solid materials in the environment may further extend the time over which at least part of the infectivity can be recovered. Early studies demonstrated that increased temperature, ammonium concentrations and pH are major factors in the natural inactivation of PV in sewage. The average time required for a 90% decrease (corresponding to approximately 1 log<sub>10</sub> unit) in enterovirus titre in sewage sludge has been reported as 180 days at 2°C and 26 days at 23°C. Factors that affect the transmission of the virus include extent of crowding, levels of hygiene, water quality, and sewage handling facilities. In general, in endemic countries and areas, WPVs (in contrast to the Sabin-like (SL) vaccine viruses from OPV) have a distinct seasonal pattern of circulation that varies by geographic area. In tropical and semitropical areas circulation tends to be year-round, or often associated with the rainy season. Prior to poliovirus immunizations, in temperate areas polioviruses were most prevalent in the summer and fall, although outbreaks could continue into the winter. SL virus should be detectable at all times in countries with routine use of OPV or detections may cluster around the time of national or sub-national supplementary immunization activities (SIAs). Not all PV shed in the faeces is readily available for detection through environmental surveillance. Populations living in dwellings with water toilets connected to a converging sewer network can be monitored by collecting representative samples from downstream major collectors or sewage treatment plants. Other systems used for the handling of human waste pose significant challenges for collection of representative environmental samples and may significantly decrease the value of environmental surveillance in monitoring for PVs.

## Materials and Method

### Extraction of Polio Virus from Sewage Samples

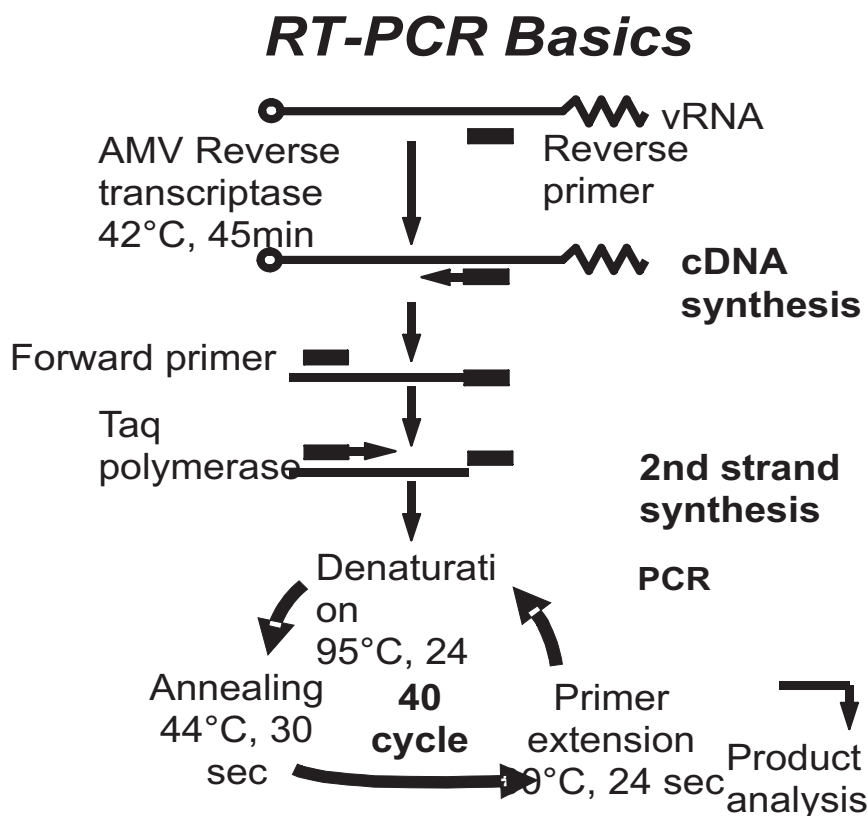
Sewage samples were concentrated using the two-

phase separation method. To 500ml of the sewage sample, the following were added: -287 ml of 29% PEG (polyetheneglycol), 39.5ml of 22% Dextran and 35ml of 5M NaCl. The mixture was mixed thoroughly by vigorous stirring for 1hr on a magnetic stirrer. During the stirring, the separation funnel was prepared and allowed to stand upright in vertical positions. The sample was poured carefully in to the separation funnel and allowed to stand overnight at 4°C. The entire lower phase and the interphase were harvested slowly drop wise into a sterile 50ml centrifuge tube. The harvested concentrate was re-suspended with the pellet, treated with 20% chloroform and centrifuged at 4°C for 20min at 1500rpm. The upper layer was carefully collected and antibiotic was added (penicillin and streptomycin to a final concentration of 100iu/ml and 100ug/ml respectively). The concentrated specimen was kept at -20°C until used (WHO, 2015).

### Inoculation of the Sewage Extract

Isolation of Polio virus was done by tissue culture technique using RD cell line (Rhabdo-myosarcoma) and L20B cell line (A genetically engineered mouse cell line expressing human polio receptors CD<sub>155</sub>) obtained from WHO National Polio Lab (UMTH Maiduguri), adopting the WHO standard procedures. Sewage extracts (0.5mls) were inoculated onto a healthy actively metabolizing monolayer of L20B and RD cells and incubated at 36°C to demonstrate in-vitro infection. The inoculated flasks (5flasks for L20B and one flask for RD) were examined daily for the presence of CPE which indicates the presence of virus. All flasks were observed daily for characteristics enterovirus CPE after inoculation, i.e., rounded, retractile cells detaching from the surface of the flasks, all observations were recorded and allowed CPE to develop until at least 75% of the cells are affected (3+ CPE). At this stage, a second passage in the other cell line was performed. CPE positive cells from duplicate cultures of the same sample in the same cell line were pooled for passage into a flask containing a monolayer of cells from the second cell line in 5ml of maintenance medium. All positive isolates from sewage samples were referred for Intratypic differentiation (ITD) using real time PCR (WHO, 2015).

Figure 1: PCR Background and Protocol Chart



**Sample preparation for RT- PCR:** 50 l virus cell culture was added into dilution tube and centrifuge (bench top micro centrifuge at 5,000 rpm) at room temperature for 2 minutes. 0.5 l of cell culture supernatant (or 1l of Control RNA) for each sample was added into the appropriate reaction strip/plate well. The strips were Place in real-time thermo cycler and run cycle. The endpoint fluorescent data was collected at the end of the anneal step (CDC, 2012).

#### Result

Tables 1 and 2 show the distribution of poliovirus serotypes from sewage samples obtained from selected settlements. Out of the 36 liters of sewage samples analyzed, 10 were found to be positive for serotypes P2 and P3 with isolation rate of 10 (27.8%).

Type 3 serotype has the highest isolation rate 6(60%) followed by type 2 serotype 4(40%). Distribution by settlements revealed highest isolation rate of 4(11.1%) from Abbaganaram site followed by Bulabulun Gidan Zana 3(8.3%) while Budum Roundabout, Gwange Sabon Layi and Zaman Clinic sites reported one serotype each 1(2.8%). Table 2 show the distributions of NPENT from IDP camps. Out of the 30 liters of sewage analyzed, only NPENT were isolated with isolation rate of 9(30%). Highest distributions were obtained from Dalori Camp1 and Teachers Village each with 2(6.7%) followed by Dalori Camp 2, Gubio Camp, Muna Custom, Muna Haruna and Farm Centre Camp each with 1(3.3%). No isolation was reported from NYSC, Bakassi and Goni-kachalla Camps.

**Table1: Distribution of Poliovirus serotypes isolated from sewage samples in some selected settlements in Maiduguri**

SITE	NoTESTED (Litres)	P1SL	P2SL	P3SL	P1SL+P3SL	TOTAL
		No POS	No POS	No POS	No POS	NoP OS (%)
Abbaganaram	3	2	0	1	1	4 (11.1)
Budum R/about	3	1	0	0	0	1 (2.8)
GamboruMarket	3	0	0	0	0	0 (0.0)
KasuwanShanu	3	0	0	0	0	0 (0.0)
ModuganariBypass	3	0	0	0	0	0 (0.0)
GwangeSabonlayi	3	0	0	0	0	0 (0.0)
Gwangebridge	3	0	0	1	0	1 (2.8)
Goniadamjunction	3	0	0	0	0	0 (0.0)
BulabulinG/Zana	3	2	0	1	0	3 (8.3)
Zamanclinic	3	1	0	0	0	1 (2.8)
Blind workshop	3	0	0	0	0	0 (0.0)
UmarariBorehole	3	0	0	0	0	0 (0.0)

**Table2: Distribution of NonPolio enteroviruses isolated from sewage samples from IDP Camps in Maiduguri**

Camp	No TESTED (Litres)	NPENT	TOTAL
		No POS	No POS (%)
Dalori Camp1	3	1	1(3.3)
Dalori Camp2	3	2	2(6.7)
Teachers Village	3	2	2(6.7)
Gubio Camp	3	1	1(3.3)
Muna Custom	3	1	1(3.3)
Muna Haruna	3	1	1(3.3)
Farm Centre	3	1	1(3.3)
NYSC Camp	3	0	0 (0.0)
Bakassi Camp	3	0	0 (0.0)
Goni Kachallari Camp	3	0	0 (0.0)
<b>TOTAL</b>	<b>30</b>	<b>9</b>	<b>9(30.0)</b>



## Discussion

Findings from this study revealed that, out of 36 litres of sewage samples from 12 settlements were analyzed (3 litres from each settlement). A total of 10 samples were found to be positive for mixture of serotypes P2 + P3 with isolation rate of 10 (27.8%). Type 3 serotype has the highest isolation rate 6(60%) followed by type 2 serotype 4(40%). Distribution by settlements revealed highest isolation rate of 4(11.1%) from Abbaganaram site followed by Bulabulun Gidan Zana 3(8.3%) while Budum Roundabout, Gwange Sabon Layi and Zaman Clinic sites reported one serotype each 1(2.8%). Isolation of NPENT and Sabin strains is consistent with the survey conducted in some cities in Nigeria even though no wild type or cVDPV was isolated from sewage in this study. Weldegebriel et al., (2015) reported in a previous study in Kano (In Gogau Fagge sewage site, the laboratory detected 21 isolates of which, 12 (57%) were Sabin viruses, 7 (33%) cVDPV2 and 3 (14%) were Non-Polio Enterovirus and another 1(5%) was wild polio virus type three; In Jakara police station sewage site, 22 viruses were isolated of which 13 (59%) were Sabin viruses, 7(32%) were cVDPV2. There was no Non-polio entero-virus or wild polio isolated from this site and In Kurna Masalashi site, 19 isolates were detected of which 12 (63%) were Sabin viruses, 4 (21%) were cVDPV2, two sample (11%) had wild polio virus type 1 and one sample (5%) had Non- polio enterovirus) and Sokoto (In Kofar Dundaye site there were 26 positive isolates of which 11 (42%) were cVDPV2, 7(27%) WPV1, 7 (27%) Sabin viruses and 1 (4%) was NPENT. Now WPV3 was detected; In Kofar Kware site, there were 25 positive isolates; of which 10 (40%) were Sabin viruses, 7 (28 %) were cVDPV2 and 3 (12%) were wild polio virus type 1 and 5 (20%) were NPENT. No WPV3 was detected; In Rubun Kawa site, there were 23 positive isolates of which 7 (30%) were cVDPV2, 5 (22%) were wild polio virus type1, 5 (22%) were Sabin and 6 (26%) were NPENT. No WPV3 was detected and In Tudun Wada Dallatu site, there were 19 positive isolates of which, 8 (42%) were cVDPV2, 5 (26%) were Sabin viruses, 1 (4%) was wild polio virus type one, 5 (26%) were NPENT. No WPV3 was detected).

Another study conducted in Ibadan (Adeniji et al., 2013), reported similar results with several group of NPENT and OPV strains isolated. Out of the 25 ES samples collected. 23 of the isolates were identified as Enteroviruses (EVs) by the PanEnterovirus-UTR assay. 13 (57%) of the 23 EVs were positive for the VP1 assay and identified as Coxsackievirus B3 (CVB3) (1 isolate), CVB6 (1 isolate), E6 (2 isolates),

E7 (5 isolates), E11 (1 isolate), E12 (1 isolate) and E13 (2 isolates). None and 2 (25%) of the remaining isolates were positive for the EV-B and EV-C assays, respectively. The 2 EV-C positive enteroviruses were isolated on MCF-7. This study describes three very productive ES sites, and documents the presence of CVB3, CVB6, E6, E7, E11, E12 and, E13 in Ibadan, Nigeria. It shows that including other cell lines in EV isolation protocols can broaden the diversity of EV types recoverable but the limitation of this study was that it didn't go further to classify the NPENT groups isolated.

Finding from this study is also consistent with the findings of Ndiaye et al. (2014), in Dakar which indicated that Sabin strains of Polioviruses and NPENTs were detected in 34,3% and 42,8% sewage samples respectively. No wild poliovirus neither circulating vaccine-derived Poliovirus (cVDPV) was detected. Neutralization assays have identified 49 non- polio enteroviruses that were subsequently classified in 13 serotypes belonging to HEV-A (22, 4%), HEV-B (12, 24%), HEV-C (26, 53%) and HEV-D (6, 12%) species. Other studies, Kargar et al. (2009), in Iran, revealed enteroviruses and non-polio enteroviruses were isolated 49 (56.98%) and 46 (53.49%) respectively. In another study, Khodae et al. (2008) reported in three Iranian provinces (Sistan & Baluchestan, Tehran and Fars) isolated enteroviruses 49(56.98%), 38(60.32%) and 11(22.92%) respectively. In Finland, Poyry et al. (1998) reported 72 enteroviruses (77.4%) were identified positives out of 93 studied from sewage. HEV-C species was predominant in his study (42, 4%), followed by HEV-A (33, 3%), HEV-B (18,2%) and HEV-D (6%). Similarly, in Japan, Nakamura et al. (2015), reported a high isolation frequency of Sabin strains of poliovirus isolates as 23 type 1 (PV1), 38 type 2 (PV2), and 22 type 3 (PV3) isolates. Sequence analysis of the full-length VP1 region showed that all isolates were Sabin-like (OPV strains), with 1% nucleotide divergence from Sabin 1 (S1) and Sabin 3 (S3) and 0.6% divergence from Sabin 2 (S2).

Isolation from IDPs camps revealed that, out of the 30 liters of sewage analyzed, only non-Polio Enteroviruses (NPENT) were isolated with isolation rate of 9(30%). The highest distribution was observed from Dalori Camp1 and Teachers Village each with 2(6.7%) followed by Dalori Camp 2, Gubio Camp, Muna Custom, Muna Haruna and Farm Centre Camp each with 1(3.3%) isolation rate. No isolation was reported from NYSC, Bakassi and Goni-kachalla Camps.

## Conclusion.

Prevailing immunity gap among children especially in IDPs camps were observed despite immunization campaigns using bivalent oral polio Vaccine (bOPV). It is expected that children will shed the virus and therefore could be isolated from sewage but only NPENT were isolated (No Sabin strains) in all the camps visited indicating non- response of the IDP's children to the vaccines. This could be attributed to malnutrition which has been reported in IDPs camps as malnourished children may have different immunological response to vaccines. In comparison to some selected settlements a number of Sabin-like strains were isolated indicating a good vaccine response and shedding by the immunized children which can as well help to boost herd immunity.

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