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Prevalence of vaccine-derived poliovirus in sewage waters in Maiduguri, Borno State, Nigeria

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SUMMARY

After a long global battle with wild poliovirus, the virus has been defeated through researches and vaccination using the oral polio vaccine and inactivated polio vaccine as well as sensitization. The issue that is now of global concern is that of vaccine-derived poliovirus which emerged from the unstable oral polio vaccine. Ninety sewage water samples were collected from slums in Maiduguri using grab method, concentrated using two phase separation method and subjected to intratypic differentiation and vaccine-derived poliovirus screening. The result revealed the presence of Sabin 1in 17 samples (61.0%) and Sabin 3 in 22 samples (79.0%), all of which were positive after vaccine-derived poliovirus screening. The presence of strains of Sabin 1 and Sabin 3 in the sewage water samples collected is an indication of virus shedding in individuals which could be as a result of vaccination or contact with the faeces infected or vaccinated individuals.

Keywords: Prevalence, vaccine-derived, poliovirus, sewage water, Maiduguri.

INTRODUCTION

Poliovirus is a human enterovirus belonging to the family Picornaviridae [1]. It is a non-enveloped icosahedra-shaped capsid of about 30 nm in diameter. Polioviruses possess an approximately 7.5 kilobase (kb) positive-sense single-stranded RNA genome and are transmitted by the faecal-oral route. They multiply in the gastrointestinal tract and are excreted in large numbers in the faeces of infected persons. Poliovirus infects sensitive cells of lymphoid tissue in the mouth, nose and throat. The incubation period lasts from 2 to 35 days. It can leads to a transient viremia and the virus spreads to the reticuloendothelial system without causing clinical symptoms [2-4]. Humans are the only natural hosts of poliovirus [5]. There are three strains of wild poliovirus (WPV). They are WPV1, WPV2 and WPV3. WPV 2 was declared eradicated by the World Health Organization on 20th September, 2015 and WPV 3 has not been detected anywhere in the world since November, 2012 and was declared eradicated by the year 2019 by global commission for certification of poliomyelitis (GCC) [6, 7]. All three are extremely virulent and produce the same disease symptoms [8]. Although there is no cure for polio, it is prevented through the administration of vaccine. Since the advent of vaccines, the global polio eradication initiative has been very successful; and since 1988, the global polio cases have decreased significantly by more than 99 % [5]. On the 20th of August 2020, Africa was certified free from wild poliovirus by the World Health Organisation with the last case of paralysis associated with wild poliovirus in north east Nigeria in August 2016 [9]. However, the transmission of WPV

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has not yet been interrupted in Afghanistan and Pakistan [10, 11].
Since the 1950s there have been two safe and effective vaccines against poliomyelitis, the inactivated (killed) polio vaccine (IPV) or Salk which is made from wild strains and administered by injection and live attenuated or weakened oral polio vaccine (OPV) or Sabin which is given by mouth. The OPV consist of the three serotypes known as Sabin 1, 2 and 3. Vaccine-derived poliovirus (VDPV) derived from each of the 3 OPV serotype are a mutated version of the attenuated Sabin-like virus used for oral vaccination [12, 13].

Although successful use of OPV has driven the virus out of most countries in the world, the live attenuated vaccine can revert to neurovirulence and get transmitted from person to person as a circulating vaccine-derived polio strain in setting of low immunization coverage [14]. Hence, this study is aimed at investigating the presence of vaccine-derived polio virus within communities perceived to have zero polio prevalence. The detection of vaccine derived polio virus within these communities became necessary to avoid imminent threats to community health in the light of claims of total eradication of polio.

METHODS

Nine sewage water samples each were collected from 10 different sewages water sites in highly impoverished and overcrowded urban area characterized by poor housing and living conditions in Maiduguri from December 2020 to April 2021. All procedures were done according to the guidelines on environmental surveillance for detection of Polioviruses, 2015 [15].

Grab Method of Sample Collection [15]
Samples were collected from mid-stream of inlet to the drainage by bucket and 1 litre of the sewage was collected in Jerry-can using a funnel and a sieve so that larger particles do not go into the jerrycan. The jerry-can was closed and sealed properly with Parafilm tape to ensure no leakage, the jerrycan was cleaned from outside by liquid bleach with the help of gauze and left to dry. The Jerrycan was marked with permanent marker with all the details of sample on it. The Jerrycan filled with sewage sample was placed properly in carrier containing ice packs, keeping the ice packs on all sides of the Jerrycan to keep the temperature inside the carrier at 4°C. The bucket, funnel and the sieve were sterilized with detergent and liquid bleach and left to dry. The samples were sent to the WHO Polio laboratory at the University of Maiduguri and then stored at 4°C before processing.

Two Phase Separation Method [15]
A volume of 500ml of each sample was transferred into labelled centrifuge bottles (1-90) and the rest were kept at 4°C as a backup. The samples were centrifuged at 1500rpm for 20 minutes at 4°C. After centrifuging, the supernatants were carefully poured into 1 litre beakers with a magnetic bar inside the beakers and the sediment remaining in the bottles were kept at 4°C to the next day. To 500ml of each sample in the beaker, 287ml of 29% Poly ethylene glycol (PEG) 6000, 39.5ml of 22% dextran, and 35ml of 5M sodium chloride (NaCl) were added and then mixed thoroughly by vigorous stirring for 1 hour on a magnetic stirrer. The samples were poured into a separating funnel. The funnels were then left to stand overnight at 4°C and the entire lower phase and the interphase were slowly harvested drop wise into a sterile 50ml tube. The harvested concentrates were used to re-suspend the pellets remaining in the centrifuge bottles by forced pipetting. The volumes were measured and recorded after re-suspending the pellets. To each sample, 12 glass beads were added and 20% chloroform was added using a glass pipette and were shaken on a mechanical shaker for 20 minutes. They were again centrifuged at 4°C for 20 minutes at 1500rpm. After chloroform extraction, the upper aqueous layers were collected carefully using a sterile pipette. The concentrates for each sample were divided into labelled two 4ml cryovials. To each concentrate in the cryovials, 500µl of penicillin-streptomycin and 100µl of gentamycin were added. One cryovial for each sample was for inoculation and the other cryovial was for laboratory inventory and was stored at -20°C.

Isolation of Poliovirus from Sewage Water Concentrates [15]
Monolayer cultures that are 2 to 3 days old were microscopically examined to be sure that the cells are healthy and at least 75% confluent. Five T25 flasks of L20B and one of RD were labelled (F1, F2, F3, F4 and F5 for L20B) and (F6 for RD) for each

concentrate to be inoculated with specimen number (1-90) and 1 T25 flask of each cell type were labelled as a negative control. Inoculation was done according to the procedure in the supplement for poliovirus isolation algorithm, 2006.

Intratypic differentiation
Real-Time reverse transcriptase polymerase chain reaction
A PCR reaction was run using an ABI 7500: real-time thermocycler and cycle as follows: At 95°C for 15 seconds, 50°C for 45 seconds, and then a 25% ramp speed to 72°C for 5 seconds for 40 cycles. The end point fluorescent data is collected at the end of the 50°C anneal step. The FAM dye was selected for each assay before starting the reaction.

RESULTS

In Table 1, the result showed that all the cell culture isolates in the nine sewage water samples collected from site 01 did not contain poliovirus. Isolation of poliovirus from the sewage water concentrates in site 02 revealed that suspected polioviruses were found in three sewage water samples whereas in the third site, isolates in five sewage water samples were shown to contain suspected poliovirus. Also, suspected poliovirus were detected in the isolates from two sewage water samples in site 04. Moreover, suspected poliovirus where found in cell culture isolates in four of the sewage water samples collected in the fifth site. Unlike the fifth site, suspected poliovirus were shown in three sewage water samples in site 06. In the seventh site, poliovirus was suspected to be present in four sewage water samples. The cell culture isolates of the samples from site 08 showed the presence of suspected poliovirus in two sewage water samples. Similarly, two sewage water samples in site 09 where suspected to have poliovirus. While in site 10, only three sewage water samples contain suspected poliovirus. In total, poliovirus was suspected to be present in twenty eight out of the ninety sewage water samples collected from all the ten sites. As such, all sewage water samples whose cell culture isolate indicated the presence of suspected poliovirus were marked as positive sewage water samples.
Table 2 indicates the results of non-polio enteroviruses (NPEVs) found in the sewage water samples. According to the result, there was no NPEV in all the nine sewage water samples in site 01. However, cell culture isolate of one sewage water sample in site 02 shows the presence of NPEV. In the third site, two sewage water samples contain NPEVs. Also two sewage water samples contain NPEV in site 04 and likewise in the fifth site. Meanwhile in the sixth and seventh site, only one sewage water sample each contain NPEV. Non polio enteroviruses were present in three sewage water samples each in the eighth, ninth and tenth sites. Generally, out of the ninety sewage water

Table 1 - Collection of Sewage Water Samples and Isolation of suspected Poliovirus from Sewage Water Concentrates.

Site Name	*Latitudes*	*Longitudes*	*Site Code*	*Number of Samples*	*Positive Sewage Water Samples*
Maduganari	11◦49′33.5″N	13◦07′55.9″E	01	9 (100%)	0 (0.00%)
Bulabulin	11◦50′15.5″N	13◦09′41.2″E	02	9 (100%)	3 (33.0%)
Blind workshop	11◦50′57.2″N	13◦10′11.7″E	03	9 (100%)	5 (55.0%)
Abbaganaram	11◦51′30.6″N	13◦09′19.8″E	04	9 (100%)	2 (22.0%)
Gamboru	11◦51′05.8″N	13◦10′12.0″E	05	9 (100%)	4 (44.0%)
Kulo gumna primary school	11◦50′27.3″N	13◦09′48.″E	06	9 (100%)	3 (33.0%)
Gadan zare	11◦50′41.1″N	13◦09′58.7″E	07	9 (100%)	4 (44.0%)
Budum	11◦50′58.5″N	13◦09′46.5″E	08	9 (100%)	2 (22.0%)
Gwange zawiya	11◦50′38.2″N	13◦10′02.3″E	09	9 (100%)	2 (22.0%)
Gwange layin mamman shitta	11◦50′24.0″N	13◦09′51.5″E	10	9 (100%)	3 (33.0%)
Total				90 (100%)	28 (31.0%)

Table 2 - Isolation of Non-polio Enterovirus (NPEV) from Sewage Water Concentrates in Borno State.

Site	*Number of Sewage Water Samples*	*Non-Polio Enterovirus Positive Samples*	*Non polio Enterovirus Negative Samples*
01	9 (100%)	0 (0.00%)	9 (100%)
02	9 (100%)	1 (11.0%)	8 (89.0%)
03	9 (100%)	2 (22.0%)	7 (78.0%)
04	9 (100%)	2 (22.0%)	7 (78.0%)
05	9 (100%)	2 (22.0%)	7 (78.0%)
06	9 (100%)	1 (11.0%)	8 (89.0%)
07	9 (100%)	1 (11.0%)	8 (89.0%)
08	9 (100%)	3 (33.0%)	6 (67.0%)
09	9 (100%)	3 (33.0%)	6 (67.0%)
10	9 (100%)	3 (33.0%)	6 (33.0%)
	90 (100%)	18 (20.0%)	72 (80.0%)

Table 3 - Suspected Sabin 1 Poliovirus obtained after Intratypic Differentiation (ITD) in the Sewage Water Samples.

Site	*Positive Sewage Water Samples*	*Sabin 1 Positive Sewage Water Samples*
02	3 (100%)	2 (67.0%)
03	5 (100%)	3 (60.0%)
04	2 (100%)	2 (100%)
05	4 (100%)	1 (25.0%)
06	3 (100%)	1 (33.0%)
07	4 (100%)	3 (75.0%)
08	2 (100%)	2 (100%)
09	2 (100%)	1 (50.0%)
10	3 (100%)	2 (67.0%)
Total	28 (100%)	17 (61.0%)

Table 4 - Suspected Sabin 3 Poliovirus obtained after Intratypic Differentiation (ITD).

Site	*Positive Sewage Water Samples*	*Sabin 3 Positive Sewage Water Samples*
02	3 (100%)	2 (67.0%)
03	5 (100%)	4 (80.0%)
04	2 (100%)	2 (100%)
05	4 (100%)	4 (100%)
06	3 (100%)	2 (67.0%)
07	4 (100%)	2 (50.0%)
08	2 (100%)	2 (100%)
09	2 (100%)	1 (50.0%)
10	3 (100%)	3 (100%)
Total	28 (100%)	22 (79.0%)

samples only eighteen sewage water samples contain NPEVs.

According to Table 3, further identification of the positive cell culture isolates of the positive sewage water samples through intratypic differentiation shows that two of the three positive sewage water samples from site 02 contain Sabin 1 poliovirus strain. The same poliovirus strain was also present in the three positive sewage water samples in site 03 as well as in both the positive sewage water samples in site 04. In the fifth site, Sabin 1 strain was present in only one out of the four positive sewage water samples from that site likewise in site 06, Sabin 1 was present in only one positive sewage water sample. Three positive sewage water samples possess Sabin 1 strain in site 07 and 100% of the positive sewage water samples in site 09 contain this Sabin strain of poliovirus. However, 67% of the positive sewage water samples in site 10 contain Sabin 1 which makes Sabin 1 poliovirus present in 17 (61%) out of the total positive sewage water samples.

The results in Table 4 shows that 67% of the positive sewage water samples in site 02 contain Sabin 3 poliovirus while four of the five positive sewage water samples in site 03 and all positive sewage water samples in site 04 contain this strain. Intratypic differentiation of the positive cell culture iso-

lates of the sewage water samples also reveal the presence of Sabin 3 strain in all the four positive sewage water samples in site 05 and two out of the three positive sewage water samples in site 06 are positive after they were screened for Sabin 3. In the seventh site, 50% of the positive sewage water samples contain the Sabin 3 strain while in site 08, all the positive sewage water samples contain this poliovirus strain. Half of the positive sewage water samples in site 09 and all the positive sewage water samples in the tenth site contain the Sabin 3 strain of Poliovirus. In total, 22 (79%) out of 28 positive sewage water samples contain Sabin 3 poliovirus strain.

Table 5 shows the result of other polioviruses which include Sabin 2, wild poliovirus type 1, poliovirus type 2, wild poliovirus type 3 African strain and wild poliovirus type 3 Asian strain that were suspected to be present in the positive sewage water samples but were proofed to be absent in the sewage water samples after intratypic differentiation.

The distribution of Sabin 1 and Sabin 3 in the positive sewage water samples as shown in table 6 indicates that out of the three positive sewage water samples in site 02, one positive sewage water sample contain only Sabin 1 in the same way the other positive sewage water sample contain only Sabin 3 likewise the third positive sewage water sample in site 02 contain mixture of both Sabin 1 and Sabin 3. However, in site 03, only 40% of the positive sewage water samples contain only Sabin

Table 5 - Other Suspected Polioviruses after Intratyic Differentiation (ITD).

Site	*Positive Sewage Water Samples*	*S2*	*WPV 1*	*WPV3-1*	*WPV3-2*	*PV2*
02	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
03	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
04	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
05	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
06	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
07	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
08	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
09	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
10	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	28(100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Notes: S2- Sabin 3, WPV 1- wild poliovirus type 1, WPV 3-1- wild poliovirus type 3 (African strain), WPV 3-2 – wild poliovirus type 3 (Asian strain).

Table 6 - Distribution of Sabin 1 and Sabin 3 Poliovirus in the Positive Sewage Water Samples.

Site	*Positive Sewage water samples*	*Positive Sewage water samples that contain only S1*	*Positive Sewage water samples that contain only S3*	*Positive Sewage water samples that contain Mixture of S1 and S3*
02	3 (100%)	1(33.3%)	1 (33.3%)	1 (33.3%)
03	5 (100%)	2 (40.0%)	2 (40.0%)	1 (20.0%)
04	2 (100%)	0 (0.00%)	0 (0.00%)	2 (100%)
05	4 (100%)	0 (0.00%)	3 (75.0%)	1 (25.0%)
06	3 (100%)	1 (33.0%)	2 (67.0%)	0 (0.00%)
07	4 (100%)	2 (50.0%)	1 (25.0%)	1 (25.0%)
08	2 (100%)	0 (0.00%)	0 (0.00%)	2 (100%)
09	2 (100%)	1 (50.0%)	1 (50.0%)	0 (0.00%)
10	3 (100%)	0 (0.00%)	1 (33.0%)	2 (67.0%)
Total	(100%)	7 (25.0%)	11 (39.0%)	10 (36.0%)

Notes: S1 – Sabin 1, S3 – Sabin 3.

Table 7 - Vaccine derived poliovirus Real- time PCR Primers and Probes Sequences.

Sabin VDPV Real-time Primer specificity	*Primer and Probe sequences 5'-3'*
S1 VDPV VP1	Sense CATGCGTGGCCATTATA Anti-Sense TAAATTCCATATCAAATCTA 22S VP1 Probe *FAM*-CACCAAGAATAAGGATAAG -*BHQ1*
S3 VDPV VP1	Sense CATTTACATGAAACCCAAAC Anti-Sense TGGTCAAACCTTTCTCAGA 12S VP1 Probe *FAM*-AGGAACAACTTGGAC-*BHQ1*

1, another 40% contain only Sabin 3 strain and mixture of both Sabin strains were found in 20% of the positive sewage samples in that site. In the fourth site, mixture of Sabin 1 and Sabin 3 strains of Poliovirus were present in all the positive sewage water samples from that site while in the fifth site 75% of the Positive sewage water samples contain only Sabin 1 and the remaining 25% contain mixture of Sabin 1 and Sabin 3 strains. Moreover, there was no sewage water sample that contain both Sabin strains in site 07 while all the positive sewage water samples in site 08 contain both Sabin 1 and Sabin 3 strains. In the ninth site, 50% of the positive sewage water samples contain only Sabin 1 and the other 50% contain only Sabin 3 strain of the virus. In site 10, one of the three sewage water sample contain only Sabin 3 and the two samples contain mixture of Sabin 1 and Sabin 3. In total, out of the twenty eight positive sewage water samples, seven samples contain only Sabin 1 strain, eleven samples contain only Sabin 3 and ten samples contain both Sabin 1 and Sabin 3 strains of poliovirus.

DISCUSSION

In this study, intratypic differentiation shows the presence of Sabin 1 strain and/or Sabin 3 strain in 9 out of the 10 sites where sewage water samples were collected, this shows that there is some level of vaccine coverage in these locations visited for the study but probably low. In a research conducted across Moscow between 2004- 2016 by Ivanova et al. using similar method, vaccine strains of all the three poliovirus serotypes were found in the sewage samples collected with 178 Sabin 3 poliovirus isolates (45%), followed by Sabin 2 with 144 isolates (36.5 %) and lastly, Sabin 1 with 73 isolates [16]. Also, Ndaiye et al., detected 29 (31.18%) samples contained Sabin 1 poliovirus, 58 (62.37%) samples contained Sabin 2 poliovirus and 28 (30.11%) contained Sabin 3 poliovirus while 22 samples contain mixtures of Sabin polioviruses out of the 93 poliovirus positive samples [17]. In both the studies by Ivanova et al. and Ndiaye et al., nucleotide sequencing of the VP1 region of the suspected isolate of the vaccine-derived poliovirus screening showed that all the poliovirus isolates obtained were Sabin-like polioviruses with little genetic variation when compared to the original Sabin strains hence agreeing that the poliovirus were from vaccine recipients [16, 17]. On the contrary, research conducted by Shariff et al. on stool samples in Afghanistan indicates that only 6 VDPV type 2 were confirmed out of the 154 positive samples detected when comparative analysis of their VP1 sequence to that of an original Sabin 2 vaccine strain was conducted [18].

The Sabin strain found in the oral polio vaccine (OPV) mutate to first become Sabin like polioviruses with genetic sequence that closely match the sequence of the originating vaccine strain (<10 VP1 mutation for type 1 and 3, <6 VP1 mutation for type 2). Sabin like polioviruses further evolve into VDPV (>10 VP1 mutation for type 1 and 3, >6 VP1 mutation for type 2) if there is sustained persistence in human population and continues evolution result in the circulation of VDPV with higher neurovirulence which are confirmed to be circulating as cVDPVs by epidemiological investigation and genetic linkage [19].

Although there were amplifications after vaccine-derived poliovirus screening of the Sabin 1 and Sabin 3 isolates, molecular sequencing is not done in this study. As such the level of genetic changes in the VP1 region of the Sabin 1 and Sabin 3 strains found in the sewage water samples is not known. However, there was no Sabin 2 strain in all the collected sewage water samples thus making the presence of Sabin 1 and Sabin 3 an indication that these viruses were from vaccine recipients, as there was a switch from the trivalent polio vaccine

that contains all the three serotypes of poliovirus to a bivalent vaccine that contains only the type 1 and the type 3 portion of the attenuated virus in 2016. This is done primarily to get rid of the highly unstable and fast mutating type 2 strain present in the trivalent vaccine as it is responsible for 90% of the global cVDPVs cases. Moreover, all the cVDPVs cases in Nigeria are of cVDPV2 [20]. In a similar work by Ivanova et al. [16], only Sabin 1 virus and Sabin 3 virus were isolated from 673 sewage samples collected after the switch to the bivalent vaccine (Sabin 1 in 27 sewage samples and Sabin 3 in 77 sewage samples). However, Adamu et al. [21] showed that 85 cVDPV2 isolates were detected in sewage samples from January 2018 to May 2019 in Nigeria which could be as a result of cVDPV2 outbreak or silent circulation of cVDPV2 strain which may be associated with low vaccination coverage and poor sanitation. A cVDPV2 strain was also found in sewage sample in London [22]. This is an indication of virus shedding in individuals. According to a study by Lai et al., out of the 1818 cVDPV cases reported from 1st January 2016 to 30th June 2021, 1728 (95%) of the cases were of cVDPV2, followed by cVDPV1 with 83 cases, then 6 cases of cVDPV3 [23-25].

In a recent outbreak of cVDPV3 in Israel, the cVDPV strain that is responsible for the outbreak is genetically linked to a cluster of VDPV3 isolated in environmental samples in Jerusalem between September 2021 and January 2022 [26]. This is evidence that the Sabin 1 and Sabin 3 strains present in environmental samples are liable to revert to neurovirulent strains that could cause devastating effect in human population if care is not taken. Though, the exact mechanism of reversion of poliovirus is still not clear, various researches have shown that the reversion is as a result of mutation because of the lack of the proofreading stage in the replication of an RNA virus or recombination [27]. In support of this, Muslin et al. in their research emphasized that mutation and recombination are parts of virus evolution mechanism [28]. Pita et al. also suggested that recombination is an important mechanism in the evolution of enterovirus genome and it occurs very often in enterovirus [29].

This study also isolated non-polio enterovirus (NPEV) in 18 (20%) sewage water samples. The presence of different Sabin strains of poliovirus and NPEV in the sewage water samples collected could also be a risk factor in the formation of new Sabin strains as recombination in enteroviruses is both intraspecies and interspecies [30-32]. A study conducted by Xu et al.,also justifies that recombination can occur among enteroviruses of the same and different species and according to Lowry et al. recombination plays a vital role in the emergence of new neurovirulent strains of poliovirus. There is also evidence of recombination between poliovirus of vaccine and wild type origin [33, 34].

The presence of strains of Sabin 1 and Sabin 3 in the sewage water samples collected is an indication of virus shedding in individuals. This could be as a result of vaccination or contact with the faeces of infected or vaccinated individuals. It is a positive progress in eradication if only Sabin strains are contained in sewage samples but a poor indicator of vaccine coverage and sanitation, a lot more caution need to be taken to sustain immunization before it becomes another public health problem.

Disclaimer

This research is original in its entirety, and to the best of our knowledge no similar work has ever been conducted within the study area or the northeast region of the country.

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Conflict Of Interest

Authors declared no conflict of interest.

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Authors contribution

The authors Sakinatu Buba Bislava, Aliyu Daja and Bamidele Soji Oderinde were involved in conceptualizing the idea, design and conduct of the research while Sani Muhammad Uzairu was involved in manuscript development and proof reading of the work.

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