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Isolation and molecular characterization of polio virus isolates from apparently healthy children in maiduguri metropolis

Abstract

Introduction: Expanded Programme on Immunization (EPI) is one of the strategic universally accepted methods for the control of child hood diseases including poliomyelitis. On the 24th May 2008 the World Health Assembly Resolution urged Nigeria to reduce the risk of international spread of poliovirus through intensified eradication strategies.

Objective: The study was carried out to isolate and characterized polio virus isolates from apparently healthy children in an effort to complement the acute flaccid paralysis surveillance for wild poliovirus in Maiduguri metropolis.

Methodology: The methods adopted for virus isolation were described in WHO Polio Laboratory Manual 2004 and the supplemental manual of 2006 for the New Algorithm Technique currently used for poliovirus isolation. In brief, stool suspensions were prepared using chloroform and phosphate buffered saline, centrifuged at 3000rpm for 30minutes. 200µl of the supernatant was inoculated onto healthy monolayer of L20B and RD cell lines. The inoculated monolayers were incubated at 36°C and observed daily for the characteristic enterovirus cytopathic effects (CPE) of rounded refractile cells and detaching from the surface of the tube. The tubes with CPE up to 75% and above were harvested and kept at -20°C to be passaged to a fresh monolayer of the opposite cell line. Sample preparation for RT- PCR: 50 µl virus cell culture was added into dilution tube and centrifuge (bench top micro centrifuge at 5,000 rpm) at room temperature for 2minutes. 0.5µl of cell culture supernatant (or 1µl 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.

Result: Out of the 400 stool samples analysed, 11samples were found to be positive given the isolation rate of 2.8%. Sex distribution shows 214 (54%) male while 186(47%) were female also out of the 11 positive serotypes isolated 4(1.9%) was obtained from Male while 7(3.8%) from female. Statistically, rate of infections is not significantly associated with sex (P=0.36). Also 263(66%) had vaccinations history while 143(36%) had no vaccinations history against any of the serotypes while Non Polio Enterovirus (NPENT) isolation rate was found to be 34(8.5%).

Conclusion: From the findings both wild and Sabin poliovirus were isolated from apparently healthy children.

Keywords: poliovirus, apparently healthy, children, Maiduguri, Nigeria

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Muhammad Talle,[1] Auwalu Uba,[2] Fatima Tahir,[2] Monilade Akinola[1]

[1]WHO National Polio Laboratory, University of Maiduguri Teaching Hospital, Nigeria
[2]Abubakar Tafawa Balewa University, Nigeria

Correspondence: Muhammad Talle, WHO National Polio Laboratory, University Of Maiduguri Teaching Hospital, P.M.B.1414 Borno State, Nigeria, Tel 0803 4722 684, Email muhammadt6@gmail.com

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Abbreviations: BSC, bio-safety cabinet; NID, national immunization day; ITD, intratypic differentiation; RRF, relative risk factor; PEI, polio eradication initiative

Introduction

Poliomyelitis was a pandemic and continues to be a major public health problem in countries where it now exists. In May 1988, the 41st World Health Assembly committed the member States of the World Health Organization (WHO) to the global eradication of poliomyelitis by the year 2000 (resolution WHA41.28).[1] The resolution specified that the polio eradication initiative should be pursued in ways that would strengthen the Expanded Programme on Immunization. In 1989, the 42nd World Health Assembly approved a general Plan of Action for Global Polio Eradication. The global effort to eradicate polio is the largest public health initiative in history. Since the initiative was launched in 1988 extraordinary progress has been made to halt transmission of wild poliovirus and achieve global certification of eradication by 2005.

Materials and method

Extraction of polio virus from stool samples

The methods adopted for virus isolation were described in WHO Polio Laboratory Manual 2004 and the supplemental manual of 2006 for the New Algorithm Technique currently used for poliovirus isolation. All centrifuge tubes were labelled with a corresponding lab I.D and to each centrifuge tube the following were added, 5ml phosphate buffer saline containing antibiotic, 0.5ml chloroform, 1gm of glass beds and about 1gm of faecal sample to each of the labeled tube and place in a mechanical shaker horizontally to align with the direction of the shaking for 30mins. All tubes were later centrifuge at 3000rpm for 30mins after which 2ml of the supernatant were aseptically transferred into 2ml cryovials and stored at -20°C until used Nadkarni et al.,2003.

Safety conditions: All safety guidelines as contained in the lab manual were strictly adhering to and all procedures were carried out

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under Bio-safety cabinet level II (BSC II).

Inoculation of the stool extract

Stool extracts were inoculated onto a healthy actively metabolizing monolayer of L20B (a genetically engineered mouse cell lines expressing human polio receptors CD_{155}) and RD cells (obtained from human Rhabdo myo-sarcoma) and incubated at 36°C to demonstrate in-vitro infection. The inoculated tubes were examined daily for the presence of CPE which indicates the presence of virus. The new algorithm test for poliovirus isolation and characterization was employed. Three days old monolayer cultures of L20B and RD cell lines were examined to be sure that the cells are healthy and attained at least 75% confluent before inoculation. Two hundred microlitres (0.2ml) of the stool extract were inoculated in to 2Label tubes of RD and L20B for each specimen with specimen number, date, passage number. A negative control tubes were set up along and incubated in the stationary sloped (5%) position at 36°C, all culture tubes were examined daily using an inverted microscope for the appearance of viral cytopathic effect (CPE). All daily observations of inoculated and control tubes were recorded, and record of CPE (if any) as 1+ to 4+ to indicate the percentage of cells infected i.e. 1+ represents up to 25% of cells; 2+ represents 50%; 3+ represent 75% and 4+ represent 100%.

Note: both cell lines were inoculated on the same day. After inoculation with each cell line, the BSC was clean with 10% hypochlorite, allow it to dry, then mop with 70% alcohol and run for 10 minutes before the second cell line was inoculated.

If no CPE appears after at least 5days of observation, a blind passage was perform in the same cell line and continue examination for further 5days. Cultures were examined for a total of at least 10days (i.e. minimum of 5days post-inoculation and minimum of 5days post- passage) before being judged as negative and discarded. All tubes were observed daily for characteristics enterovirus CPE after inoculation 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 tube containing a monolayer of cells from the second cell line in 1ml of maintenance medium. All positive isolates from stool samples were subjected to Intratypic differentiation (ITD) using real time PCR while non Sabin like isolates were referred to CDC for sequence.

Results

Table 1, shows the age distribution of children with poliovirus serotypes from apparently healthy children. Out of the 400 stool samples analysed, 11samples were found to be positive given the isolation rate of 2.8%. Of the 11serotypes isolated, their distributions were as follows: P2SL (6), P1SL (0), P3SL (4) and a mixture of P2SL+P3SL (1) while distribution by age group revealed highest prevalence among 10-12 (5.6%), 1-3 (3.3%), 4-6 (2.6%) and 7-6 (1.6%). The sex distribution of poliovirus serotypes isolated from apparently healthy children in Maiduguri is shown in (Table 2). Out of the 400 stool samples analysed, 214 (54%) were from male while 186 (47%) from female also out of the 11 positive serotypes isolated, 4(1.9%) was obtained from Male while 7(3.8%) from female and distribution of individual serotypes were as follows: P2SL (8), P3SL (2) and a mixture of P2SL+P3SL (1).Of the 34 non-polio enteroviruses (NPENT) isolated from stool samples of apparently healthy children, distribution by age group shows highest prevalence among 1-3 (60%), 4-6 (12.8%), 10-12 (5.6%) and the least 7-9 (4.2%), (Table 3).

Table 1 Age distribution of poliovirus serotypes isolated from stool samples of apparently healthy children in Maiduguri (July, 2016)

		PW1	P1SL	P2SL	P3SL	P1SL+P3SL	P2SL+P3SL	Total
AGE RANGE	NO TESTED	NO POS	NO POS	NO POS	NO POS	NO POS	NO POS	NO POS (%)
1–3	60	0	0	0	1	0	1	2(3.3)
4–6	78	0	0	2	0	0	0	2(2.6)
7–9	190	0	0	2	1	0	0	3(1.6)
10–12	72	0	0	2	2	0	0	4 (5.6)
Total	400	0	0	6	4	0	1	11(2.8)

PW1 Wild Poliovirus type 1
P1SL Sabin–like Poliovirus type 1
P2SL Sabin–like Poliovirus type 2
P3SL Sabin–like Poliovirus type 3

Table 2 Sex distribution of poliovirus serotypes isolated from stool samples of apparently healthy children in Maiduguri (July 2016)

		PW1	P1SL	P2SL	P3SL	P1SL+P3SL	P2SL+P3SL	Total
SEX	No TESTED	No POS	No POS	No POS	No POS	No POS	No POS	No POS %
F	186	0	0	5	2	0	0	7(3.8)
M	214	0	0	3	0	0	1	4(1.9)
Total	400	0	0	8	2	0	1	11(2.8)

PW1 Wild Poliovirus Type 1
P1SL Sabin–like Poliovirus type 1
P2SL Sabin–like Poliovirus type 2
P3SL Sabin–like Poliovirus type 3
OPV Oral polio Vaccine (Sabin)

Table 3 Age distribution of non polio enteroviruses (NPENT) isolated from stool samples of apparently healthy children in Maiduguri (Jan–July 2016)

		NPENT	Total
AGE RANGE	**No TESTED**	**No POS**	**No POS (%)**
1-3	60	12	12(60.0)
4-6	78	10	10(12.8)
7-9	190	8	8(4.2)
10-12	72	4	4s (5.6)
Total	400	34	34(8.5)

Figure 1, shows the phylogenic tree of the VP1 sequence of the wild isolate obtained from apparently healthy child, NIE-BOS-JRE-16-44C2 was PV1 WEAFB1 orphan virus in cluster N7B (Arrow).The closest matching viruses are NIE-BOS-DAM-13-005 and NIE-BOS-BAM-11-012 at 95.8% nt. VP1 identity (38 nucleotides different). This shows that the virus has been in circulation since 2011 indicating a surveillance gap and poor immunization coverage.

Figure 1 Sequenced phylogenic tree of polioviruses isolated in Nigeria.

Discussion

In May 2008, the World Health Assembly in its resolution WHA 61.1 urged Nigeria to undertake intensive activities to immediately stop the outbreak of poliomyelitis in the northern part of the country. In order to achieve this goal, National Immunization days (NID) including 'mopping up' and acute flaccid paralysis surveillance were executed in 2009 and in subsequent years. In spite of the rigorous vaccination exercise and surveillance, Nigeria remains one of the major reservoirs for wild poliovirus transmission globally Adewumi et al.,[2] The result of poliovirus isolation from apparently healthy children revealed an isolation rate of 2.8%. This is contrary to the findings of Oderinde & Baba et al.,[3] with the isolation rate of 0.005% in FCT and 8% in North Eastern Nigeria respectively. NPENT isolation rate among apparently healthy children was found to be 8.5%. This is similar to the findings of Baba et al.,[3] with NPENT isolation rate of 8.5%. In this study, only one wild polio virus type 1(WPV1) was isolated from apparently healthy child and this may not necessary reflect the true situation as so many local government areas (LGAs) cannot be access due to insurgency as there might be a silent circulation of wild poliovirus in such remote areas.

This continuous excretion of WPV1 from apparently healthy child is consistent with a report that substantial proportion of vaccinated individuals shed WPV1 in India Grassly et al.,[4] These researchers observed that mucosal immunity induced by OPV may wane overtime such that immunized children may be infected and excrete poliovirus although the titer of excretion among these children is reduced compare to unimmunized children. Therefore, excretion of wild poliovirus in asymptomatic individual may pose a serious setback to polio eradication initiative (PEI) in Nigeria. Furthermore, poor sanitary condition, overcrowding, and poor sewage handling facilities can help in the transmission of the virus.[5] The shedding of the 11Sabin strains of the poliovirus is advantageous to the program as unvaccinated children can as well be vaccinated in contact with the immunised children there by boosting the herd immunity. The excretion of poliovirus mixtures in this study correlated with the study from the same environment [3] showing neutralizing antibodies to mixtures of polio virus type 1+3 and 2+3. The use of bivalent OPV 1+3 predisposes children to infections by type 2 as revealed in this study, such immunity gaps may encourage the spread of type 2 strains. It was also found in this study that, in healthy children, NPENT isolation rate was high 34% (Table 3). Multiple enteric infections, poor sanitation and overcrowding could also be the reasons which prevent the desired immunity for OPV against the poliomyelitis in the endemic region of Nigeria. This interference highlights the capacity of NPENTs to remain in silent circulation in a healthy population. The excretion of Sabin strain of poliovirus from vaccinated children gives hope to polio eradication initiative in Nigeria.

According to Sutter et al., (2004), Sabin strain limits the exposure of the unvaccinated children to infection with wild-type especially the younger siblings of those children shedding the virus, thereby help to achieved herd immunity. Also, in this study, the age distribution of children presented with paralysis shows that most cases occur between six months of age and four years of age. This shows that maternal antibodies (which normally wanes out after six months of age) prevents polio occurrence. This deviates from the report by Grassly et al.,[4] It was also observed that poliomyelitis occur in boys and girls but paralysis occurred more in boys as shown, 25(10.3%) were from males while 22(13.4%) occurred in female children (Table 2). The rate of infections is not significantly associated with sex (P=0.35) and the relative risk factor (RRF) was found to be (RRF=1.30). This means sex is not a risk factor in the epidemiology of the polio virus infections. This is consistent with previous studies which reported that poliovirus infection in children occurs equally in boys and girls but boys are more commonly paralyzed. This means sex is not a risk factor in the epidemiology of the polio virus infections.

This is consistent with previous studies which reported that poliovirus infection in children occurs equally in boys and girls but boys are more commonly paralyzed. Other factors in the transmission of the virus includes, overcrowding, Poor sanitary conditions and sewage treatment facilities helps in the wide spread of the virus. Similarly, other reports in Africa revealed Wild poliovirus can replicate in well-immunized persons without causing clinical disease and spread to susceptible individuals by two routes: fecal-oral and nasopharyngeal (Ghendon & Robertson 1994). Outbreaks, due to poliovirus type 1, in Namibia van Niekerk *et al*.,[6] and in Zambia Mpabalwani *et al*.,[7] have shown that even high vaccination coverage through routine administration of OPV may be inadequate to provide protection against outbreaks of paralytic poliomyelitis. In Bombay, India Deshpande *et al*., [8] reported silent transmission of wild poliovirus in 8.2% of the studied population and in 4.4% of fully vaccinated children in Ibadan, Nigeria.[9-20]

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None

Conflict of interest

The author declares there is no conflict of interest.

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