Comparison of the Potency of Circulating Vaccine Derived Poliovirus Type 2 with Sabin like Type 2 by Micro Titration.

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Abstract: The largest recorded outbreak of the circulating vaccine derived poliovirus (cVDPV), especially type 2 occurred in Nigeria in 2005-2009. This incidence provided a unique but rare opportunity to compare the potency of both the Sabin like poliovirus type 2 and cVDPV 2 for differential diagnosis purpose. This study was carried out to establish the possible differential diagnosis in relation to the titre of both strains as a measure of virulence. In the methodology, twenty cases with ten isolates each of poliovirus type2 (Sabin like) and cVDPV 2 strains from the faeces of children with acute flaccid paralysis in Nigeria were identified and confirmed by CDC. The potency of these strains were determined according to the protocol in WHO Polio manual 2004. However, no significant difference (> 0.05) was found in the titres of both strains of poliovirus serotype 2. The mean titres of Log CCID 50 -7.26 and -7.24 were obtained for cVDPV 2 and Sabin like 2 respectively. Both strains of Poliovirus 2 are potent and capable of causing disease. Therefore molecular intratypic differentiation (ITD) and sequencing are important methods for their differentiation. Immune status of the host may determine the degree of virulence of this virus. The need for vaccine compliancy cannot be overemphasised to terminate the circulation of cVDPV and its associated problems in the environment.

Key words: Comparison, Circulating, Vaccine-derived, Sabin like, Poliovirus, Titres

INTRODUCTION

Poliomyelitis is an acute infectious systemic viral disease affecting human in widely varying severity from a non-specific illness to almost irreversible paralysis or death due to polioviruses of serotypes 1, 2, and 3 infections. In rarely severe cases, death is often due to asphyxiating especially children below the age of five years. It has remained endemic in some parts of the world including Nigeria. The eradication of type 2 Wild Polio-viruses (WPV) globally in 1999 was a remarkable achievement (Helen) but in Nigeria between 2005 to 2009, the vaccine- induced population immunity to type 2 VDPV poliomyelitis declined significantly in all the geographical zones (range, -4.7% to -8.8% annually) (Jenkins, H.E., 2010).

However, in recent years a new dimension of risk was identified with the discovery of highly divergent vaccine-derived polioviruses (VDPVs) of which the first was in September 2002, a type VDPV was isolated from an incompletely immunized 21-month-old boy from Plateau State, in north central Nigeria. The VDPV isolate differed from Sabin type 2 (Sabin 2) OPV Strain at 2.5% of VP1 nucleotides, and had 3D sequences derived from a source distinct from any of the Sabin Strains (Adu, F.D., 2007). There was increase in the number of type 2 circulating vaccine-derived polioviruses (cVDPVs) paralysis in Nigeria from 2006 to 2009 with the highest number of incidence.

A case of circulating vaccine-derived polioviruses (cVDPVs) paralysis is defined as any case of acute flaccid paralysis in which vaccine related poliovirus isolated from at least one stool sample differed from the original Sabin vaccine strain by 1 to 15% of nucleotides in the region encoding the viral capsid protein VP1 (Kew, O.M., 2005). Cases of wild-type poliomyelitis virus were defined as cases of acute flaccid paralysis in which wild-type virus was detected in at least one stool sample. While cases of nonpolio acute flaccid paralysis were defined as those in which neither wild-type nor vaccine-related poliovirus was isolated in either of two adequate stool samples collected within 2 weeks after the onset of paralysis, at least 24 hours apart.
There are three categories of vaccine-derived polio-viruses (VDPVs), immunodeficiency-associated VDPVs (iVDPVs) associated with chronic poliovirus infections (Bellmunt, A., 1999; Halsey, N.A., 2004; Kew, O.M., 1998; Khetsuriani, N., 2003; Maclennan, C., 2004; Minor, P., 2001; World Health Organization (WHO), 2004a), and (Adu, F.D., 2007) circulating VDPVs (cVDPVs) associated with polio outbreaks in areas with low rates of OPV coverage (Kew, O.M., 2002; Liang, X., 2006; Rousset, D., 2003; Shimizu, H., 2001; Yang, C.F., 2003). A third category, ambiguous VDPVs (aVDPVs) include clinical isolates from patients with un recognized immunodeficiency and not associated with an outbreak (Cherkasova, E.A., 2002; Georgescu, M.M., 1997; Korotkova, E.A., 2003) and environmental isolates whose ultimate sources have not been identified (Blomqvist, S., 2004; Centre for Disease Control and Prevention (CDC), 2005a; Shulman, L., 2000).

The emergence of a serotype 2 circulating vaccine-derived poliovirus (cVDPV) in Nigeria has complicated the epidemiology of polio as well as vaccine selection and scheduling for supplementary immunization activities (Kew, O.M., 2005). It is evident that towards the end of the eradication campaign, the role of the virus diagnostic laboratories is increasing as all poliovirus isolations from paralytic case require virological follow-up to elucidate the source of the agent (World Health Organization (WHO), 2004b). That is, whether the paralysis is due to a wild-type infection or to Vaccine-associated Paralytic Bliomyelitis, or to infection with a non-polio enterovirus causing polio-like symptoms (Melnick, J.L., 1984; Hayward, J.C., 1989). Since the beginning of 2001, all polio isolates from WHO laboratory network are tested by two intratypic differentiation (ITD) methods; one antigenic and one genomic. All isolates that are Sabin-like in one test but not Sabin-like in the other are sequenced in VP1.

**MATERIALS AND METHODS**

The methods adopted for AFP surveillance and Laboratory testing of faecal specimens’ isolation and typing of isolates, ITD and Sequencing procedures were as described in the WHO Polio Laboratory Manual, 2004 and the supplemental Manual of 2006 describing the New Algorithm Technique. In brief, surveillance officers, DSNOs and Paediatricians collected two faecal specimens per AFP case, 24 hours apart and within 14 days of the onset of paralysis, and were sent in Giostyle box with ice packs to preserve the cold chain to the National Laboratory.

Feacal samples were pre-treated with chloroform before being inoculated on a healthy monolayer of L20B Mouse and Rhabdomyosarcoma (RD) cell lines in maintenance medium (Eagle’s MEM supplemented with 2% fetal calf serum (FCS). The cells were seeded 48 hours earlier into culture tubes with growth medium (Eagle’s MEM supplemented with 10% FCS). The inoculated monolayers were observed daily for the characteristic enterovirus cytopathic effects (CPE) of rounded, refractile cells 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 second cell line, while those negative after 5 days of incubation were re-passaged on the same cell line after being negative for another 5 days is declared Negative. Positives isolates are preferred in RD cells because they retain higher titre even at destination when shipped to Global Reference Laboratories (Usually takes 5-7 days in transit) for other molecular techniques.

**Isolation and Identification of Polio and Non-polio Enteroviruses:**

**Titration of Sabin like Poliovirus Type 2 and cVDPV 2 Isolates:**

Dilution tubes with screw caps are labelled $10^{-1}$ – $10^{-9}$ per virus isolate. 1.8 ml of Maintenance medium (MM) are dispensed into the tubes. 0.2 ml of the isolate is pipetted into the first tube using a pipettor with sterile Aerosol Resistant Tips (ARTs). Cover the tube and vortex gently. Take another pipette tip, transfer 0.2 ml from the first tube to the second tube, and discard pipette tip. Cover the tube and vortex gently. Repeat dilution steps, transferring 0.2 ml at each time and always changing pipette tip between dilutions, up until tube 9. Add 100 μl of virus dilutions to 96 well microtitre plate from wells 1 to 10 in rows A to H – that is, 20 wells per dilution from $10^{9}$ to $10^{4}$. Add 100 μl of maintenance medium to wells A11 to H12 in rows for the cell controls. Label the edge of the microtitre plate appropriately, indicating the dilution e.g. $10^{9}$, $10^{8}$ to $10^{4}$ as applied in this case. Add 100 μl each of L20B cells from a cell suspension containing 2 x $10^{5}$ cells /ml to all wells in rows A to H on the plate. Cover the plate with non-toxic sealer and incubate at 36°C. Examine for development of CPE, using an inverted microscope, and record daily readings for 5–7 days. For a valid test, the cell control should have a complete monolayer of healthy cells.
Fig 1: Flow-chart for virus isolation in RD and L20B cells by New Algorithm Technique (WHO, 2006). Isolates with growth L+R+ or L-L+R+ were selected for this study.

Fig. 2: a and 2b are pictures of Rhabdomyosarcoma (RD) cells showing a healthy monolayer and typical enterovirus cytopathic effects. MDGlab2010.

Fig. 3: a and 3b are the pictures of a healthy monolayer of L20B cell and with typical enterovirus cytopathic effects. MDGlab2010.
Validity of Virus Titration Test:

The Sabin 2 NIBSC Reference Strains Number 01/530 on three separate microtitrations mean on the cell lines was carried out as recommended in WHO Polio Laboratory Manual of 2004 and the standard values of 5.1 log 10 CCID_{50}/0.1ml in RD cells and 4.8 log 10 CCID_{50}/0.1ml in L20B cells was obtained.

For the test to be valid, the following condition was observed; The first dilutions (10^{-6}) showed > 90% CPE. The last dilutions (10^{-9}) showed < 10% CPE.

Calculation of the Virus Titre by the Karber Formula:

\[ \log \text{CCID}_{50} = L - d (S - 0.5) \]

where: \( L \) = log of lowest dilution used in the test, \( d \) = difference between log dilutions steps, \( S \) = sum of proportion of ‘positive’ tests (i.e. cultures showing CPE).

RESULTS AND DISCUSSIONS

Table 1: Comparison of the potency of poliovirus serotype 2 among cVDPV and SL based on their titres.

<table>
<thead>
<tr>
<th>SN</th>
<th>cVDPV2 titre (n=10)</th>
<th>SL2 titre (n=10)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>7.30</td>
<td>7.10</td>
</tr>
<tr>
<td>2</td>
<td>7.10</td>
<td>6.95</td>
</tr>
<tr>
<td>3</td>
<td>7.15</td>
<td>7.50</td>
</tr>
<tr>
<td>4</td>
<td>7.15</td>
<td>7.40</td>
</tr>
<tr>
<td>5</td>
<td>7.50</td>
<td>7.25</td>
</tr>
<tr>
<td>6</td>
<td>7.30</td>
<td>7.30</td>
</tr>
<tr>
<td>7</td>
<td>7.35</td>
<td>7.25</td>
</tr>
<tr>
<td>8</td>
<td>6.85</td>
<td>7.35</td>
</tr>
<tr>
<td>9</td>
<td>7.30</td>
<td>7.15</td>
</tr>
<tr>
<td>10</td>
<td>7.80</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Mean titre 7.26 7.24

The mean titre of cVDPV and SL are closely related. This table shows the mean and standard deviation of group statistics for cVDPV and SL with a mean of 7.26 and 7.24.

Table 2: Mean and standard deviation (mean ± S.D) for total cVDPV and SL and correlation coefficient.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± S.D</th>
<th>P-value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>cVDPV2</td>
<td>7.26 ± 0.26</td>
<td>0.16</td>
<td>Non significant</td>
</tr>
<tr>
<td>SL2</td>
<td>7.24 ± 0.16</td>
<td>0.15</td>
<td>Non significant</td>
</tr>
</tbody>
</table>

From the value obtained in the table above, it shows that the P values (0.89) is greater than 0.05. This indicates that there is no significant difference between cVDPV and SL. They both passed normality test. Standard deviation 0.26 and 0.16 respectively. For two tailed P value is 0.89, considered not significant. \( t = 0.15 \) with 9 degrees of freedom.

The eradication of type 2 WPV globally in 1999 was a remarkable achievement, but the fragility of this achievement is glaring, given the pathogenicity and severity of diseases associated with type 2 cVDPVs. No significant differences were found in the titre of for cVDPV 2 and Sabin like 2 which is also an indication of degree of virulence and pathogenicity. This calls for the need for vaccine compliancy which cannot be overemphasised to terminate the circulation of cVDPV and total eradication of wild polioviruses and its associated problems in the environment.

The estimated average annual clinical attack rate of type 2 cVDPV per 100,000 susceptible children under 5 years of age was 2.7 (95% CI, 1.9 to 3.6) (Jenkins, H.E., 2010). This is quite high with regard to the resources being utilised under the global polio eradication initiative. In addition, the frequent use of serotype 1 and serotype 2 monovalent and bivalent oral polio vaccines has resulted in improvements in vaccine-induced population immunity against these serotypes and in declines in immunity to type 2 which gave way for the cVDPVs epidemic in Nigeria. In addition, most doses of oral polio vaccine received by children in Nigeria are delivered through supplementary immunization activities rather than routine services which is not proper for efficient vaccine coverage.

To continue progress towards more rapid detection of WPV and cVDPVs the implementation of the algorithm for laboratory testing and incorporation of new diagnostic tools (such as real-time polymerase chain reaction) should be carried through to completion. The pursuit of an active research agenda by the eradication programme through the reconstituted Polio Research Committee, using new strategies to overcome obstacles to eradication. We therefore recommend active regional and town planning that will also intervene even in the existing building structures and road networks of the congested areas to facilitate aeration and easier access.
even during Immunization Plus Days (IPDs) in the states covered by this study. In addition, provision of portable water, improved sanitation and hygiene, discouraging overcrowding will enhance the eradication of cVDPVs and wild poliovirus-generally.

REFERENCES


