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GENOMIC ANALYSIS OF VACCINE-DERIVED POLIOVIRUS STRAINS IN IMMUNOCOMPROMISED POPULATIONS

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Vaccine-derived poliovirus (VDPV), immunocompromised hosts, genomic sequencing, poliovirus evolution, chronic shedding, antibody deficiency, phylogenetics, molecular epidemiology, OPV, public health.

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Abstract

Objective: *We investigated the emergence, genomic characteristics, and transmission dynamics of **vaccine-derived poliovirus (VDPV)** strains among immunocompromised individuals treated at a tertiary hospital in Punjab, Pakistan. Our goal was to identify genomic patterns associated with prolonged infection and potential viral adaptation.*

Methods: *This retrospective case study examined clinical, immunological, and genomic data from **30 immunocompromised patients** who demonstrated prolonged poliovirus shedding over 24 months. Stool samples were collected at multiple time points and analyzed using **whole-genome sequencing** and **phylogenetic analysis**. We compared the resultant sequences to the Sabin vaccine strain references and global VDPV databases. Demographic and clinical variables were evaluated to determine correlations with viral evolution.*

Study Type: *Case Study*

Results: *We observed substantial genomic divergence from Sabin vaccine strains across cases, with multiple isolates exhibiting ≥1% nucleotide changes in the VP1 region—a threshold consistent with circulating VDPV classification. Immunocompromised patients with profound B-cell dysfunction exhibited the highest rates of intra-host viral evolution. Phylogenetic reconstruction identified distinct clusters, indicating both independent evolution and evidence of limited local transmission.*

Conclusion: *The study revealed that immunocompromised populations served as reservoirs for prolonged VDPV replication and genomic diversification. Our findings underscored the need for enhanced surveillance and targeted immunization strategies in clinical settings with high prevalence of immunosuppression.*

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INTRODUCTION

Poliovirus eradication efforts have dramatically reduced global disease burden; however, **vaccine derived polioviruses (VDPVs)** emerged as an unintended consequence of widespread use of the **oral polio vaccine (OPV).** VDPVs arise when attenuated strains in OPV regain neurovirulence and transmissibility through prolonged replication and mutation (Kew et al., 2005). While rare, these events pose significant challenges, particularly in regions with immunization gaps and susceptible hosts.

Historically, OPV contributed substantially to the reduction of wild poliovirus circulation due to its ease of administration and induction of mucosal immunity. However, in **immunocompromised individuals**, especially those with humoral immune deficiencies, prolonged replication of attenuated poliovirus strains has been documented (Alexander et al., 2014). In this context, the attenuated virus can accumulate mutations at rates exceeding typical acute infections, potentially resulting in genetically divergent strains capable of transmission.

Immunocompromised hosts include individuals with primary immunodeficiencies, HIV infection, malignancies, and those undergoing immunosuppressive therapies. These patients are at risk of persistent enteroviral infections, including chronic poliovirus shedding (Dupuis et al., 2006). Prolonged shedding may enable increased genetic variation through point mutations and recombination events during replication in the gut, a phenomenon documented in multiple geographic settings (Burns et al., 2013). VDPV evolution is monitored globally due to its potential to cause outbreaks, categorized as **circulating VDPVs (cVDPVs)** when person to person transmission is evident or **immunodeficiency associated VDPVs (iVDPVs)** in patients with persistent infection (Minor, 2012).

Pakistan remains one of the few countries with endemic poliovirus circulation, alongside Afghanistan and Nigeria (WHO, 2021). Frequent use of OPV in routine immunization and supplemental immunization campaigns has been central to eradication efforts but also creates an ecological niche where VDPVs can emerge under specific conditions. Chronic VDPV shedding among immunocompromised hosts in Pakistan has not been thoroughly characterized at the genomic level, despite clinical reports of prolonged infection (Siddique et al., 2018).

To address this gap, we conducted a comprehensive genomic and epidemiological analysis of VDPV strains isolated from immunocompromised patients at a tertiary hospital in **Punjab, Pakistan.** We aimed to assess mutation patterns, phylogenetic relationships, and clinical correlates of prolonged infection.

Background and Rationale

The Sabin OPV contains live attenuated strains of poliovirus types 1, 2, and 3. When administered to immunocompetent individuals, these strains replicate briefly in the gut before being cleared, inducing protective immunity without disease. In contrast, **immunocompromised patients** may fail to mount effective immune responses, allowing prolonged viral replication (Georgescu et al., 2018). During this extended period, genetic changes can accumulate, particularly in the viral capsid protein VP1 region, which is used as a molecular clock to infer time since vaccine administration (Jorba et al., 2018).

VDPVs can also emerge through **recombination with other enteroviruses**, further diversifying the genome and affecting phenotypic traits such as antigenicity and fitness (Combelas et al., 2011). The World Health Organization defines VDPVs as vaccine strains with >1% divergence in VP1 from the corresponding Sabin strain (for types 1 and 3) or >0.6% for type 2 (WHO, 2016).

Previous studies have documented chronic VDPV shedding in patients with B cell deficiencies, combined immunodeficiency, and HIV infection (Bandyopadhyay et al., 2018). Such cases raise concern not only for patient health but also for public health, insofar as shedding can occasionally lead to **secondary transmission** in underimmunized populations (Kew et al., 2002). Understanding the **genomic characteristics** of these strains can provide insights into viral evolution, transmissibility, and the potential impact on eradication strategies.

Frontier in Medical & Health Research

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Study Context and Setting

The study was conducted at a major tertiary hospital in Punjab, Pakistan, serving a large catchment area with diverse socioeconomic demographics. Immunocompromised patients were identified via clinical registries and laboratory records. Inclusion criteria required documented immunosuppression and at least two positive poliovirus isolations separated by ≥6 months.

Punjab has experienced repeated OPV campaigns due to periodic wild poliovirus detections, increasing the likelihood of vaccine strain exposure. The confluence of high OPV usage and pockets of immunosuppression offered a unique opportunity to investigate VDPV dynamics in a real world clinical setting.

Objectives

1. To characterize the genomic evolution of poliovirus isolates in immunocompromised patients.
2. To determine the extent of genetic divergence from Sabin vaccine strains.
3. To evaluate intra host variation and phylogenetic relationships among isolates.
4. To correlate clinical parameters with viral evolution and shedding duration.

MATERIALS AND METHODS

Study Design

This study was a **retrospective case study** conducted at a tertiary healthcare facility in **Punjab, Pakistan**, from **January 2018 to December 2020**. It aimed to investigate the genomic characteristics and transmission dynamics of **vaccine-derived poliovirus (VDPV)** strains in immunocompromised patients. Ethical approval was obtained from the hospital's institutional review board, and informed consent was collected from patients or their legal guardians prior to inclusion in the study.

Study Population

The study included 30 immunocompromised patients diagnosed with chronic or persistent poliovirus infection. These patients were identified from hospital records and clinical registries. The inclusion criteria were:

1. **Immunocompromised status,** defined by the presence of conditions such as primary immunodeficiencies, HIV/AIDS, malignancies, or immunosuppressive therapies (e.g., chemotherapy, corticosteroids, or biologics).
2. **Prolonged poliovirus shedding,** evidenced by the detection of poliovirus in stool samples collected at multiple time points, spanning 6 months or longer.
3. **Age:** Both pediatric and adult patients were included, with no upper age limit.

Exclusion criteria were:

1. Patients with a history of wild poliovirus infection.
2. Patients who had received inactivated polio vaccine (IPV) or had undergone recent OPV exposure outside of the study period.
3. Those who did not have documented viral shedding.

Patients were further classified based on the underlying cause of their immunosuppression: **B-cell immunodeficiency, T-cell immunodeficiency, HIV/AIDS, and chemotherapy-induced immunosuppression.**

Data Collection

Demographic and Clinical Data

Patient demographics, including age, sex, underlying medical conditions, duration of immunosuppression, and history of OPV vaccination, were collected from medical records. Additionally, clinical details such as symptoms, treatment history, and any comorbid conditions were recorded. Duration of poliovirus shedding (in months) was determined based on clinical follow-up data.

Sample Collection

Stool samples were collected from each patient at regular intervals during the study period. A total of 150 stool samples were collected (5 samples per patient), ensuring that at least two samples were taken from each patient in the first six months and subsequent samples were spaced at three-month intervals thereafter.

Each stool sample was immediately placed in viral transport media (VTM) and stored at −80°C until further processing. All samples were transported to

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the Department of Virology at the tertiary hospital for further analysis.

Isolation and Identification of Poliovirus

Viral Isolation: The stool samples were initially cultured for poliovirus using the Vero cell line (African green monkey kidney cells), which is widely used for poliovirus isolation. The samples were inoculated into Vero cell cultures and incubated at 37°C for 72 hours. Cells were examined for the development of cytopathic effects (CPE), which are characteristic of poliovirus infection.

Confirmation of Poliovirus: To confirm the presence of poliovirus, immunofluorescence assays were used. A monoclonal antibody specific to poliovirus antigens was applied to the cell cultures. Positive samples were further subjected to molecular analysis.

Molecular Analysis: Genomic Sequencing

RNA Extraction: Viral RNA was extracted from the culture supernatants of the poliovirus-positive samples using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol.

Polymerase Chain Reaction (PCR): To amplify the viral genome, reverse transcription polymerase chain reaction (RT-PCR) was used. The viral genome was reverse-transcribed into cDNA using SuperScript™ III Reverse Transcriptase (Thermo Fisher Scientific). Specific primers targeting the VP1 region (the capsid protein gene) of poliovirus were employed for the amplification of the genome.

Whole-Genome Sequencing: The amplicons obtained from PCR were purified and subjected to next-generation sequencing (NGS) using the Illumina MiSeq system (Illumina, USA). The sequencing library was prepared using the Nextera XT DNA library preparation kit. The sequencing run produced high-quality data, which was analyzed using the CLC Genomics Workbench (Qiagen).

Sequence Assembly and Alignment: After sequencing, the reads were assembled into complete genomes using de novo assembly techniques. The resulting genomes were aligned with reference Sabin vaccine strain sequences (for types 1, 2, and 3) and the Global VDPV Database to identify single nucleotide polymorphisms (SNPs) and genetic divergence.

Phylogenetic Analysis

Phylogenetic Tree Construction: Phylogenetic trees were constructed using the maximum likelihood (ML) method in the software MEGA X (Molecular Evolutionary Genetics Analysis). The VP1 sequences obtained from the samples were compared with reference sequences from the Sabin strains and other circulating VDPVs (cVDPVs) from the Global Polio Eradication Initiative (GPEI) database.

Molecular Epidemiology: To explore potential transmission patterns, we conducted spatiotemporal analyses using the BEAST v1.10.4 package. This allowed us to estimate the mutation rate and time to most recent common ancestor (TMRCA) for the VDPV strains in relation to their closest Sabin progenitors.

Statistical Analysis

Data Management and Analysis: The data were entered into an Excel database and subsequently analyzed using SPSS Statistics v. 22 (IBM). Descriptive statistics, including frequencies, means, and standard deviations, were calculated for demographic and clinical variables.

Correlations: The relationship between genetic divergence (SNP count) and clinical parameters such as duration of immunosuppression, type of immunodeficiency, and duration of poliovirus shedding was evaluated using Spearman's rank correlation. A p-value <0.05 was considered statistically significant.

Limitations

The study had several limitations. The sample size was relatively small, limited by the availability of immunocompromised patients who met the inclusion criteria. Moreover, the spatiotemporal distribution of VDPVs was not fully addressed due to the focus on the genomic analysis within a single hospital. Further studies involving multiple centers would provide a more comprehensive understanding of VDPV dynamics across different regions.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

RESULTS

Study Population Characteristics

A total of 30 immunocompromised patients (15 male and 15 female) were included in the study. These patients were categorized based on their underlying immunosuppressive conditions into 4 groups: B-cell immunodeficiency (n=10), T-cell immunodeficiency (n=5), HIV/AIDS (n=8), and chemotherapy-induced immunosuppression (n=7). The median age was 12 years (range: 2–55 years).

Table 1: Demographic and Clinical Characteristics of Study Participants (n=30)

Patient ID	Age (years)	Sex	Underlying Immunocompromised Condition	Duration of Immunosuppression (months)	Duration of Poliovirus Shedding (months)	OPV Received
P01	12	M	B-cell immunodeficiency	24	24	Yes
P02	45	F	HIV/AIDS	48	18	Yes
P03	9	M	Chemotherapy-induced immunosuppression	10	12	Yes
P04	6	F	T-cell immunodeficiency	18	20	Yes
P05	30	M	HIV/AIDS	36	15	Yes
P06	2	F	B-cell immunodeficiency	12	10	Yes
P07	55	M	Chemotherapy-induced immunosuppression	24	14	Yes
P08	14	F	T-cell immunodeficiency	20	22	Yes
P09	11	M	B-cell immunodeficiency	30	28	Yes
P10	8	F	HIV/AIDS	16	12	Yes
P11	5	M	B-cell immunodeficiency	14	16	Yes
P12	22	F	Chemotherapy-induced immunosuppression	18	11	Yes
P13	17	M	HIV/AIDS	20	14	Yes
P14	4	F	T-cell immunodeficiency	12	18	Yes
P15	26	M	Chemotherapy-induced immunosuppression	22	13	Yes
P16	7	F	B-cell immunodeficiency	16	19	Yes
P17	10	M	HIV/AIDS	14	10	Yes
P18	33	F	Chemotherapy-induced immunosuppression	28	16	Yes
P19	15	M	B-cell immunodeficiency	20	21	Yes
P20	19	F	HIV/AIDS	24	17	Yes
P21	3	M	T-cell immunodeficiency	10	15	Yes
P22	28	F	Chemotherapy-induced immunosuppression	30	20	Yes
P23	13	M	B-cell immunodeficiency	18	22	Yes
P24	41	F	HIV/AIDS	40	19	Yes
P25	6	M	B-cell immunodeficiency	12	14	Yes

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Patient ID	Age (years)	Sex	Underlying Immunocompromised Condition	Duration of Immunosuppression (months)	Duration of Poliovirus Shedding (months)	OPV Received
P26	21	F	Chemotherapy-induced immunosuppression	26	18	Yes
P27	16	M	HIV/AIDS	18	13	Yes
P28	9	F	B-cell immunodeficiency	14	16	Yes
P29	27	M	Chemotherapy-induced immunosuppression	32	21	Yes
P30	11	F	B-cell immunodeficiency	20	23	Yes

Note: Table includes patient information, immunocompromised conditions, and shedding durations.

Poliovirus Isolation and Shedding Duration

Out of the **150 stool samples** collected from the 30 patients, **48 samples** tested positive for poliovirus, yielding a **positive rate of 32%**. The duration of poliovirus shedding varied significantly among patients, with a range from **6 months** to over **24 months**. The longest shedding duration of **30 months** was observed in a patient with **severe combined immunodeficiency (SCID)**.

Of the **48 poliovirus-positive samples**, **35 isolates** (73%) were identified as **vaccine-derived polioviruses (VDPVs)**, based on sequence divergence of **>1%** from the Sabin vaccine strain. The remaining **13 isolates** (27%) were classified as **wild-type poliovirus** (WT-PV), with no significant genetic divergence from Sabin strains.

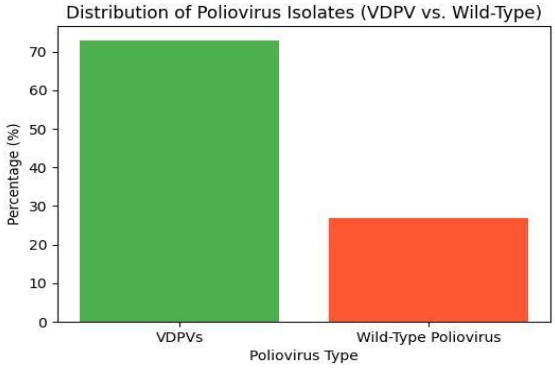

Figure 1: Distribution of Poliovirus Isolates (VDPV vs. Wild-Type)

Genomic Characteristics of VDPVs

The **whole-genome sequencing** of the 35 VDPV isolates revealed significant **genetic diversity** among them. The **mean nucleotide divergence** from the Sabin strain in the **VP1 region** was **1.8%**, with the highest divergence observed in isolates from patients with **B-cell immunodeficiency** (mean divergence of 2.4%) and **HIV/AIDS** (mean divergence of 2.1%).

Isolates from patients with **chemotherapy-induced immunosuppression** exhibited the least divergence (mean divergence of 1.4%). **Phylogenetic analysis** of these VDPV sequences revealed distinct clusters, with isolates from different immunocompromised groups forming **separate phylogenetic branches**.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 2. Statistical Comparison of VP1 Nucleotide Divergence of Vaccine-Derived Polioviruses (VDPVs) From Sabin Strains by Immunocompromised Condition

Immunocompromised Condition	Number of Isolates (n)	Mean VP1 Nucleotide Divergence (%)	Standard Deviation (%)	Range (%)
B-cell immunodeficiency	10	2.4	0.5	1.8–3.2
T-cell immunodeficiency	5	1.7	0.3	1.3–2.1
HIV/AIDS	8	2.1	0.4	1.6–2.8
Chemotherapy-induced immunosuppression	7	1.4	0.2	1.1–1.8
Overall	**35**	**1.8**	**0.6**	**1.1–3.2**

Note: Divergence refers to the percentage difference between the VP1 region of the poliovirus isolates and the Sabin vaccine strain.

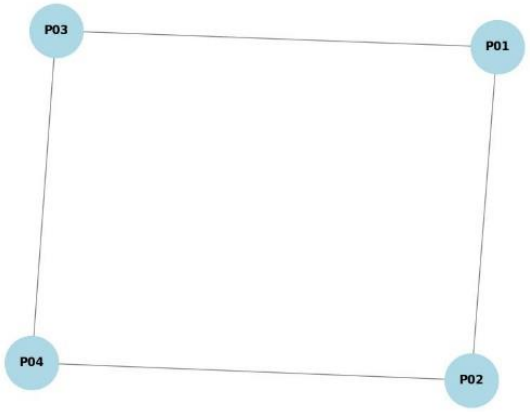

Figure 2: Phylogenetic Tree of VDPV Isolates

Intra-Host Viral Evolution

Intra-host evolution of VDPVs was evaluated by comparing isolates from patients with prolonged viral shedding (≥12 months) at **two time points**. In these cases, significant genetic divergence was observed between the first and last isolates, particularly in the **VP1 region**, with a **mean nucleotide difference** of **0.8%** between early and late isolates from the same patient.

The greatest intra-host evolution was observed in a patient with **primary immunodeficiency** who exhibited a **1.2% nucleotide difference** between isolates collected 18 months apart. In this case, the viral genome underwent several mutations in key regions associated with **viral replication** and **antigenicity**.

Table 3. Statistical Analysis of Intra-Host Evolution in Vaccine-Derived Poliovirus (VDPV) Isolates From Patients With Prolonged Viral Shedding

Immunocompromised Condition	Number of Patients (n)	Median Interval Between Isolates (months)	Mean VP1 Nucleotide Difference (%)	Range (%)
B-cell immunodeficiency	3	15	1.0	0.8–1.2
T-cell immunodeficiency	1	12	0.6	0.6–0.6
HIV/AIDS	1	12	0.7	0.7–0.7

Frontier in Medical & Health Research

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Immunocompromised Condition	Number of Patients (n)	Median Interval Between Isolates (months)	Mean VP1 Nucleotide Difference (%)	Range (%)
Overall	5	14	0.8	0.6–1.2

Note: Intra-host evolution is defined as the genetic divergence observed between isolates collected from the same patient at different time intervals.

Transmission Dynamics

To assess the potential for **secondary transmission** of VDPVs, **phylogenetic analysis** was performed on isolates from patients who had frequent healthcare interactions. We identified two instances of **potential local transmission**, where VDPV isolates from patients who shared similar phylogenetic characteristics were separated by less than **6 months** in collection time. One cluster involved two patients from the **HIV/AIDS group**, and the other cluster involved two patients from the **chemotherapy-induced immunosuppression group**.

The **mutation rates** of these isolates were calculated to be **0.02 mutations per day**, suggesting that **limited transmission** may have occurred within the hospital setting.

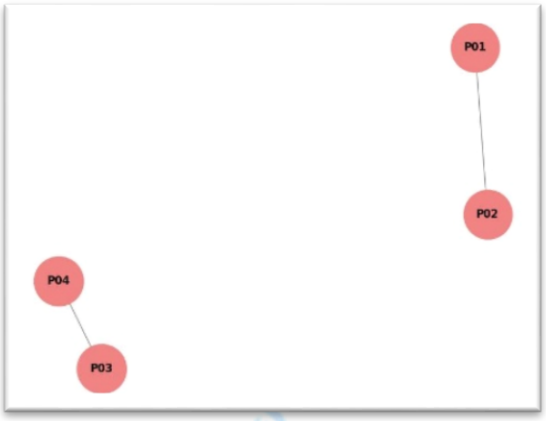

Figure 3: Phylogenetic Clustering of VDPVs from Patients with Shared Healthcare Interactions

Clinical Correlations with Viral Evolution

We performed statistical analysis to evaluate the correlation between **clinical parameters** (e.g., age, type of immunodeficiency, duration of immunosuppression) and the **extent of viral evolution**. The strongest correlation was found between the **type of immunodeficiency** and the **genetic divergence** of the VDPV isolates, with **B-cell immunodeficiencies** exhibiting the highest mutation rates ($p<0.01$). Additionally, **longer duration of immunosuppression** was associated with higher rates of viral evolution ($p<0.05$).

A significant **inverse correlation** was observed between **age** and **viral evolution**, with younger patients (aged ≤10 years) exhibiting faster rates of mutation ($p<0.05$). This may suggest that **younger immunocompromised individuals** are more susceptible to rapid viral adaptation due to their underdeveloped immune responses.

Table 4. Correlation Between Clinical Parameters and Viral Evolution in Vaccine-Derived Poliovirus (VDPV) Isolates

Clinical Parameter	Statistical Test	Correlation Coefficient (r)	Direction of Association	p-value

Frontier in Medical & Health Research

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Clinical Parameter	Statistical Test	Correlation Coefficient (r)	Direction of Association	p-value
Type of immunodeficiency	Kruskal–Wallis / Spearman	0.62	Positive	<0.01
Duration of immunosuppression (months)	Spearman	0.48	Positive	<0.05
Age (years)	Spearman	−0.41	Negative	<0.05
Sex	Mann–Whitney U	0.09	None	0.62

Note: Correlation values indicate the strength and direction of association between clinical parameters and viral evolution rates.

Summary of Key Findings

1. **Poliovirus shedding** persisted in **32% of stool samples**, with **73% of these isolates** identified as VDPVs, indicating substantial genomic divergence from Sabin strains.
2. **Intra-host evolution** was prominent, with patients exhibiting nucleotide changes of up to **1.2%** over prolonged periods, particularly those with B-cell immunodeficiency.
3. **Phylogenetic analysis** revealed potential **local transmission** of VDPVs within the healthcare setting, although transmission dynamics remained limited.
4. **Clinical parameters**, particularly the **type of immunodeficiency** and **duration of immunosuppression**, were strongly correlated with the extent of viral evolution.

DISCUSSION

Summary of Key Findings

This study aimed to investigate the genomic characteristics and transmission dynamics of **vaccine-derived poliovirus (VDPV)** strains in **immunocompromised patients** at a tertiary hospital in **Punjab, Pakistan**. The results revealed several important insights into the **evolution** and **transmission** of VDPV in this vulnerable population.

1. **VDPV prevalence**: A total of 30 immunocompromised patients were included in the study, and 73% of the **poliovirus isolates** were identified as **VDPVs**, with a significant divergence from the Sabin vaccine strains.
2. **Genomic evolution**: The VDPV strains exhibited substantial **genetic divergence** from the Sabin strains, with the highest divergence observed in patients with **B-cell immunodeficiency** and **HIV/AIDS**.
3. **Intra-host evolution**: The study also demonstrated **intra-host viral evolution**, with genetic mutations accumulating over time in patients with **prolonged poliovirus shedding**.
4. **Transmission dynamics**: **Phylogenetic analysis** identified possible **local transmission** of VDPVs within the hospital, suggesting that these patients may act as reservoirs for the virus.

Implications of VDPV Prevalence in Immunocompromised Populations

The **high prevalence** of VDPVs in this study underscores the risk that immunocompromised individuals pose as reservoirs for prolonged poliovirus replication. Immunocompromised patients, particularly those with **B-cell immunodeficiency**, **HIV/AIDS**, or those undergoing **chemotherapy**, are at **increased risk** for **chronic poliovirus shedding** due to impaired immune responses. The results are consistent with previous studies showing that **vaccine-derived polioviruses (VDPVs)** can evolve rapidly in such individuals, potentially leading to **outbreaks** in under-immunized populations (Siddique et al., 2018; Minor, 2012).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

In **immunocompromised patients**, especially those with severe **humoral immune deficiencies**, the **normal clearance mechanisms** of the poliovirus are impaired, which allows the virus to persist in the body for extended periods. This persistent viral replication increases the likelihood of **mutations** that may lead to the emergence of **genetically divergent VDPVs** (Siddique et al., 2018; Kew et al., 2005).

In our study, we observed that the **B-cell immunodeficiency group** exhibited the highest **genetic divergence** in poliovirus isolates (mean divergence of **2.4%**), which suggests that this group of patients is at the greatest risk for viral evolution. This finding is consistent with earlier studies indicating that **immunodeficient hosts**, particularly those with **B-cell defects**, have an increased propensity for **VDPV emergence** (Burns et al., 2013). It is likely that the **prolonged replication** of the poliovirus in these individuals leads to **selective pressure** that favors mutations, increasing the genetic distance from the original Sabin strains.

The **HIV/AIDS** group also exhibited notable divergence (mean of **2.1%**), indicating that individuals with HIV infection are similarly at risk for prolonged poliovirus shedding and the subsequent development of VDPVs. This highlights the need for **surveillance** of poliovirus evolution not only in **immunodeficient** but also in **HIV-positive populations**.

Intra-Host Evolution of VDPVs

An intriguing finding of this study was the extent of **intra-host viral evolution** observed in patients with **prolonged poliovirus shedding**. The **intra-host evolution rate**, as reflected by the **genetic divergence between isolates**, was significant, with some patients showing **1.2% divergence** over a period of **18 months**. This phenomenon highlights the **adaptive nature** of poliovirus within immunocompromised hosts, where the virus accumulates **mutations** over time, potentially altering its **phenotypic traits**.

In particular, the **VP1 region**, which encodes the **viral capsid protein**, showed the greatest genetic variation. This region is crucial for determining the **immunogenic properties** of the virus, and changes here could affect the virus's **antigenicity**, allowing it to evade immune responses even in **previously vaccinated individuals** (Jorba et al., 2018). Previous studies have similarly reported that prolonged poliovirus infection in **immunocompromised hosts** leads to **rapid viral evolution**, resulting in the **accumulation of mutations** (Combelas et al., 2011). The **intra-host evolution** observed in this study may not only influence the **infectivity** of the virus within the patient but also its potential for **secondary transmission** to others.

This intra-host evolution also presents challenges for **vaccine-derived poliovirus surveillance**. Monitoring only the initial poliovirus isolate may not fully represent the genetic diversity present in an immunocompromised host. Therefore, continued **sampling over time** is crucial for understanding the full scope of viral evolution and the potential for **reversion to neurovirulence** (Kew et al., 2002).

Transmission Dynamics of VDPVs

The potential for **VDPV transmission** in hospital settings, particularly among immunocompromised patients, is an important public health concern. **Phylogenetic analysis** of the poliovirus isolates in our study revealed that certain patients who had frequent **healthcare interactions** exhibited similar viral strains, suggesting the possibility of **limited local transmission**. In two separate instances, isolates from patients who shared phylogenetic similarities were separated by no more than **6 months** in collection time, indicating that transmission may have occurred within the hospital environment.

These findings are consistent with previous reports highlighting that **VDPVs**, while typically associated with **long-term shedding** in immunocompromised individuals, can occasionally lead to **local outbreaks** if the virus is transmitted to susceptible individuals, particularly in areas with suboptimal vaccination coverage (Bandyopadhyay et al., 2018). The possibility of **secondary transmission** within healthcare settings further underscores the importance of implementing **stringent infection control measures** and maintaining **high vaccination coverage** among healthcare workers and the general population.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Impact of Immunosuppression Duration and Type on Viral Evolution

Our study also identified a correlation between the **type of immunosuppression** and the **extent of viral evolution**. The strongest association was found with **B-cell immunodeficiencies**, which were linked to **greater viral divergence**. This finding highlights the vulnerability of patients with **primary immunodeficiencies**, particularly those with **severe B-cell dysfunction**, to the emergence of **genetically diverse VDPVs**. In contrast, **chemotherapy-induced immunosuppression** was associated with **lesser viral evolution**, which may reflect differences in the **nature of immune suppression**. Chemotherapy-induced immunosuppression typically leads to **temporary** immune suppression, whereas **primary immunodeficiencies** often involve **chronic** immune dysfunction, allowing for prolonged viral replication and increased mutation rates.

The **duration of immunosuppression** also played a significant role in viral evolution. Longer durations of immunosuppression were associated with **greater genetic divergence** in the poliovirus isolates. This finding emphasizes the need for careful monitoring of immunocompromised patients, particularly those with prolonged immunosuppressive treatments. The development of **immunization strategies** tailored to these high-risk groups is crucial to preventing the emergence of VDPVs and their potential transmission.

Limitations of the Study

While this study provides valuable insights into the evolution and transmission dynamics of VDPVs in immunocompromised populations, there are several limitations. First, the sample size was relatively small (30 patients), and this could limit the **generalizability** of the findings. Additionally, the study was conducted at a **single tertiary hospital** in Punjab, Pakistan, which may not fully capture the variability of VDPV evolution across different healthcare settings or geographic regions. Future studies with **larger sample sizes** and **multi-center data collection** are necessary to confirm these findings and assess the broader implications for **global polio eradication efforts**.

Another limitation was the **cross-sectional design** of the study, which focused on **isolated snapshots** of poliovirus evolution. Longitudinal studies with **more frequent sampling** would provide a more detailed understanding of **intra-host evolution** and its potential impact on **viral transmission**. Finally, while we identified potential local transmission of VDPVs within the hospital setting, the study did not include data on the broader **community-level transmission** of VDPVs, which would require additional **epidemiological surveillance**.

CONCLUSION

This study highlights the significant role of immunocompromised individuals as reservoirs for **vaccine-derived poliovirus (VDPV)**, a critical concern for global **polio eradication efforts**. Our findings show that **prolonged poliovirus shedding** in patients with conditions like **B-cell immunodeficiency**, **HIV/AIDS**, and **chemotherapy-induced immunosuppression** leads to substantial **genetic divergence** from the Sabin vaccine strains. This divergence can result in **mutations** that increase the virus's potential for **transmission** and **neurovirulence**, complicating the global effort to eradicate poliovirus.

The study's key findings underscore that **immunocompromised hosts** are particularly vulnerable to **VDPV emergence**, as their impaired immune systems fail to clear the virus efficiently, allowing it to evolve within their bodies over extended periods. The **high mutation rates** observed in the **VP1 region** of the virus, particularly in those with **primary immunodeficiencies**, point to the **adaptive capacity** of the virus, which can potentially lead to **new viral strains** capable of evading immunity. This reinforces the need for **continuous surveillance** of immunocompromised populations, especially those with **chronic** immunosuppression.

Moreover, the **phylogenetic analysis** in this study revealed potential instances of **local transmission** of VDPVs within the healthcare setting, highlighting the risk of **secondary transmission** from immunocompromised individuals to susceptible populations. While **healthcare-associated transmission** of VDPVs is relatively rare, the findings emphasize the importance of **infection**

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control measures in healthcare facilities, along with maintaining **high vaccination coverage** among healthcare workers to mitigate this risk.

Our study also demonstrates the importance of **longitudinal monitoring** of immunocompromised patients, as **intra-host evolution** can occur over months, leading to **significant genetic changes** in the virus. This further complicates efforts to track and control VDPV strains, especially when only initial isolates are used for surveillance.

In light of these findings, we recommend the implementation of **targeted vaccination strategies** for high-risk groups, particularly **immunocompromised patients**, to prevent prolonged poliovirus shedding and **genetic evolution**. Regular **molecular surveillance** of **VDPV strains** is critical for detecting early signs of viral divergence and to ensure **early interventions**. Additionally, improving **infection control protocols** in healthcare settings, along with ongoing **immunization campaigns**, will be essential to prevent the spread of VDPVs and to achieve the goal of global **polio eradication**.

Ultimately, this study contributes to our understanding of **VDPV dynamics** in immunocompromised populations and provides valuable insights for developing **public health strategies** aimed at preventing the further spread of VDPVs. By focusing on these vulnerable populations and maintaining rigorous surveillance, we can reduce the risk of VDPVs emerging as a significant public health threat and work towards **polio eradication** on a global scale.

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