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Article

Rotavirus Prevalence, Genetic Diversity, and Co-Infections during the 2023-2024 Cholera Outbreak in Zambia: Insights from Multi-Pathogen Diagnostics

Adriace Chauwa [1], Samuel Bosomprah [1,2], Bernard Phiri [1], Natasha M. Laban [1], Dhvani H. Kuntawala [1], Dennis Ngosa [1], Harriet Ng'ombe [1,3], Fraser Liswaniso [1], Charlie C. Luchen [1,4], Mutinta Muchimba [1], Innocent Mwape [1,3,5], Bertha T. Nzangwa [1], Sekayi F. Tigere [1], Kennedy Chibesa [1], Suwilanji Silwamba [1], Michelo Simuyandi [1], Nyuma Mbewe [3], Roma Chilengi [1,5] and Caroline C. Chisenga [1'] *

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Abstract

During cholera outbreaks in Zambia, diagnostic strategies that rely on single-plex or targeted assays risk overlooking concomitant infections with other clinically important enteric pathogens. We estimated the prevalence of rotavirus and described co-detected enteropathogens and rotavirus genotypes among patients admitted with clinically suspected cholera during Zambia's 2023–2024 cholera outbreak. We conducted a sub-analysis of diarrhoeal specimens collected from patients admitted to five cholera treatment centres who met the syndromic suspected cholera case definition. Stool samples were tested using the Bosphore® Gastroenteritis Panel v2, a multiplex PCR enteric panel, to detect rotavirus and other gastrointestinal pathogens. Rotavirus-positive specimen with sufficient viral load were further characterised by RT-PCR genotyping and Sanger sequencing targeting VP7 and VP4 genes**.** Among 319 suspected cholera admissions, rotavirus was detected in 18 patients, yielding a prevalence of 5.6% (95% CI 3.4%, 8.8%). Rotavirus detections occurred predominantly in children aged <5 years (87.5%) and 6-15 years (80.0%). Co-infection was common - 93.7%, (15/16) of rotavirus-positive samples showed co-infection with at least one additional enteric pathogen, primarily *Campylobacter*. Genotyping was successful in five samples and showed heterogenous circulating strains, including G1P[8], G2P[4], G3P[6], G12P[6], and a rare G1P[6] reassortant. During a large 2023–2024 cholera outbreak in Zambia, rotavirus accounted for a modest but clinically important fraction of the suspected cholera admissions and was typically identified within mixed enteric infections. These findings highlight the limitations of syndromic diagnosis in outbreak settings and support integrating multi-pathogen diagnostics and sustained molecular surveillance to improve case management, antimicrobial stewardship, and vaccine-era monitoring.

Keywords: rotavirus; diarrhoeal disease; genotype diversity; cholera outbreak; surveillance

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Introduction

Diarrhoeal diseases remain one of the leading causes of preventable morbidity and mortality worldwide, disproportionately affecting young children in low-resource settings. Each year, these illnesses are responsible for around 1.17 million deaths globally (Du et al., 2022a), with the WHO African region alone recording approximately 515,000 diarrhoeal deaths in 2020 (1). National

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statistics in Zambia show that diarrhoea is among the top killers of children under five, causing an estimated 15,000 child deaths annually (2). During declared cholera outbreaks, the singular diagnostic focus on *Vibrio cholerae* can obscure the true aetiology of diarrhoeal disease, particularly in children. This narrow approach misses co-circulating pathogens like rotavirus, limiting a comprehensive understanding of the disease burden and hindering targeted public health action.

Cholera's acute watery diarrhoea is clinically indistinguishable from that caused by other pathogens like rotavirus and *Escherichia coli* (*E. coli*) (3). The diagnostic overlap is often witnessed in outbreak settings; for example, in a cholera-endemic region of the Democratic Republic of Congo, just 38% of suspected cholera patients were PCR-positive for *V. cholerae*, while enterotoxigenic *E. coli* and Cryptosporidium were identified in 36% and 28% of cases, respectively (4). Similarly, during a large diarrhoeal outbreak in Bangladesh, rotavirus emerged as the leading cause of acute diarrhoea among children under five (26% of cases), surpassing cholera in that age group (5). Treating such non-cholera cases as cholera results in unnecessary antibiotic use that provides no benefit against viruses and instead fuels AMR (6), while this diagnostic oversight would miss an opportunity to document rotavirus genotypes, data which is crucial for evaluating vaccine effectiveness and efficacy. Therefore, the importance of detecting RV in outbreak contexts extends beyond case attribution.

Implementing multi-pathogen diagnostic panels during outbreaks yields two vital streams of data: molecular epidemiological intelligence on circulating strains and evidence of co-infection patterns with other enteric agents. Rotavirus surveillance is particularly critical in countries implementing live attenuated vaccines, where ongoing viral evolution, reassortment, and genotype replacement may influence vaccine performance. In this context, understanding rotavirus genetic diversity during large diarrhoeal outbreaks provides essential data to inform immunisation programmes and long-term disease control strategies. Zambia's recent transition from Rotarix® to Rotavac®, - both live attenuated oral vaccines but derived from different strain formulations, underscores the need for continued molecular surveillance to monitor circulating genotypes and detect shifts that may have implications for vaccine effectiveness.

The 2023-2024 cholera outbreak in Zambia provided a unique opportunity to leverage multi-pathogen diagnostics to assess rotavirus epidemiology beyond routine surveillance frameworks. In this study, we investigated the prevalence of rotavirus among clinically suspected cholera cases, characterised the genetic diversity of circulating rotavirus strains, and examined patterns of co-infection with other enteric pathogens.

Methods

Study Design: This was a laboratory-based analysis nested within a broader multi-pathogen investigation conducted during the 2023-2024 cholera outbreak in selected provinces of Zambia. The sub-study aimed to identify rotavirus cases among suspected cholera cases and understand their contributions to the burden of diarrhoeal disease during the outbreak at period of heightened clinical surveillance. In this sub-analysis, we focused specifically on evaluating the presence and molecular characteristics of rotavirus in patients initially suspected to have cholera, with the goal of assessing its contribution to the clinical case burden.

Participants: We included a total of 351 patients from five (5) cholera treatment centres across Lusaka and surrounding districts who presented with acute watery diarrhoea and met the clinical case definition for cholera.

Stool sample processing: Total nucleic acid was extracted from the participant stool samples using a published standard silica membrane-based protocol (7). Stool samples weighing approximately 150mg were homogenised in SK 38 bead-beating tubes containing easyMAG® Lysis Buffer (bioMérieux S.A., Marcy l'Etoile France). The resulting homogenate was then centrifuged at 14,000 RPM for 2 minutes, and 200µl of the supernatant was utilised for subsequent nucleic acid extraction using the Qiagen MinElute Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Eluted nucleic acid was stored at -80 °C until ready for use.

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Purified nucleic acids were screened using the Bosphore® Gastroenteritis Panel v2 (Anatolia Geneworks), a multiplex real-time quantitative PCR assay targeting 11 enteric pathogens, including astrovirus, rotavirus, norovirus G1 and GII, adenovirus, while the bacterial pathogens comprise *Clostridium difficile*, *Campylobacter spp*., *Salmonella spp*., Enteroinvasive *E. coli* (EIEC) and *Shigella spp*., verotoxigenic *E. coli* (VTEC) and *Yersinia enterocolitica*. The assay was run in a 25 µL reaction volume according to the manufacturer's instructions, using the Bosphore® Rotor-Gene® or compatible thermocycler platform. Pathogen-specific detection was based on fluorescence signal threshold cycles (*Ct*), with *Ct* <35 considered positive.

Rotavirus Testing: For this sub-analysis, rotavirus-positive stool nucleic acid extracts were selected for genotyping based on *Ct* value and sample quality. Reverse transcription PCR (RT-PCR) was performed in a 20 µL reaction volume using previously described Gouvea primers (for VP7) and Gentsch primers (for VP4), targeting the outer capsid protein genes used in G and P genotyping, respectively. The RT-PCR was carried out using the SuperScript™ III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogen), under the following cycling conditions: reverse transcription at 50 °C for 30 min, initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 42 °C for 30 sec, and 68 °C for 1 min, with a final extension at 68 °C for 5 min. Amplicons were resolved by agarose gel electrophoresis and purified using a QIAquick PCR Purification Kit (Qiagen). Sequencing was performed via Sanger sequencing at Zambia National Public Health Institute (ZNPHI) reference laboratory, and genotype assignment was conducted using RotaC v2.0.

Statistical Analysis

Demographic characteristics were summarised using descriptive statistics, with categorical variables presented as frequencies (percentages) and continuous variables as medians and interquartile intervals (IQIs). Rotavirus positivity was reported as an overall proportion. Frequencies of rotavirus-positive participants were visualised across predefined age categories. Rotavirus genotypes were described, and their distribution was examined in relation to co-detected enteric pathogens using a genotype–pathogen co-occurrence plot. All analyses were conducted using STATA 18 (StataCorp, College Station, TX, USA).

Ethics Statement

Ethical approval for the parent study was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC; Ref: 001-02-23) and the National Health Research Authority (NHRA). Written informed consent was obtained from all adult participants or from parents/legal guardians of children prior to their enrolment into the parent study. This sub-analysis on rotavirus data constitutes a secondary analysis within the scope of the original approved protocol. All data were de-identified and stored securely to ensure participant confidentiality. All procedures were performed in accordance with the ethical standards of the responsible committees and with the Helsinki Declaration.

Results

Tracing of Study Participants

The study workflow began with 351 patients who presented at the 5 cholera admission centres with clinically suspected cholera. After excluding 32 individuals who were not tested for multi-pathogens, a final study population of 319 patients who were both clinically suspected cholera cases and had multi-pathogen test results. Out of 319 participants, 18 individuals, tested positive for rotavirus. Among the 18 rotavirus-positive cases, 7 patients tested positive for cholera via a Rapid Diagnostic Test (RDT), while the other 11 individuals were not tested with a cholera RDT but were managed clinically.

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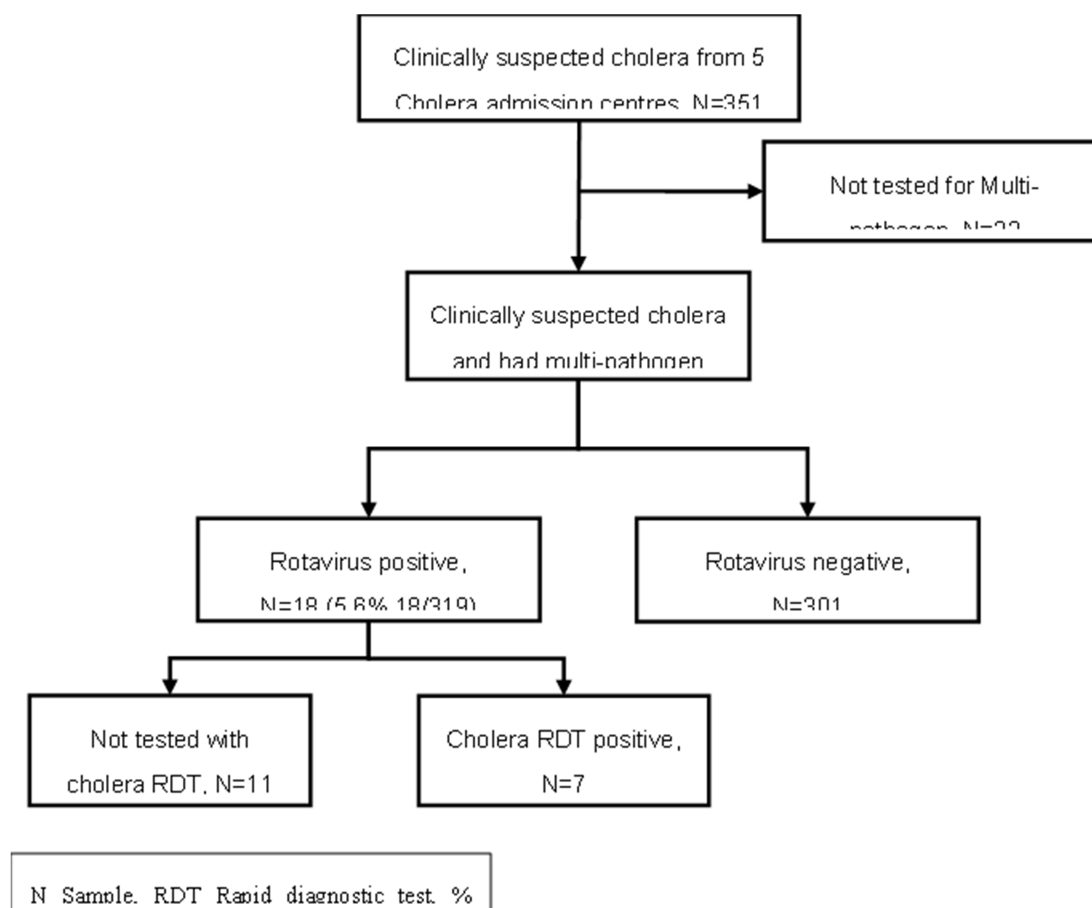

Figure 1. *Study flow chart.*

Descriptive Statistics of Study Participants

Table 1 summarises the background characteristics of study participants. The median age was 24, with 50% of the group being between 12 and 38. Participant samples came from 5 facilities, with the majority being from Matero (37%), while less than 10% came from Levy and Chipata district (9 and 8%, respectively). Less than half (44%) of the participants were male, while 31% were female, and a substantial 25% had missing data for sex. Most participants were HIV negative (89%) and had missing data on cholera vaccination status (88%).

Table 1. Background characteristics of study participants.

Characteristic	**Total N=319**
	n (% of total)
Sex	
Male	*141 (44.2)*
Female	*100 (31.3)*
Missing	*78 (24.5)*
Age (year)	
Median (IQR)*	**24 (12-38)**
Age group (years)	
Infants & Young Children (< 5)	*31 (9.7)*
Children/Adolescents (6-17)	*44 (13.8)*

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Young Adults/Adults (18-44)	*138 (43.3)*
Older Adults (45+)	*38 (11.9)*
Missing	*68 (21.3)*
Facility	
Chipata	*26 (8.2)*
George	*74 (23.2)*
Heroes	*71 (22.3)*
Levy	*29 (9.1)*
Matero	*119 (37.3)*
Vaccinated against cholera	
No	*32 (10.0)*
Yes	*5 (1.6)*
Missing	*282 (88.4)*
HIV Status	
Negative	*283 (88.7)*
Positive	*30 (9.4)*
Missing	*6 (1.9)*

N Sample, % Percentage, IQR Interquartile range

Prevalence of Rotavirus Infection Among Clinically Suspected Cholera Cases

Among 319 patients admitted to five cholera treatment centres with clinically suspected cholera during Zambia's 2023–2024 outbreak, rotavirus was detected in 18 cases, corresponding to a prevalence of 5.6% (18/319; 95% confidence interval: 3.4% to 8.8%).

Rotavirus Co-Infections with Other Enteric Pathogens

Analysis of pathogen co-detection patterns among 18 rotavirus positive samples, revealed that rotavirus was almost exclusively found in mixed infections. Only one case (6.1%, 1/18) involved a single rotavirus infection, while the majority of detections (94.5%, 17/18) involved at least one additional enteric pathogen. Dual-pathogen infections were the most common profile (38.9%, 7/18), followed by triple-pathogen combinations (22.2%, 4/18). We also identified more complex infections involving four (11.1%, 2/18) and five (5.6%, 1/18) pathogens. *Campylobacter* was the most prevalent bacterial co-pathogen, appearing alongside rotavirus in all multi-pathogen categories and frequently co-occurring with viral pathogens such as norovirus GI/GII and adenovirus. EIEC/*Shigella* and *Salmonella* were sporadically co-detected **(Figure 2).**

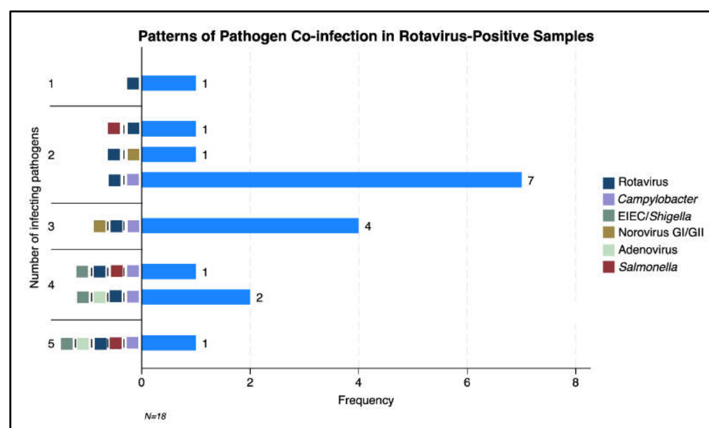

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Figure 2. The bar chart illustrates the frequency of bacterial and viral co-infections among rotavirus-positive samples (N =18) by the number of pathogens detected. The majority of rotavirus detections (93.7%, 15/16) occurred in mixed infections with other enteric pathogens.

Rotavirus Genotypes

Rotavirus Genotypes and Age-Distribution

A total of five rotavirus samples met the genotyping threshold, and all showed evidence of co-infecting enteric pathogens, highlighting the complexity of diarrhoeal aetiologies during cholera outbreaks. The identified strains exhibited significant diversity, encompassing globally common types (G1P[8], G2P[4]), regionally prevalent strains (G12P[6], G3P[6]), and a rare reassortant (G1P[6]). Co-detections were restricted to *Campylobacter* alone or *Campylobacter* in combination with Norovirus GI/GII, with no other enteric pathogens observed among the five genotyped samples. Of the genotyped cases, one was a child under 5 years co-infected with *Campylobacter* and Norovirus GI/GII, while three separate cases showed co-infection with *Campylobacter* alone. These cases were distributed across the <5, 6–17, while the rest were missing age categories, respectively (**Figure 3).**

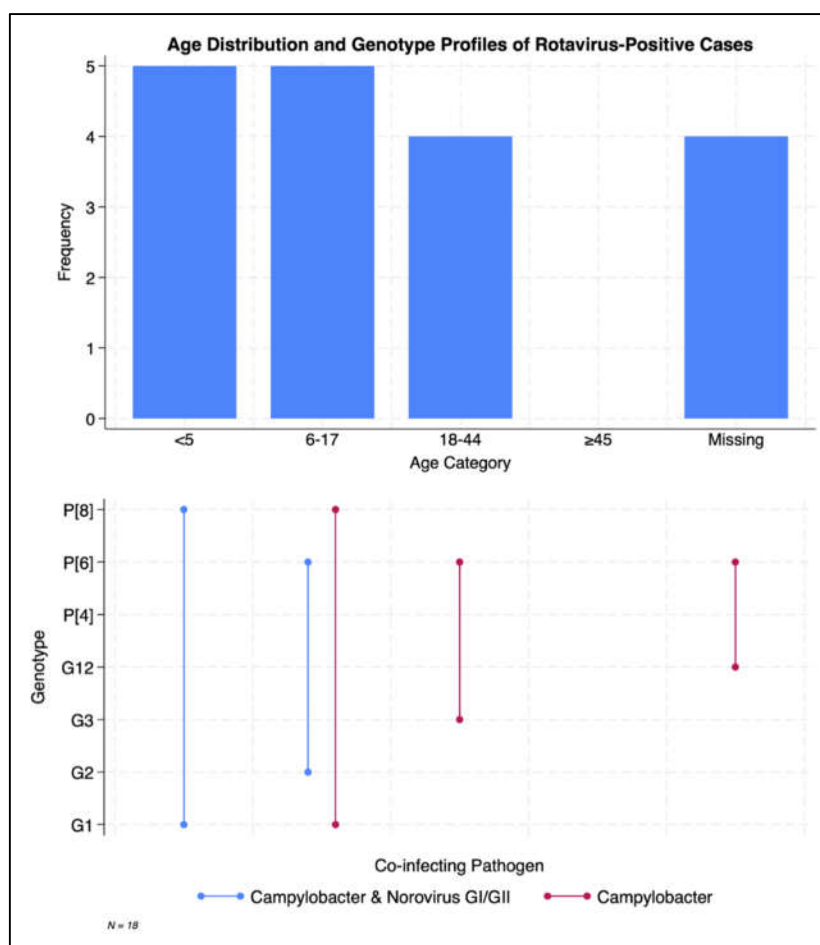

Figure 3. Distribution of cholera-negative, Rotavirus -positive samples across age categories; Rotavirus co-detected pathogens.

Discussion

We found that rotavirus accounted for a small portion of the diarrhoeal cases during the 2023–2024 cholera outbreak in Zambia. Approximately 6% of patients presenting with cholera-like symptoms were positive for rotavirus, indicating that rotavirus co-circulated amid the cholera outbreak. Notably, these rotavirus cases showed a high frequency of co-infections with other

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pathogens and considerable strain diversity. Co-detection analysis showed that rotavirus was almost always identified in mixed infections - only one case was a single-pathogen rotavirus infection, while most involved two or more additional enteric pathogens, with Campylobacter emerging as the dominant bacterial co-pathogen and recurring across the multi-pathogen profiles. Among the subset of samples with sufficient viral load for genotyping (n=5), the circulating strains were heterogeneous, spanning globally common genotypes (G1P[8], G2P[4]), regionally prevalent P[6]-associated strains (G12P[6], G3P[6]), and a rare reassortant (G1P[6]), with co-detections in these genotyped cases limited to Campylobacter alone or Campylobacter plus norovirus GI/GII. Collectively, these findings indicate that during cholera outbreaks, rotavirus contributes a modest but non-trivial burden among suspected cholera admissions, frequently within complex polymicrobial infections and alongside diverse genotypes, reinforcing the value of integrated multi-pathogen diagnostics and genomic surveillance to better characterise diarrhoeal aetiology and guide outbreak response.

An important insight from our investigation is the clinical overlap between cholera and rotavirus illness and its consequences for case management. The profuse watery diarrhoea, vomiting, and rapid dehydration characteristics are clinically indistinguishable from severe rotavirus gastroenteritis (8,9). In our cohort, this phenotypic overlap likely led clinicians to initially suspect cholera in rotavirus-infected patients, illustrating how syndromic definitions can mask the true aetiologies during outbreaks. The practical implication is that, without multiplex diagnostics, viral diarrhoeas may be misclassified as cholera, resulting in inappropriate management. Patients with unrecognised rotavirus infection might be admitted to cholera treatment centres and administered unnecessary antibiotics, which provide no benefit against viruses and instead contribute to antimicrobial resistance (12). At the same time, misattribution delays appropriate supportive care for those patients and misses opportunities to collect data on rotavirus strains, which is crucial for evaluating vaccine performance. These considerations strongly support the adoption of multi-pathogen testing in outbreak investigations, to ensure that co-circulating infections are correctly identified and treated, and that surveillance captures all major contributors to the outbreak's morbidity.

One of the most striking findings was the high rate of co-infections among rotavirus cases, underscoring the complex aetiology of diarrhoeal outbreaks. Nearly 94% of rotavirus-positive cases harboured at least one additional enteric pathogen – most often *Campylobacter* (either alone or in combination with Norovirus GI/GII). Dual infections were common, and a sizable fraction of cases had three concurrent pathogens, suggesting that clinical disease often resulted from multiple overlapping infections rather than a single-agent. These co-infections were not random – we observed Campylobacter as a recurring partner to rotavirus across different age groups, suggesting shared transmission pathways or environmental sources that facilitated their joint circulation. Our findings mirror observations from the broader outbreak investigations in Zambia where Kuntawala *et al.* (2025) reported that about 80% of suspected cholera cases in 2023-2024 actually involved mixed infections, with *Campylobacter* and Norovirus GI/GII frequently accompanying other pathogens (12). Likewise, diarrhoeal outbreaks in other settings have shown that presumed cholera cases often harbour diverse pathogens such as *E. coli* and Cryptosporidium alongside (or instead of) V. cholerae (3–5). These aetiologic complexity create challenges for treatment. For example, a patient co-infected with rotavirus (virus) and Campylobacter (bacterium) might require rehydration and careful use of antibiotics, whereas a misdiagnosis of "cholera only" could lead to suboptimal care. The convergence of these findings underscores why an integrated diagnostic and surveillance strategy is critical in outbreak settings.

Our genotyping analysis revealed a remarkable diversity of rotavirus strains circulating during the outbreak, including both vaccine-related and unusual genotypes. We detected classic human strains targeted by vaccines (such as G1P[8], G2P[4] co-circulating with less common types like G12P[6] and G3P[6], as well as a rare reassortant strain G1P[6] not typically seen in humans. Importantly, none of the rotavirus-positive patients in our study were co-infected with Vibrio cholerae, indicating that rotavirus and cholera infections occurred in parallel rather than within the

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same individuals. The presence of multiple rotavirus genotypes in this context suggests complex transmission dynamics at play. On one hand, the identification of G1P[8] and G2P[4], strains against which the Rotarix® vaccine is designed to protect, alongside other genotypes implies that routine vaccination was not fully interrupting rotavirus circulation during the outbreak. On the other hand, the appearance of unusual strains points to introduction from outside sources or virus evolution. Notably, the emergence of genotype G2P[4] as a prominent strain post-vaccine introduction has been reported in many African countries and is thought to be driven by selective immune pressure from the monovalent vaccine (Rotarix, which targets G1P[8]) (13,14). This vaccine-driven selection hypothesis is supported by antigenic studies showing that Rotarix induces stronger immunity against homotypic G1P[8] strains than against heterotypic G2P[4] (15). In practical terms, G2P[4] viruses may enjoy a fitness advantage in vaccinated populations by partially evading vaccine-derived immunity. Consistent with this, we observed G2P[4] among the outbreak strains, reinforcing the need for vigilant genomic surveillance to detect such vaccine-escape variants. Early identification of a shift toward G2P[4]-dominance (or any other non-vaccine genotype) would be crucial for assessing whether current vaccines continue to confer protection and whether vaccine compositions might need updating in the future.

Several lines of evidence from our study point to zoonotic or reassortant origins for some strains, underscoring the broader evolutionary forces shaping rotavirus diversity. In particular, the detection of a G12P[6] strain strongly suggests an animal-human transmission event. Genotype G12, especially paired with P[6], has been increasingly reported across Africa and is often associated with gene segments of porcine origin. Whole-genome analyses from Ethiopia and South Africa (16) have shown that African G12P[6] rotaviruses commonly carry internal genes (NSP2, NSP3, NSP4) derived from pig strains, providing direct evidence of porcine-human reassortment. Our finding of G12P[6] in Zambia fits this pattern, indicating a likely interspecies transmission. Such reassortment events are epidemiologically important - they can generate novel rotavirus variants with unpredictable antigenicity or virulence, potentially undermining existing immunity in the human population. This highlights the need for a One Health approach in surveillance, as the health of human populations may be directly affected by rotavirus strains circulating in livestock and other animals.

We also identified a rare G1P[6] reassortant in our sample set, which is a highly unusual genotype combination for Africa. Typically, P[6] is linked to G12 or animal strains in Africa, making G1P[6] a rare combination. To our knowledge, G1P[6] has scarcely been reported, and it was not observed in large genetic surveys from Nigeria and Nepal that did document other atypical post-vaccine genotype pairings (e.g., G2P[6], G8P[1]) (17,18). The appearance of G1P[6] during the Zambian outbreak could signify an uncommon reassortment event facilitated by the high mixing of strains in this setting. It might represent a convergence of human (G1) and animal (P[6]) rotavirus gene segments occurring in a co-infected host, possibly driven by immune pressure or ecological opportunity. Interestingly, similar novel strain mixing phenomena have been observed in other southern African locales following vaccine rollouts. For instance, Mozambique reported the emergence of unexpected genotype combinations after its rotavirus vaccine introduction (19). The identification of G1P[6] in our study is therefore an epidemiological alert. While we cannot determine definitively whether this strain arose from a local reassortment or was imported from a neighbouring region, its presence serves as a reminder that rotaviruses continue to evolve in the vaccine era. It reinforces the imperative for ongoing surveillance to promptly detect new reassortant or mutant strains. Every novel strain discovered provides insight into the virus's evolutionary trajectory and offers a chance to update vaccines or other control measures proactively.

Our findings have several important implications for public health practice and research. First, even in cholera outbreaks, other pathogens, particularly rotavirus, can contribute meaningfully to the diarrhoeal caseload. Outbreak management should therefore move beyond single-pathogen assumptions and incorporate broad-spectrum diagnostics (e.g., multiplex PCR) to improve aetiologic attribution, guide appropriate rehydration and infection control, and reduce unnecessary antibiotic use. Second, the observed rotavirus genotype diversity reinforces the need for routine genomic

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surveillance alongside clinical surveillance. In Zambia, where the national programme transitioned from Rotarix® to Rotavac® during the study period, continued strain monitoring is essential to track genotype replacement, detect potential vaccine-escape variants, and generate evidence on real-world, strain-specific vaccine effectiveness. Finally, the detection of zoonotic-associated and rare reassortant genotypes suggests ongoing viral evolution at the human-animal-environment interface, supporting a One Health approach that links clinical surveillance with animal and environmental monitoring (including wastewater or livestock sampling) to provide early warning of emerging strains. Overall, effective control will require integrated diagnostics, strengthened surveillance systems, and coordinated cross-sector action to manage the full spectrum of co-circulating enteric pathogens and safeguard vaccine impact.

A key strength of this study is that it leveraged a well-defined outbreak cohort of patients admitted with clinically suspected cholera across multiple treatment centres and applied a broad multiplex PCR platform to identify rotavirus and a wide range of co-circulating enteropathogens, thereby providing an aetiologically richer picture than routine single-pathogen surveillance and enabling description of non-random co-detection patterns. In addition, molecular characterisation of circulating strains, albeit in a subset, adds value by demonstrating genotype diversity, including uncommon and potentially zoonotic-associated types, relevant to vaccine-era monitoring and outbreak preparedness.

Important limitations should also be noted. The analysis reflects a convenience sample of treatment-centre admissions defined by syndromic criteria and therefore may not be generalisable to community diarrhoea or to milder presentations, and the small number of rotavirus-positive cases limited precision and reduced power to detect associations by demographic or clinical factors. Genotyping was possible for only a small fraction of positive samples, which constrains inference about population-level strain distribution. Finally, key clinical and exposure variables (including rotavirus vaccination history, symptom severity, antibiotic use, WASH exposures, and complete HIV-related information) were incompletely captured during the emergency response, increasing the potential for residual confounding and limiting interpretation of pathways linking pathogen detections to clinical outcomes.

Conclusion

Rotavirus contributed a modest but clinically meaningful proportion of admissions labelled as suspected cholera during Zambia's 2023–2024 outbreak, and was detected predominantly in children and adolescents and most often within mixed enteric infections. The frequent co-detection of pathogens such as Campylobacter and norovirus underscores the aetiologic complexity that can underlie cholera-like presentations and highlights the limitations of syndromic diagnosis during outbreaks. The diversity of rotavirus genotypes identified, including uncommon and potentially zoonotic-associated or reassortant strains, reinforces the importance of coupling routine case surveillance with molecular characterisation to track strain evolution and to inform vaccine-era monitoring, particularly as Zambia transitions between vaccine products. Together, these findings support a more integrated outbreak response that incorporates multi-pathogen diagnostics, judicious antimicrobial stewardship, and strengthened genomic surveillance within a One Health framework to improve clinical management and guide targeted public health action for diarrhoeal disease control.

Author Contributions: A.C: Investigation, Methodology, Visualisation, Writing – original draft; S.B.: Conceptualisation, Methodology, Supervision, Formal analysis, Visualisation, Writing—review and editing; B.P.: Formal analysis, Visualisation, review & editing; H.N.: Investigation, Writing – review & editing; F.L.: Investigation, Writing – review & editing; M.M.: Investigation, review & editing; C.C.L.: Writing – review & editing; D.N: Writing – review & editing; I.M.: Investigation, Writing – review & editing; B.T.N.: Investigation, Writing – review & editing; S.F.T.: Investigation, Writing – review & editing; K.C.: Methodology, Writing – review & editing; S.S.: Methodology, Writing – review & editing; M.S.: Supervision, Writing – review & editing;

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Data Availability Statement: All data generated and analyzed during this study are included in the published manuscript and supplementary information files. The data presented in this study are available upon reasonable request from the corresponding author. The CIDRZ Ethics and Compliance Committee is responsible for approving such request. To request data access, one must write to the Secretary to the Committee/Head of Research Operations, Ms. Hope Chinganya (Hope.Chinganya@cidrz.org). Dataset requests must include contact information, a research project title, a description of the proposed analysis, and the format in which it is expected. The requested data should only be used for the purposes related to the original research or study. The CIDRZ Ethics and Compliance Committee will normally review all data requests within 48–72 hours (Monday - Friday) and provide notification if access has been granted or additional project information is needed before access can be granted.

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