RESEARCH ARTICLE

Molecular detection and Phylogenetic Characterisation of Salivirus genotype A2 in Wastewater from Zambia's Copperbelt and Eastern Provinces

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Cite this article: *Shempela, D.M., Sikalima, J., Muleya, W., Daka, V., Kasanga, A., Sandala, D., Chipango, C., Mutinta, C., Mudenda, S., Mkandawire, E., Siwila, J., Mwenda, M., Sinyange, N., Simulundu, E., Sichinga, K., Saasa, N., & Chilengi, R. (2025). Molecular detection and phylogenetic characterisation of Salivirus genotype A2 in wastewater from Zambia's Copperbelt and Eastern Provinces. Health Press, 09(1): 7-20.*

Abstract

Salivirus (SalV), a member of the Picornaviridae family, is a novel virus associated with acute gastroenteritis. In Zambia, its prevalence and genetic diversity remain uncharacterized. We analyzed 87 raw wastewater samples from 12 sites in the Copperbelt and Eastern Provinces using next-generation sequencing. SalV was detected in 43.7% of samples, with 42.1% yielding full-length open reading frames.

Phylogenetic analysis revealed that all sequences belonged to genotype A2, closely related to human-derived strains. SalV sequences from both provinces belonged to genotype A2, and were closely related to a previous strains detected in humans.

To our knowledge, this is the first report of Salivirus genotype A2 detection in raw wastewater in Zambia highlighting the need for environmental surveillance to monitor enteric pathogens.

Keywords: Salivirus, wastewater surveillance, phylogenetic analysis

Introduction

Salivirus, formerly klassevirus, (family Picornaviridae), was first detected in pediatric gastroenteritis samples in 2009 (1). The Picornaviridae comprises 12 genera of human and animal non-enveloped viruses including enterovirus, Aichivirus, Parechovirus, Cosavirus, and Saffold virus. The genus Salivirus (SalV) has a single species; Salivirus A that has 2 genotypes, Salivirus A1 and Salivirus A2 (2). Salivirus has a linear genome whose open reading frame encodes for three structural proteins (VP0, VP1, and VP3) (3). Since its first identification, SalV has been detected in river water, sewage (4), humans (5)and primates (3). SalV has also been detected in stool (6) or sewage (7) and respiratory (8) specimens. SaIV has beein implicated in cases of acute gastroenteritis (8,9).

In Africa, SalV has been reported in children in Tunisia and Nigeria (10). There is evidence suggesting that some cases of enteric infection may arise from unrecognized causative agents that may include SalV. The virus has been associated with instances of acute gastroenteritis (11) (2) (12) (13). Studies have not yet demonstrated the significance of Salivirus in altering the course of clinical disease, however, the continued association of SalV with clinical disease has raised concern about its role in these conditions (14).

Diarrhea is a common cause of morbidity and mortality in Zambia. There are many factors that affect the outcomes of diarrheal cases especially in children under

5 years. An understanding of the causative complex of organisms and environmental factors can significantly improve the outcome of cases. These factors include age, immunity, as well as co-infecting organisms (15). It is therefore important that as many factors at play are identified and appropriate measures taken. One such source of identifying co-infecting organisms is wastewater.

This abundance of many potential pathogens in wastewater necessitates the need to understand their public health significance. In this study, we investigated the presence of important SaIV in raw, untreated sewage influent on the Copperbelt and Eastern provinces of Zambia.

Methods

2.1 Sample collection and Viral Concentration

A total of 87 raw wastewater samples were collected from various sites in the Copperbelt (Ndola, Kitwe, Luanshya) and Eastern (Chipata) Province of Zambia (Fig. 1). Sampling was conducted at the same time of the day in all sites according to guidelines issued by the water utility companies. A composite-grab wastewater sample was collected at 15-30-minute intervals and aliquoted into sterile pre-set sampling bottles. Samples were assigned a unique ID with information such as sampling location, volume, temperature, pH, time and date, recorded for each sample. The samples were transported at 4°C in a mobile refrigerator to the laboratory for processing as previously described [17]. The Bag-mediated filtration system (BMFS) was used to concentrate the samples following collection while Skimmed milk flocculation and Polyethylene glycol (PEG)/Sodium chloride precipitation were used following transportation to the laboratory.

2.2 Total RNA Assay measurement and quantification

Viral RNA was extracted from concentrated samples using the MagMAX viral isolation kit (Applied Biosystems, Foster City, CA) on Kingfisher Flex 96 Deep-well magnetic particle processor (Thermo-Fisher Scientific, USA) according to manufacturer's instructions. Briefly, 200 µL of concentrated sample was mixed with 265 µL of lysis/binding solution, followed by addition of 20 µL of bead-mix. The mixture was incubated, supernatant discarded and the remaining beads washed twice with wash buffer. Samples were eluted in 50 µL of elution buffer and stored at -80 °C until further processing. RNA was quantified using Qubit fluorometer while RNA integrity was measured by the TapeStation 4200 with a High Sensitivity RNA Screen Tape (Agilent Technologies, Santa Clara, CA).

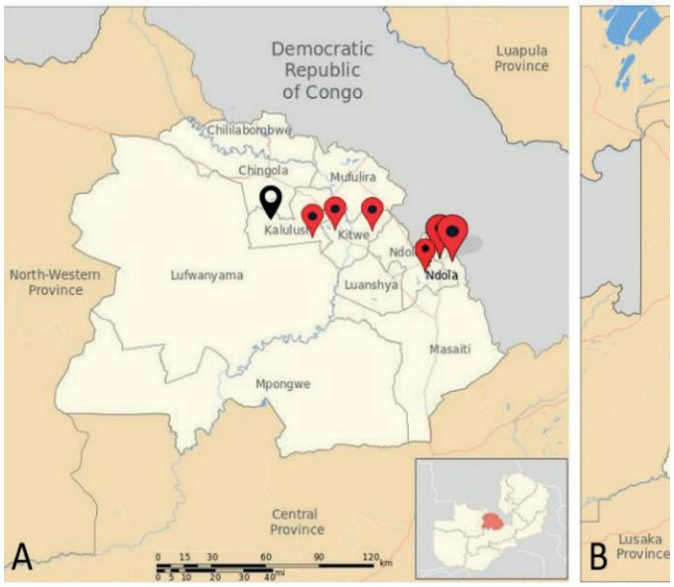

Figure 1. Map showing sampling sites for (A) Copperbelt and (B) Eastern Provinces of Zambia. The red pins with black circles represent eight sampling sites with nine and seven collections while the black pin with a light-yellow circle represent one site with one collection (16).

2.3. cDNA synthesis and Next-Generation Sequencing

cDNA synthesis and Library preparation were performed using the Respiratory Virus Oligo Panel v2 kit following the Illumina RNA Prep with Enrichment kits, which leverage bead-linked transposomes technology paired with fast enrichment (Illumina, San Diego, CA, USA). The automated Hamilton microlab 96 was used during sample processing and amplification was done on the ABI 7500 fast and the Quant studio 7. Synthesized DNA was quantified using a Qubit 4 fluorometer while DNA libraries were assessed on the TapeStation Agillant 4200. Library normalization was performed using the Qubit fluorometric quantification (ThermoFisher Scientific, Waltham, MA, USA). The starting concentration of 4nM pooled was prepared. For genomic sequencing, a 2nM pool (30µl) was used for loading in the Illumina NextSeq 2000 HT instrument through a P300 cycle cartridge loaded with a P2 flow cell. A customized version of the DRAGEN software was used for cluster generation using a pool of primer cocktail.

Sequence processing, assembly, and analysis for viral and variant detection was carried out using the customized DRAGEN Microbiology cloud-based analysis software. Generated Fast Q files were also analysed using a high computational local server (Linux) for in-house computation and verification.

2.4. Data analysis

Demographic data were entered into Microsoft Excel version 2016. The prevalence of Salivirus in wastewater was presented as proportions. The sequences were cleaned and used blastn to determine the identity. The Evolutionary analyses of Salivirus full coding, VP1 and 3D regions were conducted in MEGA7 [3].

2.5. Phylogenetic analysis

Phylogenetic analyses were performed using nucleotide sequences according to the neighbor-joining method and subjected to bootstrap analysis with 1000 replicates to determine the reliability values at each internal node. Evolutionary trees were constructed using the MEGA software.

Results

3.1 Detection of Salivirus

Of the 87 samples, SalV was detected in 38/87 (37.9%) collections. Salivirus was detected in all four districts of Ndola, Kitwe, Luanshya and Chipata. Salivirus was consistently detected at most sites (11/12) throughout the collection period. The virus was not detected at Old Kanini WWTP. The virus was also detected at two once off sites at Ndeke SP (Copperbelt Province) and Gondar Barrack SP and Tecoma WWTP (Eastern Province).

3.2. Molecular characterization of Salivirus

Out of a total of 87 collections from 12 wastewater sites, Salivirus was amplified and detected in 11/12 (91.7%) locations. Out of a total of 87 samples obtained during weekly collections, SalV genome was detected in 38/87 (43.7%). Out of the successfully sequenced samples, 16/38 (42.1%) yielded full coding regions (Table 2). The SalV sequences obtained in this study have been deposited in the GenBank database and assigned accession numbers PP943387-PP943402 (Table 2). The full genome of SalV was used for evolutionary analysis (Figure 1). The Phylogenetic analysis of the 16 SalV sequences for the full CDS (Figure 2), VP1 (Figure 3) and 3D regions (Figure 4) showed similar characteristics for the Zambia cluster. The Zambian viruses aggregated into a single cluster within the A2 genotype separate from other previously reported sequenced from within and outside Africa. There was no segregation according to province.

Discussion

We detected the presence of SalV in wastewater at several sites in the Copperbelt and Eastern provinces of Zambia. The study reveals the presence and abundance of SalV in raw wastewater during the 10-week study period. The presence of the virus suggests that the virus is discharged into the wastewater of the respective communities of the four towns under investigation.

Diarrhea is one of the leading causes of child death especially in young children under 5 years in Zambia.

Table 1: The sampling sites in the four districts of Copperbelt and Eastern province and the collections and proportion of SaIV detected.

No.	Site	Town	Province	1	2	3	4	5	6	7	8	9	10	No.	Positives
1	Chipata Motel	Chipata	Eastern	+										8	1/8
2	Gondar Barracks SP	Chipata	Eastern								+			1	1/1
3	Chipata Motel SP	Chipata	Eastern						+	+				8	2/8
4	Chipata Motel PH	Chipata	Eastern	+					+	+				8	2/8
5	Chambishi SP	Kitwe	Copperbelt	+	+	+	+	+	+					10	6/10
6	Mindolo SP	Kitwe	Copperbelt	+	+	+	+		+		+		+	10	7/10
7	Tecoma WWTP	Luanshya	Copperbelt	+										1	1/1
8	New Kanini WWTP	Ndola	Copperbelt	+	+		+		+		+	+	+	10	7/10
9	Ndeke SP	Ndola	Copperbelt										+	1	1/1
10	Nkana East WWTP	Ndola	Copperbelt	+	+		+	+			+			10	5/10
11	Lubuto WWTP	Ndola	Copperbelt	+	+	+		+			+			10	5/10
12	Old Kanini WWTP	Ndola	Copperbelt											10	0/10
														87	38/87

These infections include Rotavirus (17), Norovirus (18), Adenovirus, Salmonella, and Giardia (19). The existence of multiple agents in co-single infections makes determination of the contribution to the overall disease manifestation of each organism difficult. The finding of the current study showed for the first time that SaIV is circulating in communities serviced by these wastewater networks. Salivirus has been shown to have a worldwide distribution in Asia, Europe (20,21), USA (22), Brazil (13,23) and other African countries (10). The detection of SalV in nearly all the towns suggests that the virus is circulating in the community. Previous studies have found similar levels of the virus in wastewater (7,24,25). It would not be surprising if the worldwide distribution of SaIV could be associated with a significant portion of infections in children with diarrhea (13,26).

Table 2 The Salivirus with full coding region from Copperbelt and Eastern province.

No	Sequence ID	Date	Collection	Site	Town	Province	Concentration	Accession No.
1	SalivirusLTP1/3168/Zambia	23-Sep-23	1	Lubuto WWTP	Ndola	Copperbelt	PEG	PP943388
2	SalivirusNEB1/3188/Zambia	23-Sep-23	1	Nkana East WWTP	Kitwe	Copperbelt	BMFS	PP943397
3	SalivirusNKB1/3186/Zambia	23-Sep-23	1	New Kanini WWTP	Ndola	Copperbelt	BMFS	PP943400
4	SalivirusCMB1/3196/Zambia	10-Oct-23	1	Chambishi SP	Chambishi	Copperbelt	BMFS	PP943394
5	SalivirusMDB2/3224/Zambia	10-Oct-23	2	Mindolo SP	Kitwe	Copperbelt	BMFS	PP943391
6	SalivirusNKB2/3226/Zambia	11-Oct-23	2	New Kanini WWTP	Kitwe	Copperbelt	BMFS	PP943401
7	SalivirusNKB2/3227/Zambia	11-Oct-23	2	New Kanini WWTP	Kitwe	Copperbelt	BMFS	PP943392
8	SalivirusMDP4/3256/Zambia	27-Oct-23	4	Mindolo SP	Kitwe	Copperbelt	PEG	PP943396
9	SalivirusNEB4/3260/Zambia	28-Oct-23	4	Nkana East WWTP	Kitwe	Copperbelt	BMFS	PP943398
10	SalivirusNKB4/3265/Zambia	28-Oct-23	4	New Kanini WWTP	Kitwe	Copperbelt	BMFS	PP943402
11	SalivirusLTP5/3279/Zambia	03-Nov-23	5	Lubuto WWTP	Kitwe	Copperbelt	PEG	PP943389
12	SalivirusNEB6/3288/Zambia	10-Nov-23	6	Nkana East WWTP	Kitwe	Copperbelt	BMFS	PP943399
13	SalivirusCHP5/3275/Zambia	17-Nov-23	5	Chipata Motel SP	Kitwe	Eastern	PEG	PP943393
14	SalivirusCHP6/3277/Zambia	24-Nov-23	6	Chipata Motel SP	Kitwe	Eastern	PEG	PP943387
15	SalivirusMDP8/3526/Zambia	26-Nov-23	8	Mindolo SP	Kitwe	Copperbelt	PEG	PP943390
16	SalivirusMDP10/3544/Zambia	07-Dec-23	10	Mindolo SP	Kitwe	Copperbelt	PEG	PP943395

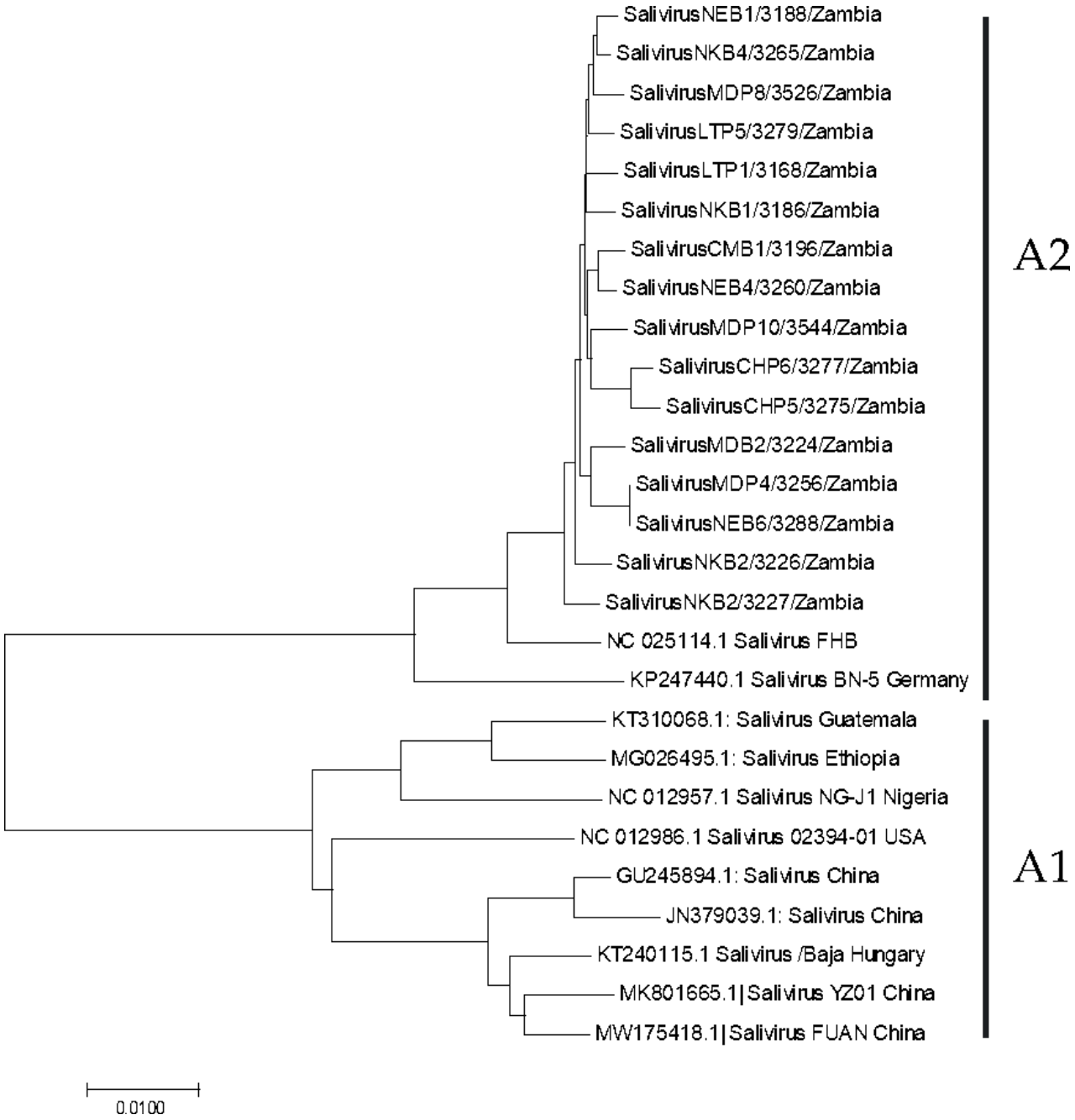

Figure 2: Phylogenetic analysis of nucleotide alignments based on the SalV full coding region (7073bp) detected in Copperbelt and Eastern province of Zambia inferred using the Neighbor-Joining method [1]. The Tree depicts the cluster of Zambia viruses among the A2 viruses. Some strains with less than the full coding regions were not included in the tree. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

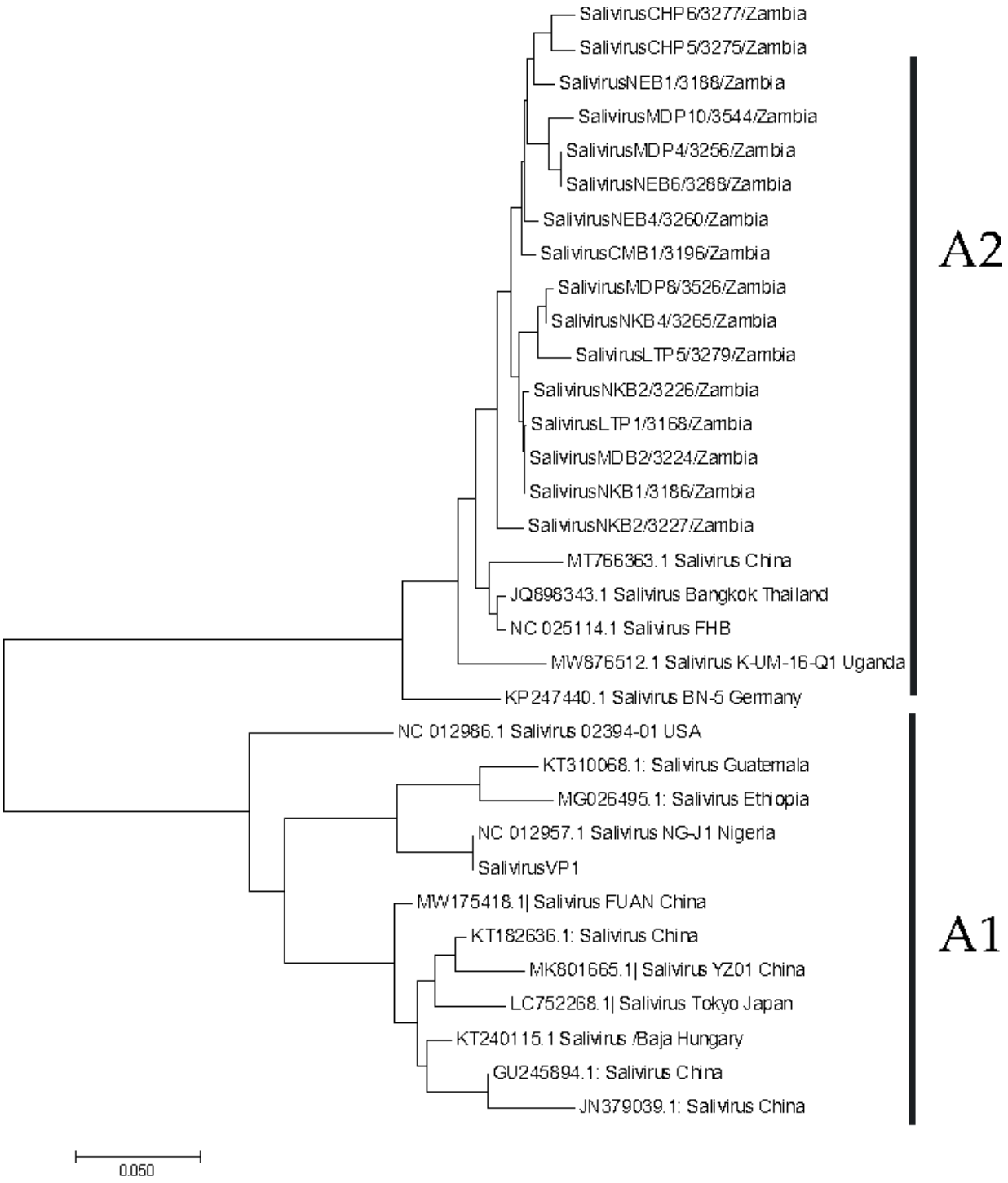

Figure 3: Phylogenetic analysis of nucleotide alignments based on the SalV VP1 region (828bp) detected in Copperbelt and Eastern province of Zambia inferred using the Neighbor-Joining method [1]. The Tree depicts the cluster of Zambia viruses among the A2 strain viruses region from Uganda, Europe and Asia.The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

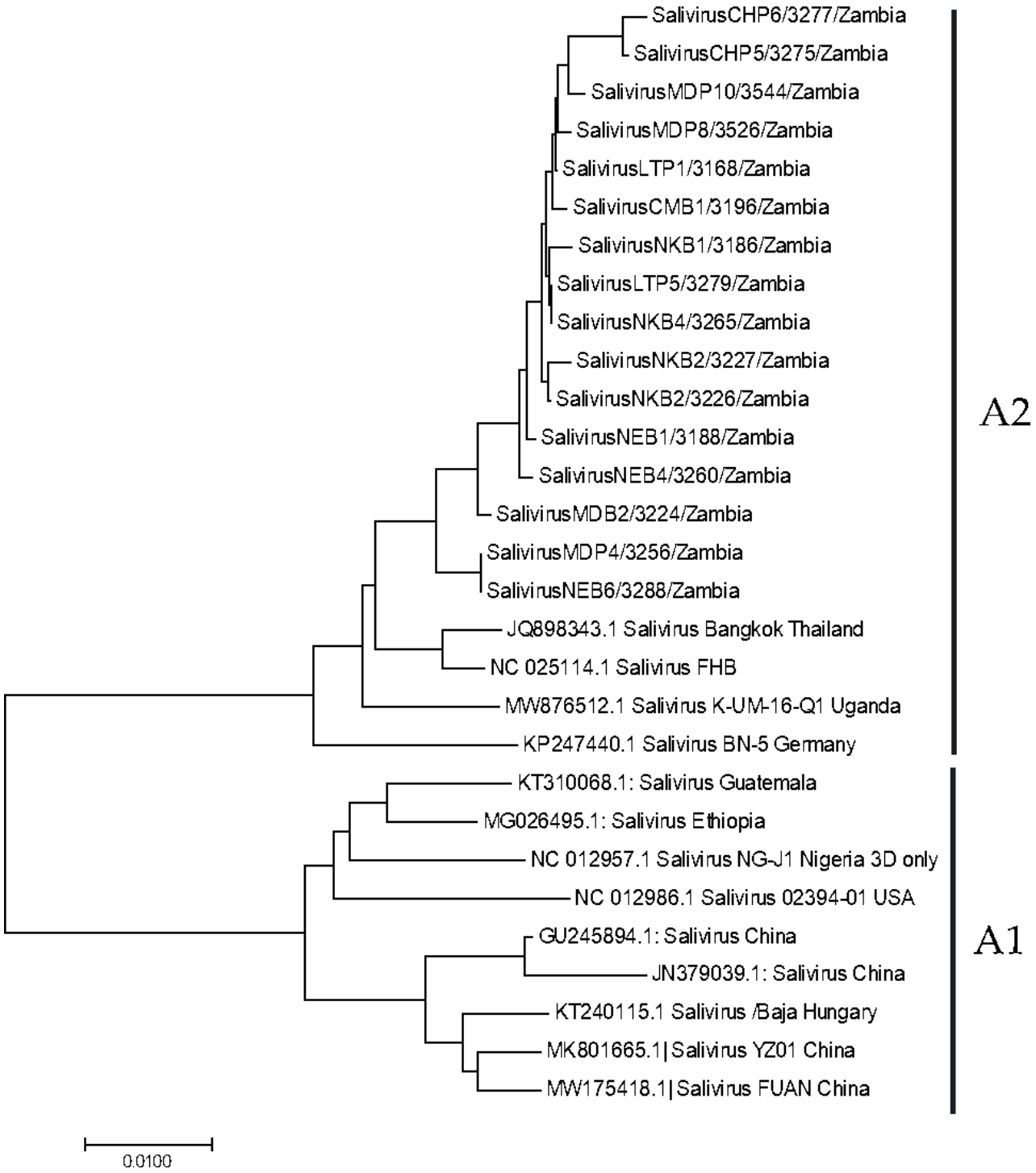

Figure 4: Phylogenetic analysis of nucleotide alignments based on the SalV 3D region (1416bp) detected in Copperbelt and Eastern province of Zambia inferred using the Neighbor-Joining method [1]. The Tree shows the cluster of Zambia viruses among the A2 strain viruses from Uganda, Europe and Asia. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Previous reports of SalV from Africa (Tunisia) and other parts of the world identified genotype A1(10) . This study identified genotype A2 as the predominant genotype. Based on the entire coding region, the virus forms a distinct cluster within the genotype A2 group viruses. It remains to be established whether the presence of A2 signifies a higher risk of infection or disease in humans. So far there have been no reports comparing the difference in infectivity or pathogenicity of the genotypes A1 and A2. Therefore, the significance of SalV detected in cases of infections in the community requires further investigation.

There is sufficient evidence to support the importance of salivirus in human infections. Previous studies have demonstrated an association of SalV with other enteric viruses such as Norovirus and Rotavirus (27). In general, whether SalV aggravates diarrhea in affected individuals remains to be determined through investigation of the virus in cases of diarrhea. The abundance of the virus in wastewater suggests an intimate closeness of the virus with humans inhabiting the communities in the sewer shed. The virus is released through feces of infected individuals in the community resulting in the presence of the virus in wastewater.

Until now, the involvement of SaIV in these infections has not been determined. A handful of studies that have attempted to establish the role of SaIV in disease causation have been conducted (28,29). Our study, like many other previous reports have focused on the detection of SaIV in new areas where the virus has not been reported before and therefore little is known regarding the involvement of the virus in disease (10,30,31).

The study had limitations that included a short 10-week period of investigation. A longer period of study would provide more information regarding the seasonality of the virus in the environment (32). The prevalence of the virus in the communities serviced by these sewer watersheds remains to be established. The work is based on wastewater samples in absence of information regarding the presence or extent of the virus distribution in the population of interest. This can be achieved by investigating the presence of the virus in clinical specimens submitted for other diarrhea-related cases of diagnosis. For instance, a larger sample size comprising cases of diarrhea would help establish the true extent of the involvement of SalV.

However, the detection of SalV is a confirmation of its existence in the wastewater and therefore possible exposure to the population in the two provinces of Zambia. This finding highlights the importance of conducting further studies on SalV to better understand the molecular epidemiology, geographical distribution, immunity and etiological role in human enteric diseases and outbreaks with unknown etiology.

Conclusions

This is the first report of SalV genome detection in raw wastewater in Zambia. The data reveals widespread circulation of SalV in most of the sites on the Copperbelt and Eastern provinces. The presence of SaIV in wastewater strongly suggests the circulation of the virus in the community. It is possible that SalV plays an important role in the illness associated with diarrheal diseases in especially in children. Therefore, clinical and routine testing for SalV would reveal the extent and significance of SalV as a contributor to illnesses in the community.

Reference

1. Greninger AL, Runckel C, Chiu CY, Haggerty T, Parsonnet J, Ganem D, et al. The complete genome of klassevirus a novel picornavirus in pediatric stool. Virol J. 2009;6.

2. Aldabbagh S, Eckerle I, Müller A, Delwart EL, Eis-Hübinger AM. Salivirus type 1 and type 2 in patients with acute gastroen- teritis,Germany. Journal of Clinical Virology.2015;72.

3. Reuter G, Pankovics P, Boros Á. Saliviruses-the first knowl- edge about a newly discovered human picornavirus. Vol. 27, Reviews in Medical Virology. 2017.

4. Mancini P, Bonanno Ferraro G, Suffredini E, Veneri C,Iaconelli M, Vicenza T, et al. Molecular Detection of Human Salivirus in Italy Through Monitoring of Urban Sewages. Food Environ Virol. 2020;12(1).

5. Daprà V, Galliano I, Montanari P, Zaniol E, Calvi C, Alliaudi C, et al. Bufavirus, Cosavirus, and Salivirus in Diarrheal Italian Infants. Inter virology. 2021;64(3).

6. Ng TFF, Magaña L, Montmayeur A, Lopez MR, Gregoricus N, Oberste MS, et al.Characterization of a salivirus (Picornavir- idae) from a diarrheal child in Guatemala. Genome Announc. 2016;4(1).

7. Adineh M, Ghaderi M, Mousavi-Nasab SD.Occurrence of Salivirus in Sewage and River Water Samples in Karaj, Iran. Food Environ Virol. 2019;

8. Pei N, Zhang J, Ma J, Li L, Li M, Li J, et al.First report of human salivirus/klassevirus in respiratory specimens of a child with fatal adenovirus infection. Virus Genes.2016;52(5).

9. Bergallo M, Daprà V, Rassu M, Bonamin S, Cuccu R, Calvi C, et al. Prevalence and Clinical Profile of Human Salivirus in Children with Acute Gastroenteritis in Northern Italy, 2014-2015. Intervirology [Internet]. 2018 Aug 15 [cited 2024 Dec 3];61(1):49–52. Available from: https://dx.doi.org/10.1159/000490568

10. Ayouni S, Estienney M, Hammami S, Guediche MN, Pothier P, Aouni M, et al.Cosavirus, Salivirus and Bufavirus in diarrheal Tunisian infants. PLoS One. 2016;11(9).

11. Nielsen ACY, Gyhrs ML, Nielsen LP, Pedersen C, Böttiger B. Gastroenteritis and the novel picornaviruses aichi virus, cosavirus, saffold virus, and salivirus in young children. Journal of Clinical Virology. 2013;57(3).

12. Gao QY, Chen YX, Fang JY. 2019 Novel coronavirus infection and gastrointestinal tract. Vol. 21, Journal of Digestive Diseases. 2020.

13. Santos N, Mendes GS, Silva RC, Pena GA, Rojas M, Amor- im AR, et al. Salivirus and aichivirus A infections in children with gastroenteritis in Brazil. Clinical Microbiology and Infec- tion. 2015;21(8).

14. Yu JM, Ao YY, Liu N, Li LL, Duan ZJ. Salivirus in children and its association with childhood acute gastroenteritis: A paired case-control study. PLoS One. 2015;10(7).

15. Hamooya BM, Masenga SK, Halwiindi H. Predictors of diarrhea episodes and treatment-seeking behavior in under-five children: a longitudinal study from rural communities in Zam- bia. Pan Afr Med J[Internet]. 2020 May 1 [cited 2024 Nov 22];36:115. Available from: https://pmc.ncbi.nlm.nih.gov/arti- cles/PMC7406458/

16. Shempela DM, Muleya W, Mudenda S, DakaV, Sikalima J, Kamayani M, et al.Wastewater Surveillance of SARS-CoV-2 in Zambia: An Early Warning Tool. Int J Mol Sci. 2024 Aug 1;25(16).

17. Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, et al. Burden and epidemiology of rota- virus diarrhea in selected african countries: Preliminary results from the african rotavirus surveillance network. Journal of Infec- tious Diseases. 2010;202(SUPPL. 1).

18. Howard LM, Mwape I, Siwingwa M, Simuyandi M, Guffey MB, Stringer JSA, et al. Norovirus infections in young children in lusaka province, zambia: Clinical characteristics and molecular epidemiology.BMC Infect Dis. 2017;17(1).

19. Chisenga CC, Bosomprah S, Makabilo Laban N, Mwila- Kazimbaya K, Mwaba J, Simuyandi M, et al. vAetiology of Diarrhoea in Children Under Five in Zambia Detected Using Luminex xTAG Gastrointestinal Pathogen Panel. Pediatric Infectious Diseases: Open Access.2018;03(02).

20. Yip CCY, Lo KL, Que TL, Lee RA, Chan KH, Yuen KY, et al. Epidemiology of human parechovirus, Aichi virus and salivirus in fecal samples from hospitalized children with gastroenteritis in Hong Kong. Virol J.2014;11(1).

21. Yu JM, Ao YY, Liu N, Li LL, Duan ZJ.Salivirus in children and its association with childhood acute gastroenteritis: A paired case-control study. PLoS One. 2015;10(7).

22. Kitajima M, Iker BC, Rachmadi AT, Haramoto E, Gerba CP. Quantification and Genetic Analysis of Salivirus/Klassevirus in Wastewater in Arizona, USA. Food Environ Virol. 2014;6(3).

23. Silva HD, Wosnjuk LAC, Santos SFO, Vilanova-Costa CAST, Pereira FC, Silveira-Lacerda EP, et al. Molecular detection of adenoviruses in lakes and rivers of Goiânia,Goiás, Brazil. Food Environ Virol. 2010;2(1).

24. Corpuz MVA, Buonerba A, Vigliotta G, ZarraT, Ballesteros F, Campiglia P, et al. Viruses in wastewater: occurrence, abun- dance and detection methods. Science of The Total Environ- ment. 2020 Nov 25;745:140910.

25. Mancini P, Bonanno Ferraro G, Suffredini E, Veneri C, Iaconelli M, Vicenza T, et al. Molecular Detection of Human Salivirus in Italy Through Monitoring of Urban Sewages. Food Environ Virol [Internet]. 2020 Mar 1[cited 2024 Nov22];12(1):68–74. Available from: https://link.springer.com/arti- cle/10.1007/s12560-019-09409-w

26. Itta KC, Patil T, Kalal S, Ghargi KV, Roy S.Salivirus in children with diarrhoea, western India. Vol. 52, International Journal of Infectious Diseases. 2016

27. Bergallo M, Daprà V, Rassu M, Bonamin S, Cuccu R, Calvi C, et al. Prevalence and Clinical Profile of Human Salivirus in Children with Acute Gastroenteritis in Northern Italy, 2014- 2015. Intervirology. 2018;61(1).

28. Lasure. N, Gopalkrishna. V. Clinico-epidemiology and genetic diversity of Salivirus in acute gastroenteritis cases from Pune, Western India: 2007–2011. Infection, Genetics and Evo- lution. 2016;44.

29. Lasure. N, Gopalkrishna. V. Clinico-epidemiology and genetic diversity of Salivirus in acute gastroenteritis cases from Pune, Western India: 2007–2011. Infection, Genetics and Evolution. 2016;44.

30. Yu JM, Ao YY, Liu N, Li LL, Duan ZJ. Salivirus in children and its association with childhood acute gastroenteritis: A paired case-control study. PLoS One. 2015;10(7).

31. Mejías-Molina C, Pico-Tomàs A, Martínez-Puchol S, Itarte M, Torrell H, Canela N, et al. Wastewater-based epidemiology applied at the building-level reveals distinct virome profiles based on the age of the contributing individuals. Hum Genomics. 2024;18(1).

32. Coutinho CRM, Cardoso JF, Siqueira JAM,Machado RS, Chagas Júnior WD das, Tavares FN, et al. Diversity of picornaviruses detected in diarrheal samples from children in Belém, Brazilian Amazon (1982-2019). J Med Virol. 2023;95(6).

33. Badru S, Khamrin P, Kumthip K, Yodmeeklin A, Surajinda S, Supadej K, et al. Molecular detection and genetic characterization of Salivirus in environmental water in Thailand.Infection, Genetics and Evolution. 2018;65.

34. Bochner AF, Makumbi I, Aderinola O, Abayneh A, Jetoh R, Yemanaberhan RL, et al.Implementation of the 7-1-7 target for detection, notification, and response to public health threats in five countries: a retrospective, observational study. Lancet Glob Health. 2023 Jun 1;11(6):e871-9.

35. Brown GW, Rhodes N, Tacheva B, Loewenson R, Shahid M, Poitier F. Challenges in international health financing and implications for the new pandemic fund.Global Health [Internet]. 2023 Dec 1 [cited 2025 May 10];19(1):97. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10696881/

36. Carter C, Anh NTL, Notter J. COVID-19disease: perspectives in low- and middle-in come countries. Clinics in Integrated Care[Internet]. 2020 Jul [cited 2025 May10];1:100005. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7261656/

37. Siedner MJ, Gostin LO, Cranmer HH,Kraemer JD.Strengthening the Detection of and Early Response to Public Health Emergencies: Lessons from the West African Ebola Epidemic. PLoS Med [Internet]. 2015 Mar 1 [cited 2025 May 10];12(3):e1001804. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4371887/

38. Weishaar H, Pozo-Martin F, Geurts B, Lopez de Abechuco E, Montt-Maray E, Cristea F, et al. Capacity-building during public health emergencies: perceived usefulness and cost savings of an online training on SARS-CoV-2 real-time polymerase chain reaction (qPCR) diagnostics in low- and middle-income -income settings during the COVID-19 pandemic. Front Public Health [Internet]. 2024 [cited 2025 May 10];12:1197729. Available from: https://pmc.ncbi.nlm.nih.gov/articlesPMC11192048/

39. Policy B on HS, Medicine I of, National Academies of Sciences E and M. StrengtheningOutbreak Management and Emergency Response Systems. Global Health Risk Frame work [Internet]. 2016 May 6 [cited 2025 May10]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK367950/

40. McGowan CR, Takahashi E, Romig L, Bertram K, Kadir A, Cummings R, et al.Community-based surveillance of infectious diseases: a systematic review of drivers of success. BMJ Glob Health [Internet]. 2022 Aug 19 [cited 2025 May 10];7(8):e009934. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9396156/

41. Alhassan JAK, Wills O. Public health surveillance through community health workers: a scoping review of evidence fromlow-income and middle-income countries.BMJ Open [Inter- net]. 2024 Apr 5 [cited 2025 May 10];14(4):e079776. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11002386/

42. Keating P, Murray J, Schenkel K, Merson L, Seale A. Elec- tronic data collection,management and analysis tools used for outbreak response in low- and middle-income countries: a sys- tematic review and stakeholder survey. BMC Public Health [In- ternet]. 2021 Dec 1 [cited 2025 May 10];21(1):1741. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8464108/

43. Dash S, Parray AA, De Freitas L, Mithu MIH, Rahman MM, Ramasamy A, et al.Combating the COVID-19 infodemic: a three-level approach for low and middle-income countries. BMJ Glob Health [Internet]. 2021 Jan 29 [cited 2025 May 10];6(1):e004671. Available from: https://pmc.ncbi.nlm.nih. gov/articles/PMC7849320/

44. Biswas RK, Huq S, Afiaz A, Khan HTA. A systematic assess- ment on COVID-19 preparedness and transition strategy in Bangladesh. J Eval Clin Pract [Internet].2020 Dec 1 [cited 2025 May 10];26(6):1599. Available from: https://pmc.ncbi.nlm.nih. gov/articles/PMC7461018/

45. Yasobant S, Lekha KS, Thacker H, Solanki B, Bruchhaus- en W, Saxena D. Intersectoral collaboration and health system resilience during COVID-19: learnings from Ahmedabad, India. Health Policy Plan [Internet]. 2024 Nov 1 [cited 2025 May 10];39(2):i29–38. Available from: https://pubmed.ncbi.nlm.nih. gov/39552345/