

Review Article

Cholera in Zambia: Examining the Multisectoral Elimination Plan, 2025

Abstract

Cholera, a highly contagious disease caused by *Vibrio cholerae*, poses a severe public health threat, especially in low- and middle-income countries with limited access to clean water and adequate sanitation. Symptoms such as acute watery diarrhoea, vomiting, and muscle cramps can lead to rapid dehydration, and without timely treatment, cholera can be fatal. Globally, cholera incidence has increased significantly, with World Health Organisation data showing a rise from 223,370 cases in 2021 to 472,697 in 2022, with the number of affected countries growing from 35 to 47. In Zambia, cholera outbreaks are recurrent, particularly during the rainy season, with 30 outbreaks recorded between 1977 and 2018. Fishing camps in rural areas and densely populated peri-urban areas, particularly in the Copperbelt and Lusaka provinces, report the highest incidences, attributed to inadequate waste management and poor drainage. This study investigates the factors influencing cholera dynamics in Zambia, focusing on climate variability, population density, and water and sanitation infrastructure. Our methodology includes a mixed-methods approach with data analysis from historical cholera records, climate variables, and socioeconomic factors from 1977 to the present. This research seeks to identify correlations between environmental and socioeconomic variables and cholera incidence, with the goal of informing effective public health interventions. The findings aim to support Zambia's efforts to eliminate cholera within its borders by 2025, aligned with the Multisectoral Cholera Elimination Plan and Vision 2030. Exploring alternative immunization strategies, including plant-derived edible vaccines, could contribute to long-term public health resilience and a cholera-free Zambia.

Keywords: *Vibrio cholerae*; Public health; Environmental parameters; Epidemiology; Edible cholera vaccines; Zambia

Introduction

In the public health sector around the world, cholera is still a major concern, heavily bearing down on inhabitants of marginalized areas [1]. The United Nations (UN) Common Analysis Update 2024 classifies Zambia as a low-income country, making most impoverished communities therein vulnerable to the weighty hurdles of infectious diseases [2]. According to the Ministry of Health, Zambia, by the last quarter of 2018 the southern African nation had undergone 30 episodes of cholera outbreaks [3], which commenced in 1977, six years after the first ever cholera case reported on the African continent [4]. The lowest recorded number of cholera cases during the Twenty-year period from 2003 to 2023 was 16 in 2022 [5], while the highest was 12,149 in 2004 [6] (Figure 1) – coupled with a Case Fatality Rate (CFR) of up

to 9.3%, which is way above the World Health Organization (WHO) recommended threshold of 1% [7]. 2006 and 2010 equally experienced severe recurrences enumerating more than 5,000 incidents in each year, with the Copperbelt and Lusaka provinces showing the highest events [8]. Mainly because the two provinces are major urban areas covered with impermeable surfaces and poor drainage systems causing blockages during the rainy season and in turn, stagnant water that breeds (pathogenic) bacteria [9]. Additionally, they are overcrowded hubs – leading to the presence of slums, namely: Bulangililo, Kamitondo, Chipulukusu, Kabushi, Matero, Chawama, Mandevu and Kanyama, among others [10]. Inadequate access to safe and clean water as well as sustainable sanitation is also a contributing factor to the epidemics in these regions [11]. However, it is noted that cholera surges do slowly propagate to other parts of the country; particularly the coastal regions that harbour fishing camps and low-lying areas that are prone to floods [12]. In Zambia, cholera flare-ups habitually emerge during the fortieth week of the year and proceeds to the twenty-third week of the following year [13]. The last crucial occurrence was from October 2023, and it is still ongoing, as of March 6, 2024, the total number of cholera cases reported was 21,007, with 702 deaths (CFR 3.3%) [14].

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(Number of deaths / Number of all cases) x 100% = Case Fatality Rate (CFR)

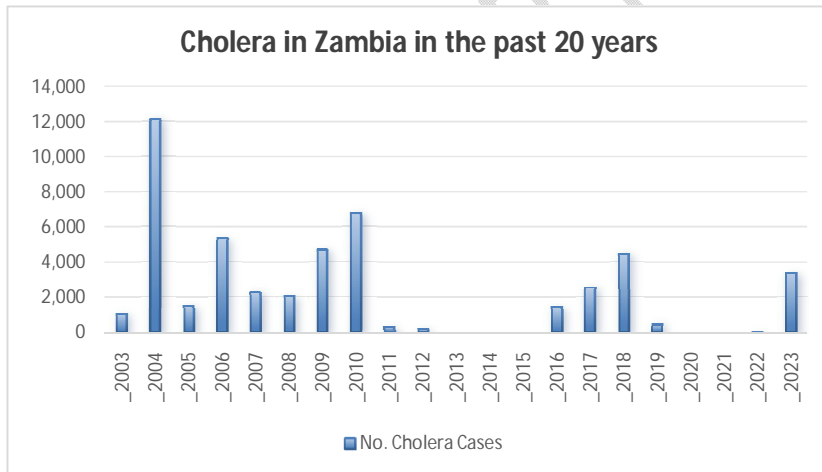


Figure 1: Yearly cholera cases in Zambia, 2003 – 2023 Ministry of Health, Zambia

Up to one hundred people occupy a square kilometre of peri-urban areas in Zambia’s capital city, Lusaka [15]. Thus, overpopulation in the country’s major economic hub is inevitable, coupled with little to no proper drainages, and unfavourable management of solid wastes[16]. During the rainy season, a large portion of the district gets heavily inundated, and streets become impassable [17]. Year after year, these environmental influences augment the likelihood of the spread of epidemic susceptible diseases such as cholera [18].

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The communicable disease outbreaks usually commence in Lusaka and circulate exponentially to other provinces due to the densely dynamic population, and this brings about further public health crises mostly on the vulnerable people of the society [19]. Additionally, according to the United Nations High Commissioner for Refugees (UNHCR) Zambia Factsheet, August 2023, approximately 90,000 refugees from other southern African nations are hosted in Zambia, although social support and community-based legal protection are provided by the government with assistance from the UNHCR alongside its partners [20]. This influx of refugees has created cramped settlements, where the camps are insufficient to house the huge numbers of people seeking shelter. Moreover, there is inadequacy of delivery of quality healthcare and unsatisfactory Water Sanitation and Hygiene (WASH) facilities [21].

Areas of high cholera incidence in Zambia

The cholera episodes are often concentrated in specific and small zones in Zambia [22]. These epicentres have a pivotal role in the transmission of the infectious disease to other districts [23]. Thus, the initiatives to control cholera in Zambia should prioritize these regions, to minimize the propagation of cholera to other areas of the country. About 4 million people are in the vulnerable cholera communities [24] (Table 1).

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Table 1 – High-Risk Cholera Areas Pinpointed in Zambia

Province	District	Environmental influences	2019 Reference population
Central	Kabwe	Slum areas	258,864
	KapiriMposhi	Intersection hub	297,484
	Ngabwe	Lukanga swamps	27,169
	Shibuyunji	Kafue flats	74,860
Copperbelt	Kitwe	Densely populated	293,612
	Ndola	Poor WASH	264,729
Luapula	Chiengi	Refugee camp	143,706
	Nchelenge	Prone to flooding	192,243
Lusaka	Lusaka	Urban areas	1,718,527
Northern	Mpulungu	International port	129,350
	Nsama	Transit point	64,142
	Mazabuka	History of Cholera	241,597

Southern		outbreaks (< 3 years)	
	Monze	Overcrowding	270,939
	Sinazongwe	Fishing camps	143,474
TOTAL			4,120,696

A mapping operation was carried out to identify endemic cholera areas in Zambia to control and possibly eradicate cholera effectively in the long run [25]. As is the country's agenda 2030 to eliminate cholera and have a cholera-free Zambia [26],[27], ~~this~~ which agrees with the African Union established movement – Partnership for African Vaccine Manufacturing [28][29]. From the exercise, up to 14 districts were highlighted as hotspots of cholera while 15 districts were prone to severe risk of cholera outbreaks (Figure 2). Contextual factors such as environmental influences coupled with geographical locations [9], were key elements in the selection of these hotspots [3]. Categorized as high rise of refugees, fishing areas, flood prone settlements, and overpopulated communities [30]. The presence of slums with inadequate WASH services [11], international borders, and districts that have experienced cholera outbreaks within the last five years, are equally such notable features of the selection process of the cholera hotspots in Zambia [31]. To further guide decision making such as administration of Oral Cholera Vaccines [32], the government through the Ministry of Health (MoH) pledges to undertake yearly surveillance [33] to analyse data collected across the country and the mitigation measures to be implemented in the cardinal situations [34].

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Fishing areas, flood prone settlements, and overpopulated communities were categorized as high rise of refugees, [30] OR

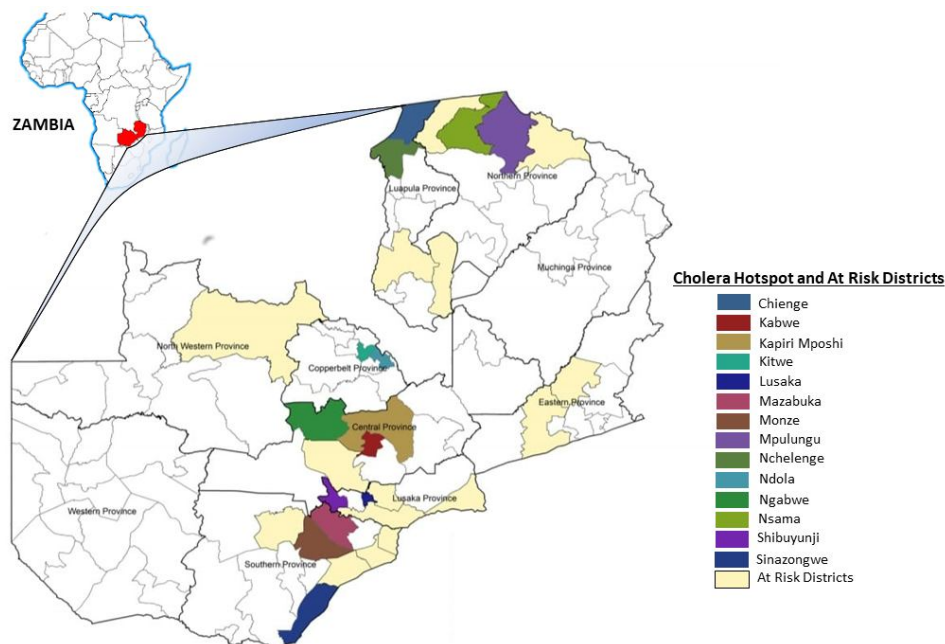


Figure 2: Zambia’s cholera hotspots and at-risk districts

The Natural World of *Vibrio cholera*

Vibrio cholera is a Gram-negative bacterium that naturally inhabits in waterlogged spaces; it is the agent that causes cholera [35], bringing about acute diarrhoea and vomiting to the infected persons. This cholera-causing population has distinct characteristics: it is primarily of serotype O1 [13], though occasionally of serotype O139. It carries a range of pathogenicity islands and virulence genes [36], notably including the CTX ϕ prophage, which encodes the cholera toxin (CT) responsible for most of the symptoms associated with cholera diarrheal syndrome [37].

From time immemorial, during a cholera outbreak, the *V. cholerae* strains have been detected via clinical laboratory tests using epifluorescent microscopy to ascertain its presence in a suspected environment [38]. Nonetheless, prior to the arrival of molecular markers culture essays were utilized to scientifically determine that a given area is colonized with *V. cholerae*[39]. These techniques had a major shortfall to appropriately detect the bacterium as it can metamorphose into a viable but non-culturable (VBNC) phase making the investigations inconclusive. Inter-outbreaks, the surroundings tend to become hostile for procreation and maturation of bacteria, hence the bacterial cells in the VBNC stage activate metabolic dormancy in their cycle making them undetectable [40]. On the other hand, when the environment becomes hospitable for the bacterium again, the cells retain their virulency and become cultivable[41]. Additionally, *V. cholerae* has the potential to go from biofilm to

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Between outbreaks, the environment becomes unfavourable for bacterial growth and reproduction,

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motile way of life and vice versa, by attaching itself to zooplankton as well as phytoplankton and in turn increasing its rate of survival in its natural setting – coastal waters [42]. *V. cholerae* is mainly hosted in copepods, hence drinking plankton contaminated water without treating it, via boiling or chlorination greatly enhances the likelihood of cholera infection [43]. Seasonal outbreaks take place every year in areas where cholera is endemic albeit, alterations in the aquatic ecosystem has the potential to affect the gravity of a cholera outbreak. Warmer months of the year tend to record higher cholera cases, especially in Sub-Saharan Africa [44] and Latin America, whereas the Bengal Delta region reports bi-modal peaks [45]. Notwithstanding, climatic changes such as temperature increase in water bodies lengthens the active period of *V. cholerae* causing serious public health concerns. Given that the bacterium plays a pivotal role in the cycling of nutrients, and it is indigenous to the aquatic milieu, complete eradication of the disease is not totally guaranteed [46]. Hence, it is imperative to fully grasp the ecology of *V. cholerae*, taking keen interest in the environmental influences that vehicle cholera, more so as a recurring virulent disease [47]. Mathematical models to predict the emergence and construct possible control measures can be designed to further strengthen the surveillance services and contain the spread of cholera. Not only that, but cholera hotspots also need advanced real-time risk monitoring platforms to proactively detect cholera threats and safeguard public health [48,49].

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Impact of Environmental Changes on Cholera

Climate changes impacts cholera transmission and prevalence significantly [50], increase in temperature for instance causes waters to be warmer creating a conducive natural habitat for *V. cholera* to breed easily and expand its niche. Secondly, during heavy downpours of the rainy seasons, or natural disasters such as cyclones the sea-levels rise drastically and cause soil erosion in the coastal regions [51]. The flood prone areas tend to harbour bacterial contaminants and the movement of water from one region to the other contributes majorly to the spread of cholera as it disrupts water sources and interferes with the sanitation infrastructure. Stagnant water equally houses *V. cholera* and people who meet it are at risk of contracting the infection [18]. Lack of water therein can also be as a result of climatic change that leads to the spread of cholera, areas susceptible to drought or little to no rainfall usually have inadequate resources for good hygiene and/or proper sanitation facilities, and this increases the chances of cholera episodes [52]. Drought often results in famine as most farmers cannot produce sufficient food crops or dairy and meat products, this which brings about a malnourished community and makes affected individuals vulnerable to illnesses [53], cholera is one such infectious disease that can arise from malnutrition and food insecurity. Due to all these aforementioned factors, people get displaced and opt to migrate to new areas [54]. However, if they already contracted the bacterium, they risk transmitting it to different people groups along their sojourn and of course where they finally decide to settle in.

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Cholera is fondly referred to as a disease of poverty, due to limited access to clean water and sanitation, coupled with underdeveloped medical facilities in endemic areas [55]. The sequence of cholera crises in Zambia suggests that it is a cholera prone country [23], with low

socio-economic pointers namely: overcrowding especially in peri-urban slums [10], lack of waste management facilities, inadequate education on the awareness of cholera, fishing camps that do not have ample WASH infrastructure [12], and weak governance policies to effectively respond to cholera prevention measures [6].

The WASH condition in Zambia

Over three quarters of the Zambian population does not have adequate access to passable solid waste management. According to the Zambia Demographic Health Survey (ZDHS) of 2013 – 2014, about 69% of people living in Zambia have unsatisfactory access to clean water (Figure 3a) [25] and 39% have little to no provision for standard sanitation services (Figure 3b.) [22]. The national population reported zero percent in the comfortably managed category for both water supply and sanitation services [31]. These incidences are majorly reported from cholera hotspots. Although close to 90% of the populace settled in urban and peri-urban localities receive payable though inconsistent water supply, the service is costly for the less privileged in slum zones. Making such vulnerable societies resort to using unsafe and most often untreated water from shallow wells that they dig up for themselves [21]. While only 6% short of half the people in rural settings can acquire basic water and sewerage amenities, the majority can at best source water from rivers, lakes, and boreholes. During Zambia's 2017/2018 cholera outbreak, the ZDHS tested the drinking water sources in Lusaka and documented 31% faecal contamination, depicting that the larger populace does not treat their drinking water [8]. Studies have shown that washing hands with antibacterial soap can lower the prevalence of diarrhoeal diseases. Hence implementing such good personal hygiene habits in tandem with practices of proper treatment of water at source level and at consumer level may lessen the cholera transmission [56].

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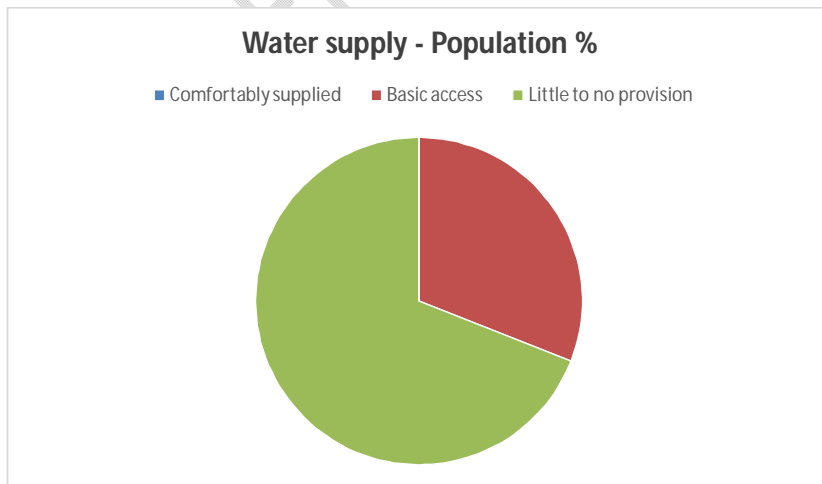


Figure 3a: Percentage of population with access to drinking water

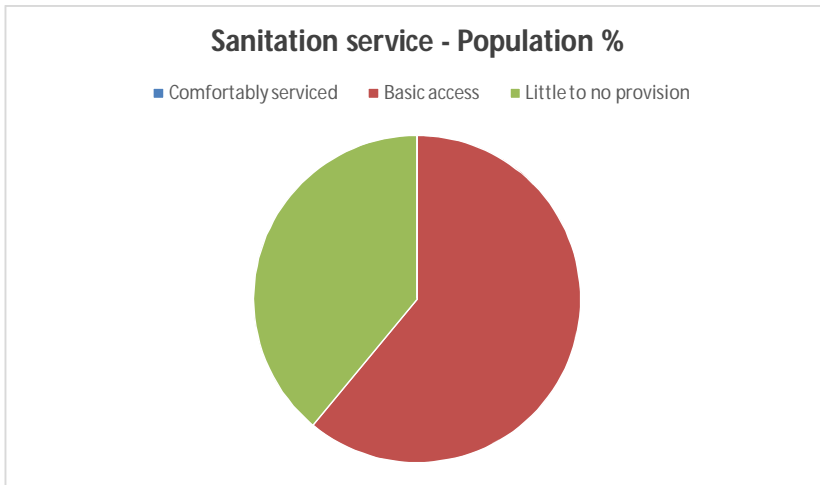


Figure 3b: Percentage of population with access to sanitation services

In 2017, United Nations International Children’s Emergency Fund (UNICEF) and WHO held a Joint Monitoring Program (JMP) for Sanitation and Water Supply, this survey illustrated that approximately 11.3 million people in Zambian households had no access to fundamental sanitation systems, while 6.4 million people lack essential requirements to acquire standard drinking water necessities, representing 69% and 39% respectively, of the then Zambian population [9]. In schools, 34% of pupils have no means to obtain sanitation amenities, whereas 21% of them have undersupply of primary water services. According to the JMP, it was reported that despite notable progress being seen regarding the betterment of core WASH infrastructure in Zambia, the slight upticks fall short of the existing void and rising requirement of these pertinent services. To attain Sustainable Development Goal (SDG) six (6) of universal WASH access by the year 2030, the southern African country has a duty to annually furnish vital hygiene services to 1.46 million people, standard sanitation to 1.16 million people and ample water supply to 660,000 people [11].

Comment [OF19]: provide

Governance structure and administrative framework

The Government of the Republic of Zambia (GRZ) in the last quarter of 2016 established the Ministry of Water Development Sanitation and Environmental Protection (MWDSEP), following the recognition of the key role coordination along with leadership play in spearheading the WASH framework and fostering advanced economic development including successful social and public health [57]. In addition, a dedicated Department of Water Supply and Sanitation was created within the ministry, whose objective was to deliver robust leadership to the WASH sector. The full-fledged body purposes to boost effective

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 In the last quarter of 2016, the Government of the Republic of Zambia (GRZ) established the Ministry of Water Development, Sanitation, and Environmental Protection (MWDSEP). This move recognized the crucial role of coordination and leadership in driving the WASH framework and promoting economic growth, social development, and public health [57].

coordination for WASH programs via forming a solid planning backbone and strengthening the financial arm [58]. This aligns with Zambia's aspirations for Vision 2030 – seeking to transform the country into a prosperous middle-income nation by 2030, besides the United Nations Sustainable Development Goals (UNSDGs) for 2030. During the 7th National Development Plan (NDP) in the period between 2017 and 2021, the government emphatically reaffirmed its plan to elevate the WASH system by primarily aiming at increasing access to Water and Sanitation for all Zambians [14].

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The full-fledged body aims to enhance coordination for WASH programs by establishing a robust planning framework and strengthening financial capabilities [58].

The National Urban and Sanitation Strategy, the Water Supply and Sanitation Capacity Building Strategy and the Open Defecation-free Zambia Strategy 2030, all support the two vital national programs to guide the provision of WASH services under the MWDSEP, namely: the 2016 to 2030 National Rural Water Supply and Sanitation Program (NRWSSP) alongside the National Urban Water Supply and Sanitation Program (NUWSSP) running from 2011 to 2030 [59]. Additionally, a legal foundation called Solid Waste Regulation and Management Act No. 20 of 2018 was developed by the Ministry of Local Government to regulate and manage solid waste services in the country. This ministry together with the MWDSEP looks forward to creating a Water Supply, Sanitation and Solid Waste Management Policy to further refine the policy framework and enhance coordination within the sector.

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The National Urban and Sanitation Strategy, the Water Supply and Sanitation Capacity Building Strategy, and the Open Defecation-free Zambia Strategy 2030 all support two vital national programs. These programs guide the provision of WASH services under the MWDSEP: the National Rural Water Supply and Sanitation Program (NRWSSP) (2016-2030) and the National Urban Water Supply and Sanitation Program (NUWSSP) (2011-2030) [59].

Zambia's cholera elimination plan

In agreement with the mission of the Global Task Force on Cholera Control (GTFCC) to eradicate cholera in endemic regions by 2030, Zambia outlined a proactive stance in the Global Roadmap 2030, by sponsoring a resolution to eliminate cholera worldwide by the year 2030 at the 71st World Health Assembly (WHA) in 2018. Furthermore, Zambia took an ambitious and bold move to end cholera within its borders by 2025, earlier than the global target date. To achieve this objective, Zambia has developed its premier Multisectoral Cholera Elimination Plan (MCEP), scheduled to run from 2019 to 2025. The main objective of this strategic plan is to decrease deaths and ailments associated with cholera, ultimately having a cholera-free Zambia, come 2025 [60]. Government ministries, donors and health partners put together collaborative efforts to form the MCEP, incorporating useful lessons learned from preceding cholera outbreaks, drawing on experiences and best practices while handling past shortfalls in the response to the public health threat. The plan was cemented on the cholera elimination and control campaigns currently running, constituting of the comprehensive situational analysis of cholera in Zambia, and an evaluation of the epidemiological status, ongoing capacities to control the infectious disease and well as technical and financial assistance from partners and donors [61].

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In line with the Global Task Force on Cholera Control's (GTFCC) mission to eradicate cholera in endemic regions by 2030, Zambia took a proactive stance by sponsoring a resolution at the 71st World Health Assembly (WHA) in 2018. This resolution aimed to eliminate cholera worldwide by 2030, as outlined in the Global Roadmap 2030.

In concordance with the Global roadmap and indeed some countries in East Africa [62–65], the MCEP outlines two pivotal goals: short-term involving the control of cholera with focus on campaigns of OCV, and the long-term goal is the elimination of cholera with emphasis on enhanced provision of WASH facilities in high-risk areas. Improved surveillance on case management and local community involvement following a well-established channel of

communication, are some additional strategies the plan has implemented [5]. To achieve these goals, the MCEP employs comprehensive three-pronged approaches (Figure 4) to attain the 90% reduction in cholera-related mortalities.

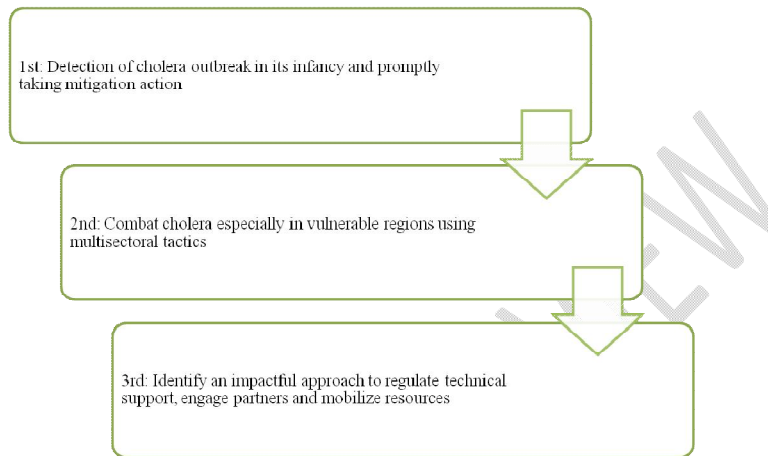


Figure 4: Strategic approaches for cholera elimination

Traditional Oral Cholera Vaccine versus Plant-based Edible Cholera Vaccines

During the 2018 cholera outbreak, Zambia used the Euvichol manufactured oral cholera vaccines, however for the 2021 campaigns [32], the Shanchol vaccine was employed covering Sinazongwe and Nsama districts of the country [66]. The survey was yielded great results as there were no cholera cases reported in 2020 and 2021 [24]. While Oral Cholera Vaccines [67] are available in the international market [68], they are very costly [69] and usually do not meet the demands especially in Sub-Saharan Africa [70].

Hence, it is imperative to diverse alternative and sustainable means of immunization. Yuki *et al.*, [71] reported that edible vaccines derived from rice carrying cholera toxin B subunit presented a groundbreaking point of view to immunization, [71]. The oral MucoRice-CTB vaccine was proven safe after a randomized clinical trial in healthy American adults [72]. Plant-based edible vaccines promise to offer potential benefits such as cost-effectiveness [73], ease mode of administration [74], low spoilage rate [75], near-user site, eradicate the need for cold chain storage and stimulation of both mucosal and systematic immunities [76]. They leverage the natural production capabilities of plants to be candidates of choice to vehicle

Comment [OF24]: During the 2018 cholera outbreak in Zambia, the Euvichol oral cholera vaccine was used [32]. In contrast, the 2021 campaigns employed the Shanchol vaccine, targeting Sinazongwe and Nsama districts [66].

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Yuki *et al.* [71] reported a groundbreaking approach to immunization, using edible vaccines derived from rice that carry the cholera toxin B subunit.

green factory vaccines in a less invasive manner and a more accessible delivery system [77]. Although, ongoing studies are important to determine the efficacy and safety of these vaccines [78], crucial points are to be considered in the scaling up manufacture, regulatory approvals on development of genetically modified crops [79] as well as how they can be integrated into the existing healthcare systems, i.e. the dosage issues and distribution strategies to ensure equal access of these vaccines, globally [80].

Comment [OF27]: Recast

They utilize the natural production capabilities of plants to develop innovative, minimally invasive, and highly accessible vaccine delivery systems, making them ideal candidates for "green factory" vaccines.[77].

Conclusion and Future perspective

Addressing cholera's public health impact requires a comprehensive and multi-layered approach. Investments in innovative technology and organized data analysis are essential to improve outbreak surveillance before, during, and after it occurs. Predictive mathematical models could serve as early warning systems, enabling timely management of health risks tied to seasonal changes. For a coordinated response, the Zambian government could collaborate closely with health organizations and local communities to advance cholera elimination efforts. Regular evaluation of the Multisectoral Cholera Elimination Plan, along with transparent program adaptations, will ensure evolving needs are met and support the goal of a cholera-free Zambia by 2025.

To promote health and prevent cholera outbreaks, Zambia must prioritize consistent adherence to water, sanitation, and hygiene (WASH) practices while advancing strategies that meet global standards. Building a climate-resilient healthcare system is key to mitigating climate-related health challenges. Integrating advanced drainage solutions into urban planning could reduce flooding risks, while targeted interventions addressing food insecurity and policies tackling social inequality would further protect vulnerable populations. Expanded public health education and personal hygiene awareness will empower individuals to adopt healthy practices and support sustainable, long-term improvements in public health.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

- [1] Dorsainvil M. Cholera: Still a Major Public Health Issue in Sub-Saharan Africa. *J Health Care Poor Underserved* 2021;32:1734–41.
- [2] 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* 2022;7:e009934.

- [3] Mwaba J, Debes AK, Shea P, Mukonka V, Chewe O, Chisenga C, et al. Identification of cholera hotspots in Zambia: A spatiotemporal analysis of cholera data from 2008 to 2017. *PLoS Negl Trop Dis* 2020;14:e0008227.
- [4] Oprea M, Njamkepo E, Cristea D, Zhukova A, Clark CG, Kravetz AN, et al. The seventh pandemic of cholera in Europe revisited by microbial genomics. *Nat Commun* 2020;11:5347. <https://doi.org/10.1038/s41467-020-19185-y>.
- [5] Mbewe N, Tembo J, Kasonde M, Mwangilwa K, Zulu PM, Seriki JA, et al. Navigating the Cholera Elimination Roadmap in Zambia—a Scoping Review (2013–2023). *MedRxiv* 2024:2024–8.
- [6] Olu O, Babaniyi O, Songolo P, Matapo B, Chizema E, Kapin’a-Kanyanga M, et al. Cholera epidemiology in Zambia from 2000 to 2010: implications for improving cholera prevention and control strategies in the country. *East Afr Med J* 2013;90:324–31.
- [7] Finger F, Heitzinger K, Berendes D, Ciglencecki I, Dominguez M, Alberti K, et al. Reporting of deaths during cholera outbreaks: case fatality ratio and community deaths. *Lancet Infect Dis* 2024;24:e353.
- [8] Mwape K, Kwenda G, Kalonda A, Mwaba J, Lukwesa-Musyani C, Ngulube J, et al. Characterisation of *Vibrio cholerae* isolates from the 2009, 2010 and 2016 cholera outbreaks in Lusaka province, Zambia. *Pan African Medical Journal* 2020;35.
- [9] Gething PW, Ayling S, Mugabi J, Muximpua OD, Kagulura SS, Joseph G. Cholera risk in Lusaka: A geospatial analysis to inform improved water and sanitation provision. *PLOS Water* 2023;2:e0000163.
- [10] Mutale LS, Winstead A V, Sakubita P, Kapaya F, Nyimbili S, Mulambya NL, et al. Risk and Protective Factors for Cholera Deaths during an Urban Outbreak—Lusaka, Zambia, 2017–2018. *Am J Trop Med Hyg* 2020;102:534.
- [11] Ladan MT, Okukpon I, Maduekwe NC. Realising Sustainable Access to Water and Sanitation in Africa: Role of Critical Institutions. *SDGs in Africa and the Middle East Region*, Springer; 2023, p. 1–24.
- [12] Murebwa-Chirambo R, Mwanza R, Mwinuna C, Mweene-Ndumba I, Mufunda J, Mazaba ML. Occurrence of cholera in Lukanga fishing camp, Kapiri-Mposhi district, Zambia. *Health Press Zambia Bull.*; 2017.
- [13] Mwaba J, Debes AK, Murt KN, Shea P, Simuyandi M, Laban N, et al. Three transmission events of *Vibrio cholerae* O1 into Lusaka, Zambia. *BMC Infect Dis* 2021;21:570. <https://doi.org/10.1186/s12879-021-06259-5>.
- [14] Muyebe ASJ. The Politics of Cholera Epidemics in Zambia 2024.
- [15] Kateule E, Nzila O, Ngosa W, Mfume F, Shimangwala C, Gama A, et al. Multisectoral approach for the control of cholera outbreak—lessons and challenges from Lusaka district—Zambia, October 2023–February 2024. *Pan Afr Med J* 2024;48.

- [16] Hutchings P, Willcock S, Lynch K, Bundhoo D, Brewer T, Cooper S, et al. Understanding rural–urban transitions in the Global South through peri-urban turbulence. *Nat Sustain* 2022;5:924–30. <https://doi.org/10.1038/s41893-022-00920-w>.
- [17] Hassan WI, Joseph I, Abubakar S. ASSESSMENT OF EFFECT OF FLOODING AND FLOOD RISK REDUCTION STRATEGIES IN URBAN CENTRES. *Journal of Built Environment and Geological Research* 2024.
- [18] Bastin A, Knabe L, Simpson M. Climate Change and Cholera: A Review Exploring the Association Between Storm Severity and Global Human *Vibrio* spp. Incidence. *Georgetown Medical Review* 2024;8.
- [19] Merrill RD, Chabi AIB, McIntyre E, Kouassi JV, Alleby MM, Codja C, et al. An approach to integrate population mobility patterns and sociocultural factors in communicable disease preparedness and response. *HumanitSocSciCommun* 2021;8:23. <https://doi.org/10.1057/s41599-020-00704-7>.
- [20] Casati P, Fumagalli E, Baldi D, Moner-Girona M. Understanding the drivers of electricity access and willingness to pay for reliable electricity in African refugee settlements: Evidence from Zambia, Malawi, and Uganda. *Energy Res Soc Sci* 2024;113:103546.
- [21] Yasmin T, Dhesi S, Kuznetsova I, Cooper R, Krause S, Lynch I. A system approach to water, sanitation, and hygiene resilience and sustainability in refugee communities. *Int J Water ResourDev* 2023;39:691–723.
- [22] Ashraf N, Glaeser E, Holland A, Steinberg BM. Water, health and wealth: The impact of piped water outages on disease prevalence and financial transactions in zambia. *Economica* 2021;88:755–81.
- [23] Thelma CC, Chitondo L, Phiri EV, Gilbert MM. Understanding and addressing the cholera outbreak in Zambian communities. *International Journal of Science and Research Archive* 2024;11:526–34.
- [24] Ng'ombe H, Simuyandi M, Mwaba J, Luchen CC, Alabi P, Chilyabanyama ON, et al. Immunogenicity and waning immunity from the oral cholera vaccine (ShanChol™) in adults residing in Lukanga Swamps of Zambia. *PLoS One* 2022;17:e0262239.
- [25] Reaver KM, Levy J, Nyambe I, Hay MC, Mutiti S, Chandipo R, et al. Drinking water quality and provision in six low-income, peri-urban communities of Lusaka, Zambia. *Geohealth* 2021;5:e2020GH000283.
- [26] Organization WH. Programme Budget Performance Assessment: 2018–2019. World Health Organization. Regional Office for South-East Asia; 2020.
- [27] UNICEF. Global annual results reports 2019: communication for development (C4D). UNICEF; 2020.
- [28] Mathebula L, Malinga T, Mokgoro M, Ndwandwe D, Wiysonge CS, Gray G. Cholera vaccine clinical trials: A cross-sectional analysis of clinical trials registries. *Hum VaccinImmunother* 2023;19:2261168.

- [29] Sinumvayo JP, Munezero PC, Tope AT, Adeyemo RO, Bale MI, Nyandwi JB, et al. Advancing Vaccinology Capacity: Education and Efforts in Vaccine Development and Manufacturing across Africa. *Vaccines (Basel)* 2024;12.
- [30] Chibwe W, Mbewe A, Hazemba AN. The health effects of Chunga dumpsite on surrounding communities in Lusaka, Zambia. *MedRxiv* 2021:2012–21.
- [31] Libanda B, Rand E, Gyang GN, Sindano CT, Simwanza L, Chongo M. Recent and future exposure of water, sanitation, and hygiene systems to climate-related hazards in Zambia. *Journal of Water and Climate Change* 2024;15:958–77.
- [32] Mukonka VM, Sialubanje C, Matapo BB, Chewe O, Ngomah AM, Ngosa W, et al. Euvichol-plus vaccine campaign coverage during the 2017/2018 cholera outbreak in Lusaka district, Zambia: a cross-sectional descriptive study. *BMJ Open* 2023;13:e070796.
- [33] Xu H, Zou K, Dent J, Wiens KE, Malembaka EB, Bwire G, et al. Enhanced cholera surveillance to improve vaccination campaign efficiency. *Nat Med* 2024;30:1104–10.
- [34] Matenga TFL, Zulu JM. From the Plague Horrors of Cholera, what Partnership Lessons can be Learnt in Addressing COVID-19 in Zambia. *International Journal of Oral Health Dental Management* 2023;1:1–12.
- [35] Montero DA, Vidal RM, Velasco J, George S, Lucero Y, Gómez LA, et al. *Vibrio cholerae*, classification, pathogenesis, immune response, and trends in vaccine development. *Front Med (Lausanne)* 2023;10:1155751.
- [36] Raskin DM, Mishra A, He H, Lundy Z. Stringent response interacts with the ToxR regulon to regulate *Vibrio cholerae* virulence factor expression. *Arch Microbiol* 2020;202:1359–68.
- [37] Mavhungu M, Digban TO, Nwodo UU. Incidence and Virulence Factor Profiling of *Vibrio* Species: A Study on Hospital and Community Wastewater Effluents. *Microorganisms* 2023;11:2449.
- [38] Islam MS, Zaman MH, Islam MS, Ahmed N, Clemens JD. Environmental reservoirs of *Vibrio cholerae*. *Vaccine* 2020;38:A52–62.
- [39] Kang T-J, Kim B-G, Yang J-Y, Yang M-S. Expression of a synthetic cholera toxin B subunit in tobacco using ubiquitin promoter and bar gene as a selectable marker. *Mol Biotechnol* 2006;32:93–100.
- [40] Wu B, Liang W, Yan M, Li J, Zhao H, Cui L, et al. Quorum sensing regulation confronts the development of a viable but non-culturable state in *Vibrio cholerae*. *Environ Microbiol* 2020;22:4314–22.
- [41] Giroto CD, Behzadian K, Musah A, Chen AS, Djordjević S, Nichols G, et al. Analysis of environmental factors influencing endemic cholera risks in sub-Saharan Africa. *Science of The Total Environment* 2024;926:171896. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.171896>.
- [42] Martinelli Filho JE, Colwell RR, Queiroz AFS, Rivera ING, Lopes RM. *Vibrio cholerae* O139 attached to zooplankton: reservoir diversity and distribution over an estuarine-coastal gradient. *J Coast Res* 2020;95:92–6.

- [43] Feng J, Mazzei M, Di Gregorio S, Niccolini L, Vitiello V, Ye Y, et al. Marine copepods as a microbiome hotspot: Revealing their interactions and biotechnological applications. *Water (Basel)* 2023;15:4203.
- [44] Perez-Saez J, Lessler J, Lee EC, Luquero FJ, Malembaka EB, Finger F, et al. The seasonality of cholera in sub-Saharan Africa: a statistical modelling study. *Lancet Glob Health* 2022;10:e831–9.
- [45] Kopprio GA, Neogi SB, Rashid H, Alonso C, Yamasaki S, Koch BP, et al. *Vibrio* and bacterial communities across a pollution gradient in the bay of Bengal: unraveling their biogeochemical drivers. *Front Microbiol* 2020;11:594.
- [46] Armando CJ, Rocklöv J, Sidat M, Tozan Y, Mavume AF, Bunker A, et al. Spatial-temporal analysis of climate and socioeconomic conditions on cholera incidence in Mozambique from 2000 to 2018: an ecological longitudinal retrospective study. *BMJ Open* 2024;14:e082503.
- [47] Usmani M, Brumfield KD, Jamal Y, Huq A, Colwell RR, Jutla A. A review of the environmental trigger and transmission components for prediction of cholera. *Trop Med Infect Dis* 2021;6:147.
- [48] Baltazar CS, Baloi LD, Luiz N, Chitio JE, Capatine I, Siteo M, et al. Conditions to eliminate cholera in Mozambique-the pathway for the development of the national cholera plan. *Pan African Medical Journal* 2022;42.
- [49] Miggo M, Harawa G, Kangwerema A, Knovicks S, Mfuno C, Safari J, et al. Fight against cholera outbreak, efforts and challenges in Malawi. *Health Sci Rep* 2023;6:e1594.
- [50] El-Sayed A, Kamel M. Climatic changes and their role in emergence and re-emergence of diseases. *Environmental Science and Pollution Research* 2020;27:22336–52.
- [51] Christaki E, Dimitriou P, Pantavou K, Nikolopoulos GK. The impact of climate change on cholera: a review on the global status and future challenges. *Atmosphere (Basel)* 2020;11:449.
- [52] Asadgol Z, Badirzadeh A, Niazi S, Mokhayeri Y, Kermani M, Mohammadi H, et al. How climate change can affect cholera incidence and prevalence? A systematic review. *Environmental Science and Pollution Research* 2020;27:34906–26.
- [53] Obasohan PE, Walters SJ, Jacques R, Khatab K. Risk factors associated with malnutrition among children under-five years in sub-Saharan African countries: a scoping review. *Int J Environ Res Public Health* 2020;17:8782.
- [54] Gabutti G, Rossanese A, Tomasi A, Giuffrida S, Nicosia V, Barriga J, et al. Cholera, the current status of cholera vaccines and recommendations for travellers. *Vaccines (Basel)* 2020;8:606.
- [55] Hsiao A, Ramani E, Seo H-J, Pak G, Vuntade D, Ngwira B, et al. Economic impact of cholera in households in rural southern Malawi: a prospective study. *BMJ Open* 2022;12:e052337.
- [56] Sikder M, Deshpande A, Hegde ST, Malembaka EB, Gallandat K, Reiner RC, et al. Water, Sanitation, and Cholera in Sub-Saharan Africa. *Environ Sci Technol* 2023;57:10185–92.

- [57] Mogasale V, Ngogoyo SM, Mogasale V V. Model-based estimation of the economic burden of cholera in Africa. *BMJ Open* 2021;11:e044615.
- [58] Ramamurthy T, Ghosh A. A re-look at cholera pandemics from early times to now in the current era of epidemiology. *Journal of Disaster Research* 2021;16:110–7.
- [59] Ko M, Cherian T, Groves HT, Klemm EJ, Qazi S. Application of the Child Health and Nutrition Research Initiative (CHNRI) methodology to prioritize research to enable the implementation of Ending Cholera: A global roadmap to 2030. *PLoS One* 2022;17:e0264952.
- [60] Maity B, Saha B, Ghosh I, Chattopadhyay J. Model-Based Estimation of Expected Time to Cholera Extinction in Lusaka, Zambia. *Bull Math Biol* 2023;85:55.
- [61] Chowdhury F, Ross AG, Islam MT, McMillan NAJ, Qadri F. Diagnosis, management, and future control of cholera. *Clin Microbiol Rev* 2022;35:e00211-21.
- [62] Debes AK, Shaffer AM, Ndikumana T, Liesse I, Ribaira E, Djumo C, et al. Cholera hot-spots and contextual factors in Burundi, planning for elimination. *Trop Med Infect Dis* 2021;6:76.
- [63] Bwire G, Sack DA, Lunkuse SM, Ongole F, Ngwa MC, Namanya DB, et al. Development of a scorecard to monitor progress toward national cholera elimination: its application in Uganda. *Am J Trop Med Hyg* 2023;108:954.
- [64] Kiama C, Okunga E, Muange A, Marwanga D, Langat D, Kuria F, et al. Mapping of cholera hotspots in Kenya using epidemiologic and water, sanitation, and hygiene (WASH) indicators as part of Kenya's new 2022–2030 cholera elimination plan. *PLoS Negl Trop Dis* 2023;17:e0011166.
- [65] Hussen M, Worku Demlie Y, Edosa M, Kebede M, Wossen M, Mulugeta Chane A, et al. Ethiopia National Cholera Elimination Plan 2022–2028: experiences, challenges, and the way forward. *Clinical Infectious Diseases* 2024;79:S1–7.
- [66] Qadri F, Clemens JD, Holmgren J. Cholera immunity and development and use of oral cholera vaccines for disease control. *Mucosal Vaccines*, Elsevier; 2020, p. 537–61.
- [67] Song KR, Lim JK, Park SE, Saluja T, Cho S-I, Wartel TA, et al. Oral cholera vaccine efficacy and effectiveness. *Vaccines (Basel)* 2021;9:1482.
- [68] López-Gigosos RM, Plaza E, Díez-Díaz RM, Calvo MJ. Vaccination strategies to combat an infectious globe: oral cholera vaccines. *J Glob Infect Dis* 2011;3:56.
- [69] Saluja T, Mogasale V V, Excler J-L, Kim JH, Mogasale V. An overview of Vaxchora™, a live attenuated oral cholera vaccine. *Hum Vaccin Immunother* 2020;16:42–50.
- [70] Trolle H, Forsberg B, King C, Akande O, Ayres S, Alfvén T, et al. A scoping review of facilitators and barriers influencing the implementation of surveillance and oral cholera vaccine interventions for cholera control in lower-and middle-income countries. *BMC Public Health* 2023;23:455.
- [71] Yuki Y, Nojima M, Hosono O, Tanaka H, Kimura Y, Satoh T, et al. Oral MucoRice-CTB vaccine for safety and microbiota-dependent immunogenicity in humans: a phase 1 randomised trial. *Lancet Microbe* 2021;2:e429–40.

- [72] Yuki Y, Nojima M, Kashima K, Sugiura K, Maruyama S, Kurokawa S, et al. Oral Mucorice-CTB vaccine is safe and immunogenic in healthy US adults. *Vaccine* 2022;40:3372–9.
- [73] Vijayakumar VE, Vijayalakshmi MA, Lacroix-Desmazes S, Venkataraman K. The use of *Bacillus subtilis* as a cost-effective expression system for production of Cholera Toxin B fused factor VIII epitope regions applicable for inducing oral immune tolerance. *Folia Microbiol (Praha)* 2024:1–11.
- [74] Bahramnejad B, Moradi E, Naji M, Salehi H. Transfer and expression of exendin-4, stimulant of insulin secretion, fused to cholera toxin B subunit (CTB) in lettuce. *Genet EngBiosaf J* 2023;12:170.
- [75] Loc NH, Thinh LT. Investigation of agrobiological properties of *ctb* (cholera toxin B subunit) transgenic tomato under in vivo condition. 2020.
- [76] Beenzu S, Emmanuel E, Justus O, Steven R, Ngotho M. Potential of plant-derived edible vaccines: a vial or a potato? *African Journal of Biological Sciences (South Africa)* 2024;6:3696–709.
- [77] Kim M, Vergara E, Tran A, Paul MJ, Kwon T, Ma JKC, et al. Marked enhancement of the immunogenicity of plant-expressed IgG-Fc fusion proteins by inclusion of cholera toxin non-toxic B subunit within the single polypeptide. *Plant Biotechnol J* 2024;22:1402–16.
- [78] Zandinava N, Bahramnejad B, Salehi H. Specific expression of exendin-4 gene fused to cholera B toxin subunit (CTB) in carrot root (*Daucus carota* L.) under regulation of MLL promoter. *Agricultural Biotechnology Journal* 2024;16:87–114.
- [79] Kokei S, Bahramnejad B. Transient Expression of CTB-Exendin Fused Genes in *Nicotiana tabacum* via *Agrobacterium tumefaciens*. *Journal of Agricultural Science and Technology* 2020;22:1603–12.
- [80] Morris DA, Reeves MA, Royal JM, Hamorsky KT, Matoba N. Isolation and detection of a KDEL-tagged recombinant cholera toxin B subunit from *Nicotianabenthamiana*. *Process Biochemistry* 2021;101:42–9.

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