Wastewater and Environmental Surveillance Summary for Cholera

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World Health Organization

This document provides information on wastewater and environmental surveillance (WES) for pathogenic *Vibrio cholerae* (*V. cholerae*) that causes cholera. Other *Vibrio* species and non-pathogenic *V. cholerae* are not included. It should be used together with the accompanying *WES Guidance for one or more pathogens*, which includes general and cross-cutting information (available here). Except where cited otherwise, information has been drawn from existing WHO and US CDC publicly available sources, current at the time of writing.

WES for Cholera at a glance

- Overall, there is inadequate evidence to determine the optimal contribution of WES to cholera disease surveillance and response. Further research is required.

Table 1 : At a glance assessment of key WES criteria for V. cholerae (sewered and non-sewered)[a,b]

Setting	Categorical Assessment (CA) / Strength of Evidence (SoE)	Public Health Significance	Actionability / Relative value	Technical Feasibility	Operational Feasibility	Acceptability	Optimisation	
							Integrated disease response	Multitarget WES
Sewered	CA							
	SoE							
Non-sewered	CA	not separated by sewered category						
	SoE							

Key:

1. Categorical Assessment (CA) of criteria

Category	Code	Description
High		Criteria is evaluated as met at the highest level
Intermediate		Criteria is evaluated as met at an intermediate level (it may be that not all sub-components of the criteria are met)
Low		Criteria is evaluated as low
Not-supported		Criteria is evaluated as not supported
Not applicable		Criteria is not applicable OR cannot assessed due to inadequate evidence

2. Strength of evidence (SOE)

Evidence level	Code	Description
Strong		High quality consistent evidence, including from multiple relevant studies/settings, at scale, over a prolonged period, with evidence from program settings, not only from research studies or short projects.
Moderate		Relevant evidence is available but does not meet criteria for 'Strong' classification.[c]
Inadequate evidence		Evidence is inadequate and further study/evaluation is needed

[a] Further description of the criteria used to assess the applicability of WES for a specific pathogen, as well as the methods used to evaluate them, is included in *WES Guidance for one or more pathogens*. The assessment in Table 1 provides a snapshot at the global level, but country level assessment may differ.

[b] Sewered settings refers to closed reticulated sewage systems. Non-sewered settings refers to the diverse settings which are not 'sewered', including open drains and community sampling points. Individual small septic tanks at residential or building level are not viable to sample individually and are not considered here separately. Most WES evidence to date is reported from reticulated sewered settings, often from high-income settings. Yet much of the global population is on heterogenous non-sewered systems and this has implications for assessment of various WES categories.

[c] Evidence classified as 'Moderate' meets one or more of the following criteria: not from numerous settings, for a short period, without program-level evidence, and/or where findings are not consistent or of high quality.

Summary

- Cholera is an acute diarrheal disease that can be fatal within hours if left untreated. Therefore, timely population-level information about infections is relevant and **actionable** by local public health authorities.
- The causal agent, toxigenic *V. cholerae* serogroup O1 or serogroup O139, remains a pathogen of ongoing **global health importance** and a priority for surveillance. Globally there are millions of cases of cholera, causing tens of thousands of deaths, annually.
- The disease is primarily associated with **low-income** settings lacking access to and use of safe water, sanitation and hygiene. Therefore, the priority for enhancing surveillance methods is in those contexts.
- A global strategy on cholera control, "Ending cholera: a global roadmap to 2030", with a target to reduce cholera deaths by 90% was launched in 2017. Enhanced surveillance can potentially support **monitoring and action** to achieve that goal.
- *V. cholerae* WES demonstrates **technical feasibility** for a range of methods, including culture and molecular, with options for enrichment, quantitative detection, qualitative detection, and sequencing. However, there are limitations in interpretation for public health action due to other naturally occurring vibrios and the need for multiple targets to confirm pathogenic *V. cholerae* with the potential to cause cholera. Therefore, high quality pilot studies are urgently required to refine WES for *V. cholerae* and narrow critical knowledge gaps in multiple contexts.
- The value of *V. cholerae* WES to estimate infection and disease burden is **limited by the autochthonous environmental reservoir** for the pathogen. It is necessary to use primers and probes targeted at virulence genes (e.g., the toxin gene) to improve the value of WES for *V. cholerae*, whilst noting that the toxin gene can be present in non-cholerae vibrios.
- There is **very limited global practical experience** utilizing WES programs to meet cholera related surveillance objectives in operational contexts, with work to date being limited to pilot studies and research work.
- WES responses are limited to geographically targeted and system levels and are not patient specific. This may theoretically include promoting primary and booster vaccinations, establishing cholera treatment centers (CTCs) or oral rehydration points (ORPs), enhanced surveillance for symptoms, and encouraging good WASH practices through education.

Contents

1. General information

1.1. The pathogen, associated disease and risk factors

Vibrio cholerae is a species of Gram-negative bacteria of which some strains are pathogenic to humans. Acute watery diarrhea is the classical symptom of infection with *V. cholerae* toxigenic serogroups O1, and less commonly O139. The seventh pandemic *V. cholerae* serogroup O1 El Tor lineage (7PET) has been particularly pathogenic. Symptoms are usually self-limiting, persisting for a few days. Severe dehydration can occur, leading to hospitalization and even death. Treatment includes rapidly rehydrating the patient to reduce the risk of fatality. These acute gastroenteritis symptoms are associated with a diverse range of causes and are not specific to *V. cholerae*. Therefore, confirmation of a cholera diagnosis is only made after clinical testing of suspected cholera cases through culture and/or PCR on stool samples or rectal swab samples.

1.2. Global burden, geographic distribution and seasonality

The current (seventh) cholera pandemic has continued since the 1960s, and WHO's situation reports noted that in 2022, >30 countries, including several with no cases in at least the three years prior, had large, resurgent outbreaks of cholera.

There are strong climatic, seasonal, and environmental factors that are associated with increased risk of cholera outbreaks. Temperature plays a major factor, with geographical areas with warmer climates, along with warmer periods of the year, and El Nino years, being more strongly associated with increased cholera outbreaks. Major events that compromise WASH systems (e.g. climatic events such as floods and high winds; and traumatic events such as war and displacement), are strongly associated with cholera outbreaks in sub-tropical and tropical climate zones.

1.3. Routes of transmission

Since *V. cholerae* spreads via the fecal-oral route of transmission, primarily via contaminated water and food, controlling transmission of *V. cholerae* focuses on access to and use of water, sanitation and hygiene (WASH) , food safety programs, and targeted use of vaccines.

1.4. Zoonotic hosts and potential reservoirs

Humans are the primary natural host of pathogenic *V. cholerae* strains that cause cholera symptoms. Symptomatic disease is only associated with a subset of *V. cholerae* strains that can exist in the environment. Human pathogenic and non-pathogenic *Vibrio* spp. can be naturally present in brackish aquatic systems, both freely suspended in water, and associated with algae, zooplankton, and other aquatic animals, and these can infiltrate low-lying sewers, open drains, and rivers. This widespread environmental presence of closely related vibrio species is a compounding factor in WES that may present a complication when using WES to gather data on cholera within an associated community. In addition, the detection of toxigenic *V. cholerae* in environmental samples does not necessarily indicate

that clinical cases are occurring in associated communities [1] since the bacteria may be harbored in the environment in the absence of ongoing infection. Cholera is only associated with a small proportion of the many species and serotypes of *V. cholerae* that exist in the environment. Therefore, to be health-relevant, WES for *V. cholerae* must be undertaken in the context of seeking to identify whether cholera is present in the community due to locally circulating pathogens, rather than merely whether the pathogen is present.

1.5. Human pandemic potential

V. cholerae has a high disease burden, can cause death in otherwise healthy individuals, particularly the young, has frequent, recurrent outbreaks in many areas, and can reemerge in areas with no outbreaks. The disease can cause ongoing outbreaks in any areas lacking adequate WASH due to fecal-oral transmission.

2. *V. cholerae* and wastewater and environmental waters

2.1. Potential inputs to wastewater and environmental waters

Toxigenic *V. cholerae* bacteria are shed via feces and vomitus into wastewater and environmental water. The pathogen can be readily detected in laboratory samples of feces from infected persons for approximately 1-10 days post infection.

Of particular importance for WES, *V. cholerae* can also naturally be present and replicate in environmental waters (in the absence of feces or vomitus shed from infected persons). Therefore, *V. cholerae* can theoretically enter wastewater systems from natural waters entering as inflow and infiltration into the wastewater system both intentionally (combined stormwater and sewage systems) or unintentionally (uncombined systems). Factors such as warmer temperatures, more brackish salinities, higher tides, and elevated rainfall, can theoretically increase such risks.

For symptomatic infections, it can take up to five days for symptoms to become evident. The pathogen can be shed at very high levels in symptomatic stools, from 10 billion to 1 trillion culturable *V. cholerae* per liter from symptom onset, with shedding continuing for 1 to 2 weeks.

Most *V. cholerae* infections (approximately 80%) are asymptomatic and only about 1 in 10 infected experience symptoms severe enough to visit healthcare settings. Shedding is much lower in asymptomatic persons, approximately 1,000 culturable *V. cholerae* per liter of stool, and only routinely detectable for approximately 1 day.

[2]These factors mean that infections can rapidly become widespread before cholera cases are laboratory-confirmed, and even then, most infections and mild illness cases are unlikely to be documented.

2.2. *V. cholerae* target persistence in wastewater and the environment

V. cholerae are moderately persistent in wastewater, and can be routinely detected in raw sewage and environmental waters contaminated with human fecal waste. As noted above, *V. cholerae* can also naturally inhabit and replicate in the environment, particularly in warm brackish waters in tropical areas, and can survive for many weeks in fresh and marine waters even outside of their optimal growth conditions [3].

Given the many biotypes, serogroups, and subtypes of *V. cholerae* that can be present and detected, with varying presence of the key genes that characterize an outbreak-relevant strain, interpretation of WES results is challenging. Given that target genes can be present in different cells, to improve the potential utility of *V. cholerae* WES, cultivation of single isolates can be followed by genetic characterization by PCR to elucidate whether the key genes are all present in the same isolate.

Whilst cultivation of *V. cholerae* is so important in WES to determine whether single isolates carry the genes that permit them to be pathogenic, a complication with isolating and detecting *V. cholerae* in raw wastewater and environmental waters is that the pathogen can enter a dormant 'viable non-culturable' physiological state. In this state the bacteria are not recoverable using routine cultivation methods.

2.3. *V. cholerae* WES experience

The potential for utilization of WES for *V. cholerae* has been theoretically demonstrated, but has, to date, largely been limited to research projects, and mostly with an environmental monitoring objective to assess potential risks of infection from exposure to water, rather than a WES objective [4–8]The Global Task Force on Cholera Control (GTFCC) briefly discussed WES for community disease surveillance purposes but did not recommend its routine use at this stage [1]. Examples of possible areas of interest for research have been proposed [9].

3. Cholera surveillance

3.1. Overall *V. cholerae* surveillance and response

Despite decades of research and a growing understanding of the disease, prediction of cases, outbreaks, and optimizing containment remains challenging. Surveillance remains critical to providing reliable and timely data on the circulation of human pathogenic *V. cholerae* in the population. The key features of *V. cholerae* of relevance to surveillance programs include:

- The pathogen is found naturally in brackish waters in tropical areas.
- Cases can arise in any areas into which the pathogen is introduced or where the pathogen is endemic, if lacking adequate WASH due to fecal-oral and environmental transmission pathways.
- The proportion of toxigenic serotypes that grows in brackish environments is higher in tropical climates.
- The principal transmission pathway is contaminated water, and to a lesser extent, food. Direct person-to-person and fomite transmission is relatively rare due to the high median infectious dose of the pathogen.
- WHO pre-qualified vaccines are available. They are usually only used on a targeted, rather than population-wide, basis, such as targeting higher-risk groups, workers, travelers, and populations in areas with high disease incidence. In the recent past, and currently, the global vaccine supply is extremely limited due to use in outbreaks (reactive vaccination), leaving little vaccine available for preventive vaccination campaigns.

3.2. Existing surveillance systems and data sources

The GTFCC provides the global normative reference for existing surveillance (https://www.gtfcc.org/resources/) including minimum recommendations for routine monitoring and outbreak detection, and offers adaptive surveillance strategies for the application of core functions based upon the current, local situation. However, many countries face surveillance challenges for cholera preparedness, response, and prevention, and whilst there are rapid tests, these have limitations with respect to their sensitivity [2].

The goal of lab testing for public health surveillance for *V. cholerae*, including testing stool samples from persons with acute watery diarrhea using RDTs, culture-based detection, and qPCR, in accordance with the recently developed target product profile for molecular testing, is to:

- Confirm cholera cases and inform a multi-sectoral intervention to control spread.
- Understand transmission and disease burden to inform preventive use of OCV.
- Assess the effectiveness of interventions, such as vaccination and WASH programs, both in areas where the bacterium remains endemic and in outbreak situations.
- Identify antimicrobial resistance (AR) genes carried by the *V. cholerae* bacteria present.
- Identify the cholera toxin genes and sequence types (ST) of pathogens present to help understand their significance for public health, including epidemic potential and lineage and associated transmission pathways.

- PCR to detect *V. cholerae* with epidemic potential if the test includes specific gene targets for serogroups (i.e., O1 and/or O139 antigens) and the cholera toxin genes (e.g., *ctxA*).
- Detect the emergence of the bacterium in countries where it is not endemic, to enable a rapid response to contain that emergence.
- Identify cases imported by persons from endemic countries into countries where it is not endemic.
- GTFCC encourages whole genome sequencing for further characterization in some situations (e.g., confirmed cases with unknown origin), to provide more detailed information to definitively identify epidemic *V. cholerae*. By analyzing sequence data, the same gene targets can be detected (e.g., for the O1 antigen, or *ctxA*), but the additional genetic information determines evolutionary lineages, epidemic ST, emerging resistance, and provides insights on global transmission patterns.

There are limitations with this conventional public health surveillance approach:

- In situations where individuals have asymptomatic carriage or mild infections, these individuals will have no reason to present and be tested and can only be captured by extensive stool surveys or serosurveys, e.g. the intensive serosurvey in Haiti cholera outbreak in 2011 revealed approximately three times the clinical defined attack rate, [10], or other nonconventional methods.
- In the early phase of an outbreak, the appropriate culture-based tests may not be ordered or reagents not available, thus preventing laboratory confirmation of cases. Similarly, there are supply constraints for RDTs outside of the Gavi system, and PCR tests are not yet widely available.
- It may take some time after an infection occurs for symptoms to be observed, for stool samples to be collected, and for *V. cholerae* to be confirmed, which leads to a delay between infection and action, during which the infected person can infect others.
- There are supply chain issues for stool sample transport media that may limit the ability to transfer samples efficaciously to laboratories.
- The use of antibiotics in patients prior to sampling stools could reduce the sensitivity of culture-based methods.
- Even using RDTs, the time needed to test multiple suspected cases and identify a probable outbreak could be a cause for delay between infection and action, particularly noting the moderate sensitivity of RDTs, and lack of operational guidance on their rapid deployment when suspected cases arise.
- Some countries are concerned about the possible stigma associated with reporting ‘cholera’ cases and outbreaks and hence prefer to report "acute watery diarrhea" not cholera.

4. WES objectives and related public health actions

Note, WES is always considered in the context of local, multi-modal surveillance which should be integrated and complement other available data to provide actionable intelligence (not stand alone). WES must have potential to provide additional value in the local context to be considered for implementation.

4.1. Routine WES for *V. cholerae*

Routine WES involves consistent sampling at the same sites using consistent methods

In locations with limited capacity for surveillance (including limited availability of RDTs), there is a theoretical use case for routine WES for *V. cholerae* for early warning of outbreaks through capturing the presence of individuals with asymptomatic carriage or mild infections in the community that do not present at healthcare facilities, or who present to healthcare facilities but are missed due to surveillance gaps, atypical symptoms, or other reasons.

WES can also theoretically be used to obtain evidence of ongoing, inter-outbreak transmission, which could inform preventive use of WASH interventions and vaccines.

In countries without active cholera outbreaks or where cases have not been detected in ≥ 3 years, toxigenic *V. cholerae* detections from WES that are drastically different than background baseline concentrations could trigger further investigation and public health intervention.

In alignment with the methods used to follow up detections of the bacterium in stool samples, PCR detection, and if warranted sequencing, of *V. cholerae* genes from isolates cultivated from wastewater could permit characterization of the serotypes and lineages in circulation and for phylogenetic analyses to inform spatio-temporal transmission, and hence, control strategies.

4.2. Agile WES for *V. cholerae*

Agile WES means that it is time-limited surveillance with a specific trigger to initiate and is differentiated from routine surveillance; agile WES involves establishing new time-limited activities or purposive changes in the existing WES program, e.g. sampling more frequently or in different locations, reducing the turn-around time to results, and/or performing new of different analyses.

In locations with limited surveillance capacity and with WES routine testing, there is a theoretical use case for WES during outbreaks to inform the responses noted above.

4.3. Potential public health actions arising from the addition of WES for *V. cholerae*

Public health actions in response to cholera outbreaks include:

- Responses to toxigenic *V. cholerae* detection can include promoting primary and booster vaccinations, establishing cholera treatment centers (CTCs) or oral rehydration points (ORPs), enhanced surveillance for symptoms, and encouraging good WASH practices, including basic actions such as chlorination of water supplies.
- The results of WES can potentially be used to inform vaccination, intervention, and education responses.

The utility of WES for *V. cholerae* may be theoretically different according to the incidence of the pathogen in the community of interest; however, to-date there are no documented public health applications of WES for cholera.

For countries that have been cholera-free for ≥3 years and have universal access to basic WASH, ongoing WES for *V. cholerae* may not be considered warranted if sufficient surveillance (including access to and use of RDTs) exists.

Depending on whether an outbreak has been confirmed by the surveillance unit, WES may be of theoretical future use in contexts with high burdens of disease or active outbreaks and insufficient conventional public health surveillance for *V. cholerae*. This includes agile capacity for testing of stools from all or a subset of persons presenting with symptoms, or adaptive testing of stools. If WES can provide additional community-scale data to fill gaps in conventional public health surveillance data or can be used in areas where the outbreak has not yet been confirmed to estimate movement of the outbreak, it may have added value as an interim measure on the path to improved surveillance. In addition, WES can provide a useful tool to understand spatial distribution and track trends over time [6].

Such evidence could assist with targeting and evaluating interventions, such as WASH initiatives and vaccination, and in establishing how embedded the disease is within the population, and in settings with displaced populations, or population with WASH adversely impacted by natural disasters or conflict.

5. WES additional methodological considerations for V. cholerae

This section should be read in conjunction with general methodological consideration in Section 5 of *Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration* (available here). There is no global normative document for WES for pathogenic *V. cholerae* and no established process or guidance. A brief discussion has been given [1], and examples of successful applications of WES for research and investigative purposes have been published [4–8]. There is no standard protocol for WES detection of *V. cholerae* currently provided by CDC or WHO.

5.1. Sampling methods

There are no special considerations for sampling beyond those used for conventional microbiological sampling for environmental monitoring and WES. However, *V. cholerae* must be stored and transported at ambient temperature since the microorganisms become less culturable if cooled to refrigeration temperatures (such as 4˚C). In addition, as is the case for most sampling, it is preferable not to freeze samples during transport, particularly if there is an intent to isolate the microorganism through cultivation. A range of sampling methods, such as conventional liquid grab samples, composite samples, and passive/trap samples (e.g. Moore swabs), and ultrafiltration, have all been successfully utilized in WES, and there is the potential to test for *V. cholerae* specific phage as part of microbial source tracking[7,8,11–13]. However, there is no standard or preferred method that has emerged.

5.2. Laboratory methods

Laboratory methods for accurate detection and characterization of pathogenic *V. cholerae* in WES sample matrices are needed. WES tests for *V. cholerae*, as with clinical testing, can involve culture-based testing. Presumptive identification of *V. cholerae* requires culture using *Vibrio*-specific selective agar followed by seroagglutination tests with O1 and/or O139-specific antisera of an isolated colony. This approach is sufficient for cholera identification in a human specimen when paired with presentation of cholera symptoms and in consideration with epidemiological information, and is feasible since individuals are usually not co-infected with multiple *Vibrio* species (i.e., only one isolated colony needs to be tested). As an alternative or complement to culture-based methods, PCR may be used to confirm *V. cholerae* in clinical specimens and can provide additional information on the pathogen's epidemic potential if a cholera toxin gene target is included in the assay (e.g., *rfbO* and *ctxA*, respectively). Sequencing is required to definitively link an isolate/pure culture to seventh pandemic cholera.

WES samples will likely contain mixed populations of *Vibrio* spp. Because of this, application of gold standard culture-based clinical approaches to WES samples presents technical and feasibility challenges:

- Culture followed by biochemical and serological testing alone lacks specificity. For example, non-toxigenic *V. cholerae* O1 could not be distinguished from toxigenic *V. cholerae* O1.
- *Vibrio*-selective media produces identical colonies for multiple *Vibrio* spp. PCR testing of a potentially extensive number of presumptive colonies would be required to confirm the presence of toxigenic *V. cholerae*. This approach, along with sequencing of isolates, would provide the most

definitive confirmation of toxigenic *V. cholerae*, though it could be cost-prohibitive and logistically difficult.

A range of extraction and concentration methods have all been successfully utilized for culture- and/or molecular-based testing of environmental samples[7,8,11–13]. However, as was the case for sampling, there is no published standard or preferred method that has emerged as a gold-standard test for *V. cholerae* in WES samples. Notably, most of the research to-date has been via the use of culture followed by molecular methods.

A culture-independent molecular approach (i.e., conducting PCR or amplicon-based sequencing on raw or concentrated wastewater samples) would circumvent issues with detection of *V. cholerae* that enter a viable but non-culturable state in the environment and would eliminate a large isolate testing load. However, culture-independent approaches are not confirmatory of toxigenic *V. cholerae* since multiple gene targets are required from one colony/pure culture and these targets can exist independently in different *Vibrio* species, possibly present in a single WES sample. The above challenges will be exacerbated in WES samples with substantial environmental contributions, as other *Vibrio* spp., including non-toxigenic lineages of *V. cholerae* O1 and non-cholera *Vibrio* spp. that contain the toxin gene, are often present in the environment.

To better operationalize the utility of direct PCR, it would be necessary to establish a routine "baseline" or "background" level of PCR targets during non-outbreak periods, per surveillance location, and determine whether appreciable increases in direct PCR targets are observed during an outbreak and that these are correlated with clinical cases. Therefore, at this stage, multiple genes and sequence confirmation from an isolate is preferred for toxigenic *V. cholerae* and cholera of epidemic concern. However, in locations where toxigenic and virulence genes are not typically detected, the finding of such genes can be of public health interest and trigger follow up investigative testing. There may be value in some settings in testing for targets beyond those used for clinical detection (e.g., pathogenicity island genes).

5.3. Reporting and communications

WES data are most useful when used with other data.

WES results for pathogenic *V. cholerae* are more easily interpreted in effective sanitation systems receiving only or majority human feces (e.g. sewered wastewater systems), and less easily interpreted in environmental waters as they can contain high levels of genetically similar vibrio species.

5.4. Acceptability of WES for V. cholerae

As a pooled population sample, individuals are not identified in WES. There does not appear to be any specific acceptability or ethical concerns raised by population-level WES for *V. cholerae* or other gastrointestinal pathogens. However, the emotive and concerning nature of cholera outbreaks may result in fear, stigma, and economic consequences for areas that test positive for *V. cholerae*. Given these sensitivities around reporting cholera cases in some locations, integrating WES and clinical cholera data will require close, trusted partnerships between the WES and clinical data generators. Cross-cutting ethical issues are discussed in the WES overview document.

6. Integrated surveillance and multitarget WES considerations

6.1. Integration of *V. cholerae* WES into existing *V. cholerae* surveillance and response

- There is limited operational experience with integrating WES for *V. cholerae* into integrated surveillance and this remains a research gap.
- Improved integration of data management, sharing and bioinformatics, could enable more timely access and ease of interpretation for WES together with other relevant information for public health action.

6.2. Integration of multi-target WES surveillance together with *V. cholerae*

- There is limited operational experience of integrating WES for V. cholerae into multi-target WES surveillance and this remains a research gap.
- The likely geographical priority areas for WES for *V. cholerae* overlap with those for poliovirus WES. Furthermore, both pathogens are vaccine-preventable (albeit immunity is comparatively short-lived (3-5 years with two doses) compared with polio vaccination), and have their incidence strongly related to gaps in WASH coverage. Therefore, there may be opportunities to leverage and integrate the two pathogens for WES programs. However, the frequency of sampling for WES to provide early warning of cholera outbreaks would be at least weekly due to the rapid spread of the disease in areas where local circulation becomes established.
- Both liquid grab and passive trap samples can be used for V. cholerae WES, and thus, V. cholerae WES workflows should be able to be integrated to some extent with WES for other pathogens.
- Further research is required to understand what other pathogens can be concentrated alongside V. cholerae, and how assays to detect/sequence V. cholerae from WES samples can be multiplexed with assays for other pathogens.

7. Key knowledge gaps and applied research priorities

There are several applied research priorities to advance actionable application of WES for Cholera. Key knowledge gaps and recommended areas of applied research include:

- Feasibility and public health applications in contexts not well studied such as lower-income countries and non-sewered settings, e.g. the wide variety of non-sewered, low-middle income, settings from which population-level sampling is challenging.
- Strategies to design WES with assays that target toxigenic V. cholerae O1 (and O139) serotypes that would eliminate the interfering influence of environmental V. cholerae and other vibrios, not shed by infected persons but present in the environment, or amplifying in the environment after being shed, to obviate challenges in relating results from WES to infection in populations. Good proof-of-concept studies of the public health value of WES for early warning of cholera should address this question.
- Value of direct molecular analyses rather than first culturing *V. cholerae*, both in relation to sensitivity and noting that the virulence factors may be dispersed between separate bacterial populations rather than within any one cell.
- Context-specific value addition of routine and agile WES to current cholera surveillance priorities considering environmental dissemination.
- Resource requirements for initiation and maintenance of routine and agile WES for *V. cholerae*.
- Combining with other targets, particularly if culture-based methods are first required for *V. cholerae* but not for other targets.

References

General information:

The general information on cholera was drawn from CDC and WHO open-source guidance, which should be consulted for the most current approved summary of the evidence:

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