SURVEILLANCE-LABORATORY WORKING GROUP / WASH WORKING GROUP

GLOBAL TASK FORCE ON CHOLERA CONTROL

Technical Note
Environmental Surveillance for Cholera Control
October 2022

Summary of minimal environmental testing strategy

During a suspected or confirmed cholera outbreak, the primary objective of environmental testing is to determine if sources of drinking water (surface and stored) are contaminated with feces. If the water source is part of a chlorination system or program, testing of free residual chlorine is also warranted. Thus, two[a] tests are essential:

1. **Free Residual Chlorine (FRC) level testing** in all water sources that are already supposed to be chlorinated. Initial testing must be followed by systematic monitoring during outbreaks. **FRC** levels should be maintained as follows:
   - ✓ **0.5 mg/L** at all points in the supply chain;
   - ✓ **1 mg/L** at stand-posts and wells;
   - ✓ **2.0 mg/L** in tanker trucks at filling point.
2. **Fecal indicator bacteria (FIB)** (*Escherichia coli*, thermotolerant coliforms) testing for closed water sources that are not regularly chlorinated (i.e., boreholes, protected wells, etc.). Results should be as follows:
   - ✓ **Zero FIB** detected in any 100 mL sample of water that is directly intended for drinking.

For open water sources and sources with low or no FRC, assume contamination (requiring appropriate chlorination). FIB testing in these situations is not essential but may still be used as a secondary test if time and resources permit.

Chlorine dosing (with a contact time of at least 30 minutes) should be performed as follows:
- ✓ **2 mg/L** to clear water (<10 nephelometric turbidity units (NTU))
- ✓ **4 mg/L** to turbid water (>10 NTU but <20 NTU)

Context-specific testing and treatment strategies:

- ***High risk settings*** (refugee camps, natural disasters, etc.)
  Objective: outbreak prevention
  - ✓ Presume fecal contamination and chlorinate immediately while initiating systematic monitoring of FRC levels for the duration of the exposure risk.
  - ✓ Initiate microbiological indicator testing (thermotolerant coliforms (preferred), *E. coli*, and/or fecal streptococci) in water sources deemed safe and supplied for domestic use. If positive, chlorinate immediately.
- ***Active cholera outbreaks***
  Objective: outbreak mitigation, prevention of extension
  - ✓ Monitor FRC levels throughout the entire water supply chain (sources, distribution, collection and points of use).
  - ✓ Test for microbiological indicators (thermotolerant coliforms) at the points of water distribution to individuals or shared water supplies. If positive, chlorinate immediately at points of distribution or stored water and perform regular FRC level assessments.
- ***Between outbreaks and for long-term control interventions***
  Objective: monitoring of efficiency of water service delivery and thereby ensuring supply of safe water for consumption
  - ✓ Test for microbiological indicators and/or FRC levels in the drinking water network. If FIB testing results are positive or FRC levels are below recommended values, chlorinate immediately.

To date, environmental surveillance for *Vibrio cholerae* has been used neither routinely to generate epidemiological alerts anticipating cholera epidemics nor has it been used to track the evolution of outbreaks.

[a] Effective dosage of chlorine may be affected by water turbidity. Nephelometric testing may be needed prior to dose determination.

Objective

To provide guidance to Ministries of Health and other public health actors on the essential environmental surveillance testing for prevention, control, and monitoring of cholera.

Cholera and environment

Epidemic cholera is a diarrheal disease transmitted through the ingestion of an infectious dose of the bacterium *Vibrio cholerae* of O1, and more rarely O139, serogroups. The individual infectious dose varies widely and generally ranges from 10^4 to 10^8 organisms, depending on several host factors. Ingestion can occur directly due to lack of personal or domestic hygiene (i.e., hand washing) or through the consumption of contaminated water or food.

There are over 200 serogroups of *V. cholerae*, several of which can cause sporadic cases of diarrhea or local collective foodborne illness, but do not create widespread epidemics. Among *V. cholerae* of the O1 serogroup, only certain populations of strains, producing the cholera toxin and belonging to the Seventh Pandemic El Tor lineage (7PET), are responsible for current cholera epidemics and the seventh pandemic. Other local strains of *V. cholerae* O1 El Tor, although toxigenic, do not contain all the virulence elements of the Seventh Pandemic strains and are not associated with widespread outbreaks; these strains belong to non-7PET lineages[1]. Cholera toxin-producing *V. cholerae* serogroup O139, which emerged in Asia in 1992, caused major epidemics in some Asian countries but is now rarely isolated[2].

V. cholerae spreads through water contaminated with feces. The aquatic environment, especially surface waters and stored drinking water, is a key component of the cholera transmission chain. Studies have demonstrated that *V. cholerae* can be present in drinking water stored in the houses of cholera patients and a program encouraging the treatment of this water with chlorine tablets demonstrated reduced household transmission of cholera[3,4]. Several examples point to the risk of transmission of cholera through river water or water flows recently contaminated with infected fecal matter (for example, through open defecation, latrine leakage, floods, or river overflows). In Haiti, cholera broke out in the Artibonite river basin in 2010, likely from upstream inadequate sewage disposal[5]. Resurgence of cholera has also been associated with natural events such as hurricane Matthew in Haiti in 2016[6], as well as with seasonality, for example in Ecuador in 1998[7]. In the African region of the Great Lakes, some authors have pointed out intensification in cholera outbreaks associated with heavy rainfall during epidemic periods[8].

V. cholerae can be found in aquatic biotopes such as brackish waters of river estuaries either as free-swimming bacilli or associated with other organisms such as zooplankton (e.g., copepods), cyanobacteria, phytoplankton, water hyacinths, alga protozoa, crabs, bivalves, etc[9]. It can also be isolated from intestines of fish, dolphins and aquatic birds[10]. However, the role of these biotopes as a persistent reservoir of current pandemic *V. cholerae* (O1 El Tor) strains and as a generator of epidemics has never been formally demonstrated, including in Africa[11]. Genomic analyses are in favor of a human-to-human transmission of this disease: several studies using whole genome sequencing of the strains implicated in recent cholera epidemics have all strengthened the hypothesis that epidemic spread and reemergence are due to pandemic *V. cholerae* El Tor strains brought by travelers[12-14]. Moreover, genomic studies conducted on a global scale have shown that all of the explosive epidemics in Africa and the Americas since the beginning of the 7th cholera pandemic arose after the arrival of new strains that had evolved in Asia[15,16]. In areas where cholera cases are recorded throughout the year, like parts of South Asia and Haiti between 2010 and 2019[17-20], it is impossible to know whether the presence of *V. cholerae* in the aquatic environment is the result of contamination by the stools of cholera patients or whether it reflects the permanent presence of the bacterium in the environment that infects the first patients.

To date, environmental surveillance for *V. cholerae* has neither been used routinely to generate epidemiological alerts anticipating cholera epidemics, nor has it been used to track the evolution of outbreaks. Instead, epidemics are detected by identifying the pathogen in patients' stools and then tracked through counting suspected cases and deaths with a subset of them being microbiologically confirmed. The presence of toxigenic *V. cholerae* in the environment does not necessarily indicate that clinical cases are still occurring. The absence of toxigenic *V. cholerae* in a specific water source at a specific time may not be indicative of other locations and other times, thus making environmental sampling results difficult to interpret for public health action. The surveillance of *V. cholerae* in the environment as a practical public health tool to address the risk of cholera transmission is, in most circumstances, neither useful nor necessary. There is no need to test specifically for *V. cholerae* in environmental samples, including in food or drinking water.

The primary exception would be in contexts where there is no recent or historical evidence of cholera, or as part of a food or water safety investigation when the epidemiologic description points to an outbreak from a single source where removal of that source would likely control the outbreak. Another exception would be in the scope of research projects, aiming at better understanding the role of the environment in the resurgence of cholera in endemic countries, or in areas where natural events such as hurricanes occur, as well as studies to better characterize the linkages between the human-to-human and human-to-environment cycles and how they may interact.

Simplified approach to environmental testing for public health response in cholera-risk settings

It is critical to monitor fecal contamination of all sources of drinking water, regardless of the source (e.g., urban water supply networks, water trucking, private sellers, boreholes). Preferred indicators of fecal contamination include thermotolerant coliforms, *Escherichia coli*, and fecal streptococci. Surveillance of those indicators is simpler to implement and more effective in characterizing an overall transmission risk of fecal pathogens compared to direct searches for *V. cholerae*. Particularly, in the context of an ongoing epidemic, evidence of fecal contamination of a drinking water source indicates a risk of cholera transmission, even if *V. cholerae* has not been found in the specific sample analyzed.

During cholera epidemics or in settings with a high risk of cholera, the objective is to ensure that at-risk or affected populations have access to safe, potable water in sufficient quantity. Identification of specific pathogens is usually of secondary importance. Only two tests are essential in this context:

- Free Residual Chlorine (FRC)[21-23] level testing in all water sources that are already supposed to be chlorinated. Initial testing must be followed by systematic monitoring during outbreaks.

  Free chlorine residuals should be maintained as follows in an outbreak setting: 0.5 mg/L at all points in the supply chain; 1.0 mg/L at standposts and wells; 2.0 mg/L in tanker trucks at filling points.

  Note: Effective dosage of chlorine may be affected by water turbidity. Recommendations are to dose with free chlorine at about 2 mg/L to clear water (<10 nephelometric turbidity units (NTU)) and twice that i.e. 4 mg/L to turbid water (>10 NTU but <20 NTU)[b], with a contact time of at least 30 minutes. However, even low turbidity water can have high chlorine demand due to the total organic carbon load that is not detected by nephelometric testing. Regular testing of FRC and dose adjustment is essential.

- Fecal indicator bacteria (FIB) (*Escherichia coli*, thermotolerant coliforms) testing is mainly reserved for closed water sources that are not regularly chlorinated (i.e., boreholes, protected wells, etc.). For open water sources and those sources with low or no FRC, it should be assumed that the sources are contaminated and therefore

[b] Water pH and temperature may also affect chlorine requirement, but to a significantly lesser degree than total organic carbon. Again, regular testing for free residual chlorine is necessary.

require appropriate chlorination. FIB testing in these situations is not essential but may still be used as a secondary test if time and resources permit.

The World Health Organization (WHO) guidelines for drinking water quality indicate that FIB should not be detected in any 100 mL sample of water that is directly intended for drinking. In the case of an active outbreak, any drinking water should be made safe for consumption by adequate chlorination.

For common water-borne diarrheal pathogens, insuring that drinking water is safe[c] relies on i) immediate treatment (i.e., chlorination) while testing for FRC levels[d] or ii) assuring that the water is free of fecal contamination (i.e., by testing for fecal indicator bacteria). These measures must be followed by regular testing to confirm adequate chlorine levels and/or absence of fecal indicator bacteria is maintained.

For testing methodology, please refer to WHO Guidelines for drinking-water quality.

- **High risk settings (refugee camps, natural disasters, etc.)**
  - Objective: outbreak prevention
  - Following site assessment:
    - Presume fecal contamination and chlorinate immediately while initiating systematic monitoring of FRC levels for the duration of the exposure risk.
    - Initiate microbiological indicator testing (thermotolerant coliforms (preferred), *E. coli*, and/or fecal streptococci) in water sources deemed safe and supplied for domestic use. If positive, chlorinate immediately.

- **Active cholera outbreaks:**
  - Objective: outbreak mitigation, prevention of extension
    - Monitor free residual chlorine (FRC) throughout the entire water supply chain (sources, distribution, collection and point of use)[24].
    - Test for microbiological indicator (thermotolerant coliforms) at the points of water distribution to individuals or shared water supplies. If positive, chlorinate immediately at points of distribution or stored water and perform regular FRC level assessments.

- **Between outbreaks and for long-term control interventions:**
  - Objective: monitoring of efficiency of water service delivery and thereby ensuring supply of safe water for consumption
    - Test for microbiological indicators and/or FRC levels in the drinking water network. If FIB testing results are positive or FRC levels are below recommended values, chlorinate immediately.

Monitoring environmental presence of *V. cholerae*

To date, sampling of *V. cholerae* in environmental and/or sewage water has been used in different contexts applying various methods[24-30]. These methods can be used to alert authorities to the presence or increased concentration of *V. cholerae*, though they are not easy to implement and are often costly. *V. cholerae* testing has been used in surface waters, wells and boreholes, home water stores and even in sewage discharge.

[c] Safe drinking water must also be free of elevated levels of toxic chemicals at all times. Testing for chemical contamination (arsenic, lead, etc.) is beyond the scope of this document

[d] Water turbidity can affect chlorine requirements. Chlorination adapted to resulting FRC levels done on sequential water samples

The current methods of identification of *V. cholerae* in the environment are based on the isolation and identification of strains of *V. cholerae* in water samples by culture. Some authors have recommended the filtration of a large volume of water per sample (up to 30L)[28, 30], while others propose to start with an enrichment step by adding alkaline peptone water to the sample, making samples easier to handle but preventing the quantification of *V. cholerae*[30, 31]. In all cases, samples should be sent as quickly as possible to the laboratory and should not be frozen during transport[28, 30]. To distinguish non-toxigenic environmental strains from toxigenic strains circulating during an outbreak, identification should include O1 serogroup determination and be completed by Polymerase Chain Reaction (PCR) identification of the cholera toxin genes[27, 32, 33] or by the enzymatic method[30].

Beyond the technical difficulties of detecting epidemic strains of *V. cholerae*, there is the issue of interpretation and whether the water sources in which *V. cholerae* was detected acted as an original and on-going infectious source or was simply contaminated recently due to cholera transmission. In addition, water testing for specific pathogens provides only a snapshot of the microbes' presence on the day of sampling. False-negative cholera tests may minimize the need to secure the water source and any positive test could be an insignificant finding or misinterpreted.

In summary, during an outbreak, water sources must be safe to drink. An adequate assessment for water safety can be accomplished by measuring residual free chlorine levels and/or testing for fecal coliforms. Additional testing for *V. cholerae* adds little to the public health objective of ensuring availability of safe water.

Specific testing for *V. cholerae* in water sources and the environment would be reasonable for research purposes, such as:

The main objective of identifying and/or monitoring for toxigenic *V. cholerae* of the Seventh Pandemic Lineage is to support research efforts that broaden our basic understanding of the biology, life cycle, and role and interaction of pandemic and non-pandemic *V. cholerae* strains, including how new pandemic strains might arise. A priority research agenda would include the following:

- The study of aquatic ecosystems and the dynamic interactions that govern population balance between bacteria, phages, and their biome to investigate their role in the promotion and maintenance of distinct cholera strains that go on to affect Asia, Africa and the Americas[34].
- A systematic search for presence or absence of pandemic *V. cholerae* strains in between outbreaks to understand if they persist or not in aquatic reservoirs and contribute to new outbreaks, if they constitute an infectious dose for humans in free water and/or concentrate into an infectious source in food products harvested from the environments for human consumption (fish, shellfish), and determine if their isolation has predictive value in an imminent outbreak.
- Improving knowledge of the public health impact of evolving aquatic ecosystems under the influences of human activity, globalization, and climate change, which could inform policies on land and aquatic habitat management and exploitation.
- The tracking of current pandemic *V. cholerae* strains in the aquatic environment over time in countries that are working toward or have achieved sustained elimination, to determine whether aquatic persistence signals on-going cholera transmission in humans that falls below the detection of the public health surveillance system.
- Investigating the role of non-pandemic strains of *V. cholerae* O1 and non O1-non O139 strains in diarrheal disease outbreaks that may confound the true epidemiology of the pandemic strain.
- The development of new specific, effective and cost-effective methodology for the detection of *V. cholerae* in targeted environmental settings.

Given the above agenda items, it is vital that investigations utilize methods that genotypically distinguish current pandemic O1 from non-pandemic O1 and non O1-non O139 strains and that those strains are clearly reported as such.

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