Wastewater and Environmental Surveillance Summary for Typhoid and Paratyphoid

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World Health Organization

This document provides information on wastewater and environmental surveillance (WES) for *Salmonella enterica* serovars Typhi (*S*. Typhi) and Paratyphi A and B (*S*. Paratyphi A and B). Other *Salmonella* spp. and serovars are not covered. 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 World Health Organization (WHO), United States Centers for Disease Control and Prevention (US-CDC), the Coalition Against Typhoid (CaT) and the Typhoid Vaccine Acceleration Consortium (TyVAC), current at the time of writing.

WES for Typhoid and Paratyphoid at a glance

- Overall, there is inadequate evidence to determine the optimal contribution of WES to typhoid (and paratyphoid) disease surveillance and response. Multiple research studies are underway.
- Periodic updates are required to integrate emerging evidence.

Table 1: At a glance assessment of key WES criteria for S. Typhi and S. Paratyphi A and B (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

- *S.* Typhi and *S.* Paratyphi A and B are human pathogens which cause enteric fever and are of **ongoing global health importance** with substantial disease burden in endemic and outbreak prone settings where access to improved water, sanitation and hygiene (WASH) is poor.
- Multidrug resistance is widespread and extreme drug resistance has emerged, limiting therapeutic choices and resulting in increased clinical complications and program costs.
- Transmission is principally by fecally-contaminated water or food. There is no zoonotic source.
- Fecal shedding can occur at high levels in acute infection. A small proportion (2-5%) of infected persons have chronic carriage in the gall bladder with intermittent long-term shedding.
- WHO recommends facility-based **public health surveillance** in endemic settings, as a minimum, with suspected cases laboratory-confirmed through culture or molecular methods.
- There is a need for improved surveillance to define disease burden and antimicrobial resistance patterns. Such evidence would directly inform vaccination strategies, WASH priorities and access to GAVI support for typhoid conjugated vaccine for eligible countries.
- There is no current use of WES for enteric fever at scale. However, there is recognized potential for WES to cost-effectively address current gaps and strengthen surveillance.
- Routine WES is not recommended for S. Typhi or Paratyphi A and B at the present time.
- Clear research priorities, and an active coordinated research portfolio, can provide additional evidence in relation to the value of WES, which will in turn feed into regular review and updates of this document.
- However, routine WES could potentially complement and strengthen existing surveillance drawing on research underway to investigate use cases to identify local circulation and disease burden and inform country prioritization for vaccine introduction. Other potential uses cases are improved characterization of antimicrobial resistance emergence and spread and detection of importation of the pathogen through testing at ports of entry.
- Agile (non-routine) WES response to outbreaks may not add value given the relatively slow spread of typical S. Typhi and S. Paratyphi A and B outbreaks and well-established use of environmental monitoring of water sources to assess risk of fecal contamination. Time-limited use for WES may have potential in the context of vaccine introduction, to help assess the change of burden of disease before and after introduction of the vaccine.

Contents

1. General information

1.1. The pathogens and associated disease

Salmonella enterica subspecies *enterica* is a species of Gram-negative bacteria, of which some serovars – most notably Typhi and Paratyphi A and B – are highly pathogenic, causing enteric fever, typhoid fever and paratyphoid fever respectively. Symptomatic acute infections result in non-specific symptoms including fever, headache and malaise within 7-14 days of infection. Infections may also be mild or asymptomatic [1,2]. Severe disease includes intestinal perforation and death, with a case fatality rate of approximately 1% [3]. Chronic carriage of *S*. Typhi serovar with colonization of the gallbladder occurs in approximately 2-5% after acute infection, with higher incidence in older women and with an increased risk of hepatobiliary cancer [4,5].

1.2. Global burden, geographic distribution and risk factors

Despite a progressive reduction in incidence and disease burden, typhoid and paratyphoid fevers remain of concern, particularly in areas with poor access to adequate sanitation and safe water and food. Enteric fever is endemic and causes outbreaks in multiple African and South Asian countries and island nations in Oceania. Children are at highest risk of infection and death. Further progress is threatened by the rapid global spread of multiple drug resistance and emergence of extreme drug resistance in Pakistan [6,7]. Virtual elimination of locally transmitted *S.* Typhi and *S*. Paratyphi A and B has been achieved in countries with good WASH coverage. In such contexts, cases are imported from countries with ongoing local circulation or from local cases with chronic carriage.

In 2017, there were an estimated 14.3 million enteric fever cases (including 10.9 million (76%) with typhoid fever and 3.4 million (24%) with paratyphoid fever), associated with 135,900 deaths and a loss of 9.8 million Disability Adjusted Life Years [8]. However, estimates of infection incidence vary widely by region and are likely under-estimated by the lack of reliable data, particularly from sub-Saharan Africa. There are various approaches to improve disease burden estimates [2,8–12]. Passive surveillance is incomplete, as all asymptomatic and many symptomatic cases do not seek clinical testing and widely available rapid diagnostic tests have poor sensitivity and/or specificity. Recommended microbiological testing with blood culture has high specificity, but only 40-60% sensitivity (lower in the presence of antibiotics) and are expensive and not widely accessible [13].

Environmental risk is principally related to the lack of access to improved sanitation, safe water and safe food handling practices, particularly in densely populated areas near open wells or sewers. Climate change and seasonal factors such as rainfall and temperature also influence risk, through increased rainfall and flooding, as well as lack of rain and agricultural use of recycled wastewater in different contexts [14].

1.3. Hosts and routes of transmission

S. Typhi and *S*. Paratyphi are human pathogens and are transmitted through fecally-contaminated water and food or less commonly direct human-to-human or via other contaminated objects. These pathogens are not spread via animal or other hosts or via environmental amplification.

2. S. Typhi and S. Paratyphi and wastewater and environmental waters

2.1. Potential inputs to wastewater and environmental waters

S. Typhi and *S*. Paratyphi A and B are shed predominantly in the feces of infected humans, while urinary shedding also occurs. Zoonotic non-human sources are not significant. *Salmonella* Typhi shedding occurs after clinical or subclinical acute infection, with wide variability in shedding patterns between individuals in terms of timing, duration and peak [15,16]. Shedding may start as early as three days after infection and is usually self-limited within a few weeks. However, chronic carriage of S Typhi in the gall bladder with intermittent shedding over many years occurs in 2-5% of individuals . Acute and chronic carrier-shedding patterns are not well characterized in the context of dynamic antimicrobial resistance and antimicrobial and vaccine use [1]. As local elimination is close to being achieved chronic shedders become the main source of input.

2.2. Target persistence and degradation in wastewater

S. Typhi and *S*. Paratyphi A and B are fastidious organisms and are difficult to culture. While there has been extensive documentation of *S*. Typhi detection in contaminated water sources, there is limited evidence to evaluate its persistence and the factors determining the rate of degradation in wastewater and other environmental samples. *S*. Typhi is highly sensitive to chlorine. In the absence of chlorine, infectious bacteria and biomarkers are moderately persistent over a period of days to weeks in a variety of aqueous media including water, wastewater, fecal sludge, and environmental waters [22,23]. *S*. Typhi shows adaptive changes to survive in water as well as prolonged persistence in the presence of amoebae, suggesting complex biological interactions [24,25].

2.3. *S.* Typhi and *S.* Paratyphi WES experience

To date, there has been no at-scale use of WES for *S.* Typhi and/or *S.* Paratyphi.

WES is considered as a potential surveillance tool to help fill the current substantial enteric fever surveillance gaps. Specific WES-related research priorities have been identified and a substantive research agenda initiated [26]. Research studies are underway at the time of writing in settings with varying rates of *S.* Typhi and antimicrobial resistance incidence and in different phases of conjugate vaccine implementation; including in Bangladesh, Fiji, Ghana, India, Indonesia, Malawi and Nigeria. These studies are providing data on environmental prevalence of *S*. Typhi and assessing correlation with available population-level estimates of disease burden. This data complements other research efforts to improve surveillance and disease burden estimates [27]. In addition the WES research studies are evaluating methods for sampling, sample site validation, and analysis, and interpretation [21,28,29].

3. S. Typhi and S. Paratyphi surveillance

3.1. Overall *S.* Typhi and *S.* Paratyphi surveillance and response

Control of the transmission of *S.* Typhi and *S.* Paratyphi A and B is focused on prevention, including safe water, sanitation, and hand and food hygiene (WASH) practices, and vaccination with typhoid conjugate vaccine in countries with a highest burden of typhoid disease or high burden of antimicrobial resistant *S.* Typhi, as recommended by WHO (WHO, 2018b). Gavi, the Vaccine Alliance can provide support for TCV introduction in eligible countries [30]. A vaccine is under development for *S.* Paratyphi A. Diagnosis and treatment of acute and chronic carrier cases with judicious use of appropriate antimicrobials in the context of rapidly evolving antimicrobial resistance is an important therapeutic pillar [31].

The goal of public health surveillance [32] is to:

- Determine the epidemiology and disease burden for typhoid fever and paratyphoid fever to facilitate and support control strategies.
- Facilitate the rapid detection of outbreaks and response to outbreaks.
- Guide the promotion of WASH interventions, the introduction of vaccination programs, or other control strategies, in a country, given significant heterogeneity in disease burden across geographic areas and populations.
- Monitor impact of interventions on disease and potential changes in epidemiology.
- Monitor antimicrobial resistance patterns among *Salmonella* isolates, which can inform treatment practices and, in some instances, the need for vaccination campaigns.
- In non-endemic settings, identify imported cases among returned travelers or migrants (which can provide indirect measures of risk in countries visited) and support pre-travel vaccine advice or contact tracing as needed.

3.2. Existing surveillance systems and data sources

The mainstay of acute *S.* Typhi and *S.* Paratyphi surveillance is passive clinical surveillance based on blood culture results. Other non-specific clinical surveillance provides additional data including syndromic surveillance, hospitalizations, mortality as well as non-traumatic intestinal perforations. Special studies include active sentinel surveillance, serosurveys and multifaceted research studies with modelling. Surveillance for chronic carriers may include *S.* Typhi testing during gall bladder removal.

S. Typhi and *S.* Paratyphi A antimicrobial resistance emergence and geographic spread is monitored through the Global Typhoid Genomics Consortium (GTGC) and other collaborative partnerships [6,7].

Conventional clinical testing for acute *S.* Typhi and *S.* Paratyphi A and B infections involves selecting an isolated single suspect colony from a blood culture. Then advanced tests can be applied, such as antimicrobial susceptibility testing, or biochemical or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to determine species. Further genetic analysis using qPCR or sequencing can distinguish *S.* serovars.

As noted, clinical surveillance based on blood cultures has several substantive limitations, with known under-reporting of disease incidence and very wide regional variations:

- Lack of timely blood culture
  - Persons with asymptomatic and mild acute infections do not seek diagnostic testing

  - Many infected persons will not seek testing as not accessible and affordable and preferentially choose to access over-the-counter medications.
  - Blood culture capacity is not available noting limited capacity in many settings
  - A blood culture may not be ordered: blood culture is expensive and not widely available while enteric fever presents with non-specific symptoms. Use of cheaper, non-sensitive and non-specific Widal serologic tests and other rapid diagnostics remains widespread.

- Lack of timely result
  - It takes time from infection, to symptom onset, sample collection, blood culture result, and reporting of results and action by public health authorities, during which time the infected person can infect others.

- Lack of accurate result with false negative result
  - Poor sensitivity of approximately 40–60%
  - Lower in infants and young children due to their smaller blood volumes (and difficulty in taking blood samples)
  - Lower if masked by prior antibiotics

4. WES objectives and related public health actions

Note, WES is always considered in the context of local, multi-modal surveillance. WES should be integrated to complement other data and 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 *S.* Typhi and *S.* Paratyphi

Routine WES is not recommended for *S.* Typhi or Paratyphi A and B at the present time.

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

Routine WES for *S.* Typhi and *S.* Paratyphi A and B could potentially complement and strengthen existing surveillance modalities**.** Research is underway to investigate the use cases described below [33].

- **Identification of local circulation and disease burden**
    - Consistent detection of the pathogens from WES showing geographic distribution over time could potentially be used with other data to improve disease burden estimates and inform country prioritization for vaccine introduction as well as eligibility for GAVI support in addition to WASH promotion and other interventions.
    - This can also be used to monitor success of intervention strategies including at different phases of local elimination from highly endemic to when only chronic carriers remain and after introduction of conjugate typhoid vaccines.

Other potential use cases:

- **Improved characterization of antimicrobial resistance emergence and spread**
    - Phenotypic antimicrobial susceptibility testing and genomic characterization of existing and/or emerging antimicrobial resistance genes or profiles of interest such as those associated with S. Typhi multidrug resistance (MDR) and extreme drug resistance (XDR). Use of targeted or extended sequencing methods could potentially be used to triangulate with existing genomic data and improve global coverage (noting inequitable coverage of existing genomic data with low coverage in west and central Africa and pacific island nations).
- **Detection of importation of the pathogen** through testing at ports of entry (airports or land and sea border crossings). This includes detection of importation of the pathogen, as well as of importation of new strains through sequencing analysis.

4.2. Agile WES *S.* Typhi and *S.* Paratyphi

Use of WES for agile (non-routine) response to outbreaks may not add value given the relatively slow spread of typical *S*. Typhi and *S*. Paratyphi A and B outbreaks and well-established use of environmental monitoring of water sources for residual chlorine to assess adequacy of water chlorination and for coliforms to assess fecal contamination. Further clinical samples would likely provide antimicrobial resistance patterns in a clonal outbreak.

Agile WES means that it is time-limited surveillance with a specific trigger to initiate. 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 or different analyses.

One time-limited use for WES may be in the context of vaccine introduction, to help assess the change of burden of disease before and after introduction of the vaccine. This use case is being evaluated in Fiji as part of a multimodal research study.

4.3. Potential public health actions from use of WES for *S.* Typhi and *S.* Paratyphi

If information provided by WES could assist to estimate disease burden and/or antimicrobial resistance of concern this would meet a key typhoid surveillance need (addressing the gap associated with weak blood culture-based surveillance). Improved surveillance is particularly important given WHO has recommended the introduction of typhoid conjugate vaccine (TCV) to be prioritized in countries with the highest burden of typhoid disease or a high burden of antimicrobial resistance among isolates [31]. Countries require information to prioritize which vaccines to include in routine immunization while GAVI requires evidence in order to provide financial support to low-income countries to access the TCV (Gavi 2019). Thus there is an urgent need for improved data on disease burden particularly in low-income settings.

Other actions include the targeting and evaluation of WASH, vaccination, and other interventions, and in the case of AMR, providing evidence to inform antibiotic recommendations and improved antimicrobial stewardship.

5. WES additional methodological considerations for *S.* Typhi and *S.* Paratyphi

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 *S.* Typhi and *S*. Paratyphi A and B and no established process or guidance. However, several reviews have been published[34–36], as well as an estimate of the costs of setting up a program, and how those costs scale with implementation[37]. There is no standard protocol for WES detection of *S.* Typhi and *S*. Paratyphi A and B, but current research studies are underway in multiple regions[38] to help guide the development of normative documents that are in draft stage.

5.1. Sampling methods

There are no special considerations for sampling beyond those used for conventional microbiological sampling for environmental monitoring and WES. Conventional grab samples, composite samples, and passive/trap samples, have all been successfully utilized[39].

Due to the intermittent nature of shedding (in part being linked to the use of toilets), the deployment of passive/trap samples to accumulate the target over time has been the preferred method of sampling. Compared to grab samples, it likely has better sensitivity from wastewater or environmental water where shedding is occurring at relatively low levels and is feasible for field deployment, unlike automated composite sampling[35,40].

5.2. Laboratory methods analysis

Standardized laboratory methods for detection and characterization of *S.* Typhi and *S.* Paratyphi in WES samples are needed. In the absence of standard methods, the choice of methods selected for each WES application depends on the context and information sought[33]. Consideration should be given to the method specificity and sensitivity for concentrations of pathogens anticipated in the context and in relation to the surveillance objective. This requires consideration of want information is sought: e.g. concentrations, presence of specific serovars, sequence types, and/or antimicrobial resistance profiles. Practical considerations include the capability and capacity of the analytical facility and human resources as well as available resources[20,21].

The purpose of WES testing is to identify shedding of the pathogen into wastewater or environmental water and information on pathogen viability isn't necessary. Nevertheless, WES testing for *S.* Typhi and *S*. Paratyphi A and B, as with clinical testing, can involve culture-based testing which has benefits for identification (e.g., via subsequent traditional typing methods) or assessment of antimicrobial resistance phenotypes. However, a recent study showed that methods involving cultivation of *S*. Typhi from the environment have low sensitivity and standardized molecular methods for confirmation of isolates are needed. Biological Safety Level (BSL)-2 is required for processing samples where cultivation of the pathogen is part of the analytical method. Of note, selenite-based media often used for *Salmonella* culture poses high risks to laboratory staff and the environment; this should be considered when selecting a method.

For *S.* Paratyphi, rapid antigen tests have not been demonstrated for environmental testing. Such tests would have lower sensitivity than culture-based and/or molecular tests and are not coupled to allow follow up testing of antimicrobial resistance properties.

In general, the limited sensitivity and poor timeliness of culture-based tests means that highly sensitive and specific qPCR-based tests are often preferred. Culture-independent direct qPCR can also circumvent issues with detection of bacteria that enter a viable but non-culturable state in the environment. To date, there is no one single primer pair that can detect *S.* Typhi or *S.* Paratyphi directly from environmental samples. Because more than one target is required for detection, and these can exist in multiple organisms, a discrete isolate is required for confirmation. Currently, a combination of enrichment of the *S.* Typhi or *S.* Paratyphi target, which provides enhanced sensitivity, followed by molecular analysis of isolates, which provides enhanced specificity, shows the most promise for WES.

When analytical technology and bioinformatics capability are available, genomic sequencing of *S.* Typhi and *S*. Paratyphi A and B isolates from WES samples can permit the sequence types and resistomes in circulation to be characterized. This can assist in identifying clusters to help pinpoint outbreaks, and to inform recommendations on first and later line antimicrobial treatments. More research on sequencing *S.* Typhi and *S*. Paratyphi directly from environmental samples is needed to determine specificity and cost-effectiveness.

5.3. Reporting and communications

Reporting of WES results alongside clinical cases within the routine surveillance system data flow could help to provide integrated and accessible information to key end users. Examples of such systems include the Integrated Disease Surveillance and Response (IDSR) and the Surveillance Outbreak Response Management and Analysis System (SORMAS). Should genomic surveillance from WES be proved useful, reporting should be integrated alongside existing typhoid clinical genomic surveillance data to promote optimal timely use for decision making.

5.4. Acceptability of WES for S. Typhi and S. Paratyphi

General and cross-cutting acceptability or ethical concerns related to population-level WES are covered in the overarching WES Overview document. As a pooled population sample, individuals are not identified in WES. There does not appear to be any specific acceptability concerns raised by population-level WES for *S.* Typhi and *S*. Paratyphi or other gastrointestinal pathogens. However, the emotive and concerning nature of typhoid and paratyphoid outbreaks may result in fear, stigma, and economic consequences for areas that test positive for *S.* Typhi and *S*. Paratyphi, noting that WES has proven its ability to detect locally circulating pathogens in areas not reporting clinical cases [33].

Two inter-related ethical concerns of equity and cost are pertinent given enteric fever burden is concentrated in lower income countries and there is an ethical imperative to provide surveillance tools which are accessible and affordable. WES should be designed to be maximally cost-efficient and their costs and benefits considered including allocative efficiency between surveillance activities and/or disease mitigation measures such as WASH-related interventions and vaccinations.

6. Integrated surveillance and multitarget WES considerations

6.1. Integration of S. Typhi and S. Paratyphi A and B WES into existing enteric fever surveillance and response

- As WES for these pathogens is not yet a proven use case, there is no operational experience with integration of WES into existing enteric fever surveillance and response.
- However the various *S*. Typhi research studies use multimodal data collection and will provide some insights into how future integration can be planned and optimised.

6.2. Integration of multi-target WES surveillance together with S. Typhi or S. Paratyphi

- There is no operational experience of integrating WES for S. Typhi or S. Paratyphi A and B into multi-target WES surveillance. However there are several opportunities which may promote integration in one or more workflows with other targets. For example:
    - The likely geographical priority areas and sampling frequency for WES for enteric fever pathogens overlap with those for poliovirus WES. Furthermore, both pathogens are vaccine-preventable, and their incidence and disease burden correlate with gaps in WASH coverage. Opportunities to leverage existing polio ES for enteric fever pathogens are likely to be substantial (while recognizing some aspects including site location, sampling and laboratory analysis are likely to diverge and remain distinct).
    - Further research is required to understand what other pathogens can be concentrated alongside S. Typhi or S. Paratyphi A and B, and if and how assays to detect/sequence targets of interest from S. Typhi or S. Paratyphi A and B from wastewater can be multiplexed along with assays for other pathogens.
    - The use of sentinel surveillance sites at air travel hubs (airport and/or aircraft) may lend itself to multitarget surveillance to monitor incursion threats including for enteric fever with considerations of current emerging threats and their dynamic epidemiology.

7. Key knowledge gaps and recommendations for applied research priorities

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

Research underway with anticipated results within the next 12 months:

- The context specific, value add of WES to help characterize:
  - incidence of *S*. Typhi infection and relationship to disease burden.
  - changes in pathogen prevalence as a result of typhoid conjugate vaccine introduction.
  - AMR patterns with relevance to S. Typhi and other enteric fever pathogens.
- Feasibility and public health applications in relevant contexts not well studied, such as lower-income countries, non-sewered settings in tropical/sub-tropical climate zones.
- Sensitivity, specificity and other attributes of direct molecular genetic analysis compared with culture-based methods for *S*. Typhi.
- Scalable, standardized, validated methods for both sampling and laboratory analysis for *S*. Typhi.

Additional recommended research priorities:

- Thresholds for action from results.
- Resource requirements for initiation and maintenance of routine and agile WES for *S*. Typhi and *S*. Paratyphi.
- Combining testing with poliovirus and other WES targets and any trade-off considerations.
- Coverage of *S*. Paratyphi A and B as well as *S.* Typhi (noting to date, most WES work has focused on *S*. Typhi).
- Optimal reporting, dashboard and communication approaches – including definition of end users and their needs and tailored communications to meet those needs.

References

General information:

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

- WHO. Typhoid. WHO - Fact Sheets. 2024. Accessed December 3, 2024. https://www.who.int/news-room/fact-sheets/detail/typhoid
- WHO. Paratyphoid fever. WHO - Immunization, Vaccines and Biologicals. 2024. Accessed December 3, 2024. https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/paratyphoid-fever
- CDC - Centers for Disease Control & Prevention. About Typhoid Fever and Paratyphoid Fever. CDC -Typhoid Fever and Paratyphoid Fever. 2024. Accessed December 3, 2024. https://www.cdc.gov/typhoid-fever/about/index.html

Other information on typhoid global initiatives and access to GAVI support including evidence requirements can be found at:

- Coalition Against Typhoid (CaT). Take on Typhoid. CaT - Take on Typhoid. 2018. https://www.coalitionagainsttyphoid.org/
- GAVI. GAVI Vaccine Alliance. GAVI Vaccine Alliance. 2024. Accessed December 3, 2024. https://www.gavi.org/

Generic protocols:

- **Large volume wastewater:** https://www.protocols.io/view/ultrafiltration-methods-for-concentrating-and-dete-buvinw4e
- **Moore swab samples of wastewater:** https://www.protocols.io/view/moore-swab-methods-for-concentrating-and-detecting-x54v9jw3qg3e/v1
- **Environmental water**: https://www.protocols.io/view/protocol-for-detection-of-salmonella-typhi-and-sal-e6nvw54y7vmk/v1

Cited literature follows:

1. Crump JA. Progress in Typhoid Fever Epidemiology. *Clinical Infectious Diseases*. 2019;68(Supplement_1):S4-S9. doi:10.1093/cid/ciy846

2. Aiemjoy K, Seidman JC, Saha S, et al. Estimating typhoid incidence from community-based serosurveys: a multicohort study. *The Lancet Microbe*. 2022;3(8):e578-e587. doi:10.1016/S2666-5247(22)00114-8

3. Marchello CS, Birkhold M, Crump JA. Complications and mortality of typhoid fever: A global systematic review and meta-analysis. *Journal of Infection*. 2020;81(6):902-910. doi:10.1016/j.jinf.2020.10.030

4. Dougan G, Baker S. Salmonella enterica Serovar Typhi and the Pathogenesis of Typhoid Fever. *Annual Review of Microbiology*. 2014;68(Volume 68, 2014):317-336. doi:10.1146/annurev-micro-091313-103739

5. World Health Organisation. Typhoid vaccines: WHO position paper - March 2018. 2018. Accessed November 27, 2024. https://www.who.int/publications/i/item/whio-wer9313

6. Carey ME, Dyson ZA, Ingle DJ, et al. Global diversity and antimicrobial resistance of typhoid fever pathogens: Insights from a meta-analysis of 13,000 Salmonella Typhi genomes. Bonten MJ, van der Meer JW, Bonten MJ, Cowley LA, eds. *eLife*. 2023;12:e85867. doi:10.7554/eLife.85867

7. Browne AJ, Chipeta MG, Fell FJ, et al. Estimating the subnational prevalence of antimicrobial resistant Salmonella enterica serovars Typhi and Paratyphi A infections in 75 endemic countries, 1990–2019: a modelling study. *The Lancet Global Health*. 2024;12(3):e406-e418. doi:10.1016/S2214-109X(23)00585-5

8. Stanaway JD, Reiner RC, Blacker BF, et al. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet Infectious Diseases*. 2019;19(4):369-381. doi:10.1016/S1473-3099(18)30685-6

9. Mogasale V, Maskery B, Ochiai RL, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. *The Lancet Global Health*. 2014;2(10):e570-e580. doi:10.1016/S2214-109X(14)70301-8

10. Antillón M, Warren JL, Crawford FW, et al. The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. *PLOS Neglected Tropical Diseases*. 2017;11(2):e0005376. doi:10.1371/journal.pntd.0005376

11. Stanaway JD, Atuhebwe PL, Luby SP, Crump JA. Assessing the Feasibility of Typhoid Elimination. *Clinical Infectious Diseases*. 2020;71(Supplement_2):S179-S184. doi:10.1093/cid/ciaa585

12. Chen W, Chen Y, Cheng Z, et al. Global patterns of syphilis, gonococcal infection, typhoid fever, paratyphoid fever, diphtheria, pertussis, tetanus, and leprosy from 1990 to 2021: findings from the Global Burden of Disease Study 2021. *Infect Dis Poverty*. 2024;13(1):66. doi:10.1186/s40249-024-01231-2

13. Hancuh M, Walldorf J, Minta AA, et al. Typhoid Fever Surveillance, Incidence Estimates, and Progress Toward Typhoid Conjugate Vaccine Introduction — Worldwide, 2018–2022. *MMWR Morb Mortal Wkly Rep*. 2023;72(7):171-176. doi:10.15585/mmwr.mm7207a2

14. Saad NJ, Lynch VD, Antillón M, Yang C, Crump JA, Pitzer VE. Seasonal dynamics of typhoid and paratyphoid fever. *Sci Rep*. 2018;8(1):6870. doi:10.1038/s41598-018-25234-w

15. Dougan G, Baker S. Salmonella enterica Serovar Typhi and the Pathogenesis of Typhoid Fever. *Annual Review of Microbiology*. 2014;68(Volume 68, 2014):317-336. doi:10.1146/annurev-micro-091313-103739

16. Waddington CS, Darton TC, Jones C, et al. An Outpatient, Ambulant-Design, Controlled Human Infection Model Using Escalating Doses of Salmonella Typhi Challenge Delivered in Sodium Bicarbonate Solution. *Clinical Infectious Diseases*. 2014;58(9):1230-1240. doi:10.1093/cid/ciu078

17. Gonzalez-Escobedo G, Marshall JM, Gunn JS. Chronic and acute infection of the gall bladder by Salmonella Typhi: understanding the carrier state. *Nat Rev Microbiol*. 2011;9(1):9-14. doi:10.1038/nrmicro2490

18. Vogelsang ThM, Bøe J. Temporary and chronic carriers of *Salmonella typhi* and *Salmonella paratyphi* B. *J Hyg*. 1948;46(3):252-261. doi:10.1017/S0022172400036378

19. Gopinath S, Carden S, Monack D. Shedding light on *Salmonella* carriers. *Trends in Microbiology*. 2012;20(7):320-327. doi:10.1016/j.tim.2012.04.004

20. Matrajt G, Naughton B, Bandyopadhyay AS, Meschke JS. A Review of the Most Commonly Used Methods for Sample Collection in Environmental Surveillance of Poliovirus. *Clin Infect Dis*. 2018;67(suppl_1):S90-S97. doi:10.1093/cid/ciy638

21. Matrajt G, Lillis L, Meschke JS. Review of Methods Suitable for Environmental Surveillance of Salmonella Typhi and Paratyphi. *Clinical Infectious Diseases*. 2020;71(Supplement_2):S79-S83. doi:10.1093/cid/ciaa487

22. Guo Y, Sivakumar M, Jiang G. Decay of four enteric pathogens and implications to wastewater-based epidemiology: Effects of temperature and wastewater dilutions. *Science of The Total Environment*. 2022;819:152000. doi:10.1016/j.scitotenv.2021.152000

23. Zhou J, Wang XC, Ji Z, Xu L, Yu Z. Source identification of bacterial and viral pathogens and their survival/fading in the process of wastewater treatment, reclamation, and environmental reuse. *World J Microbiol Biotechnol*. 2015;31(1):109-120. doi:10.1007/s11274-014-1770-5

24. Kingsley RA, Langridge G, Smith SE, et al. Functional analysis of Salmonella Typhi adaptation to survival in water. *Environmental Microbiology*. 2018;20(11):4079-4090. doi:10.1111/1462-2920.14458

25. Douesnard-Malo F, Daigle F. Increased Persistence of Salmonella enterica Serovar Typhi in the Presence of Acanthamoeba castellanii. *Applied and Environmental Microbiology*. 2011;77(21):7640-7646. doi:10.1128/AEM.00699-11

26. Shaw AG, Troman C, Akello JO, et al. Defining a research agenda for environmental wastewater surveillance of pathogens. *Nat Med*. 2023;29(9):2155-2157. doi:10.1038/s41591-023-02457-7

27. Carey ME, MacWright WR, Im J, et al. The Surveillance for Enteric Fever in Asia Project (SEAP), Severe Typhoid Fever Surveillance in Africa (SETA), Surveillance of Enteric Fever in India (SEFI), and Strategic Typhoid Alliance Across Africa and Asia (STRATAA) Population-based Enteric Fever Studies: A Review of Methodological Similarities and Differences. *Clin Infect Dis*. 2020;71(Suppl 2):S102-S110. doi:10.1093/cid/ciaa367

28. Liu P, Ibaraki M, Kapoor R, et al. Development of Moore Swab and Ultrafiltration Concentration and Detection Methods for Salmonella Typhi and Salmonella Paratyphi A in Wastewater and Application in Kolkata, India and Dhaka, Bangladesh. *Front Microbiol*. 2021;12:684094. doi:10.3389/fmicb.2021.684094

29. Uzzell CB, Abraham D, Rigby J, et al. Environmental Surveillance for *Salmonella* Typhi and its Association With Typhoid Fever Incidence in India and Malawi. *The Journal of Infectious Diseases*. 2024;229(4):979-987. doi:10.1093/infdis/jiad427

30. WHO. Recommendations to assure the quality, safety and efficacy of typhoid conjugate vaccines, Annex 2, TRS No 1030. 2020. Accessed November 27, 2024. https://www.who.int/publications/m/item/tcv71-recommendations

31. WHO. *Typhoid Vaccines: WHO Position Paper – March 2018*. World Health Organization; 2018. https://iris.who.int/bitstream/handle/10665/272272/WER9313.pdf?sequence=1

32. WHO. *Typhoid and Other Invasive Salmonellosis*. World Health Organization; 2018. https://cdn.who.int/media/docs/default-source/immunization/vpd_surveillance/vpd-surveillance-standards-publication/who-surveillancevaccinepreventable-21-typhoid-r2.pdf?sfvrsn=993904a6_10&download=true

33. Uzzell CB, Abraham D, Rigby J, et al. Environmental Surveillance for *Salmonella* Typhi and its Association With Typhoid Fever Incidence in India and Malawi. *The Journal of Infectious Diseases*. 2024;229(4):979-987. doi:10.1093/infdis/jiad427

34. Andrews JR, Yu AT, Saha S, et al. Environmental Surveillance as a Tool for Identifying High-risk Settings for Typhoid Transmission. *Clinical Infectious Diseases*. 2020;71(Supplement_2):S71-S78. doi:10.1093/cid/ciaa513

35. Matrajt G, Lillis L, Meschke JS. Review of Methods Suitable for Environmental Surveillance of Salmonella Typhi and Paratyphi. *Clinical Infectious Diseases*. 2020;71(Supplement_2):S79-S83. doi:10.1093/cid/ciaa487

36. Zhou N, Ong A, Fagnant-Sperati C, et al. Evaluation of Sampling and Concentration Methods for Salmonella enterica Serovar Typhi Detection from Wastewater. *The American Journal of Tropical Medicine and Hygiene*. 2023;108(3):482-491. doi:10.4269/ajtmh.22-0427

37. Hagedorn B, Zhou NA, Fagnant-Sperati CS, et al. Estimates of the cost to build a stand-alone environmental surveillance system for typhoid in low- and middle-income countries. Farooqui HH, ed. *PLOS Glob Public Health*. 2023;3(1):e0001074. doi:10.1371/journal.pgph.0001074

38. Uzzell CB, Troman CM, Rigby J, et al. Environmental surveillance for Salmonella Typhi as a tool to estimate the incidence of typhoid fever in low-income populations. *medRxiv*. Published online 2021:2021-05.

39. Liu P, Ibaraki M, Kapoor R, et al. Development of Moore Swab and Ultrafiltration Concentration and Detection Methods for Salmonella Typhi and Salmonella Paratyphi A in Wastewater and Application in Kolkata, India and Dhaka, Bangladesh. *Front Microbiol*. 2021;12:684094. doi:10.3389/fmicb.2021.684094

40. Zhou N, Ong A, Fagnant-Sperati C, et al. Evaluation of Sampling and Concentration Methods for Salmonella enterica Serovar Typhi Detection from Wastewater. *The American Journal of Tropical Medicine and Hygiene*. 2023;108(3):482. doi:10.4269/ajtmh.22-0427