World Health Organization

WHO BacPREP

R&D Roadmap for Priority Bacteria

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R&D Blueprint

Powering research
to prevent epidemics

WHO BacPREP R&D Roadmap

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BacPREP led by:

- London School of Tropical Health and Medicine (LSHTM)
- Aga Khan University
- Amsterdam University Medical Center, Department of Global Health

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I. Introduction

Background

Following the release of the WHO Pathogen Prioritization Report at the 2024 Global Pandemic Preparedness Summit in Rio de Janeiro, the R&D Blueprint is moving forward with a new framework for research preparedness and response. This framework promotes a broad-based research approach across all pathogen families, irrespective of their future epidemic or pandemic potential.

A key action within this strategy is the establishment of independent Collaborative Open Research Consortia (CORCs) for each pathogen family. As this strategy is implemented, WHO is partnering with research institutions worldwide to establish these CORCs, each anchored by a WHO Collaborating Centre. Each CORC serves as a coordinating mechanism to establish unified global R&D agendas for pathogen families. Functioning through a peer-reviewed process, each consortium prioritises research areas critical for the development of medical countermeasures. Its primary role is to advance scientific knowledge and guide research efforts, fostering equitable contributions to ensure inclusive and balanced global collaboration.

The Bacterial Pathogen Research and Emergency Preparedness (BacPREP) CORC was established to bring together a globally representative, multidisciplinary group of researchers to identify key research gaps and conduct supportive activities to accelerate the development and deployment of medical countermeasures directed against priority bacterial pathogens: *Vibrio cholerae*, *Yersinia pestis*, *Shigella spp.*, non-typhoidal *Salmonella enterica* serovars Enteritidis and Typhimurium, and *Klebsiella pneumoniae*.

Purpose

To outline the role of the WHO BacPREP (Bacterial Priority Research and Emergency Preparedness) initiative as part of the WHO R&D Blueprint, supporting accelerated research, development, and deployment of countermeasures for bacterial pathogens with epidemic potential of regional and/or global importance. The roadmap defines the near- and medium-term priorities (3–5 years) to strengthen global R&D preparedness and response capacity, focusing on product development while also considering enabling platforms and readiness activities.

Vision Statement

To accelerate the development of efficacious, programmatically suitable, cost-effective, and rapidly deployable medical countermeasures against priority bacteria for epidemic preparedness and response by 2030.

Scope

This roadmap provides a unified framework for five priority bacterial pathogens: *Yersinia pestis*, *Vibrio cholerae*, *Shigella spp.*, *Klebsiella pneumoniae*, and invasive *Salmonella enterica* serovars Enteritidis and Typhimurium (iNTS). Within this framework, BacPREP focuses on accelerating the development of medical countermeasures for epidemic-prone bacterial pathogens through product development, enabling platforms, and readiness activities. The roadmap integrates pathogen-specific R&D priorities with cross-cutting enablers including surveillance, diagnostics, vaccine development, regulatory science, manufacturing readiness, digital infrastructure, and community engagement. It aims to guide global, regional, and national partners, including WHO, academic institutions, product developers, funders, and implementing agencies, toward a coordinated and sustainable approach for bacterial epidemic preparedness.

II. Pathogen-Specific Research Priorities

A. *Yersinia pestis*

Introduction/Problem statement

Yersinia pestis (*Y. pestis*), the causative agent of plague, remains a persistent global health threat due to the rapid clinical progression of the disease, high case fatality rates (CFR), and continued circulation in animal reservoirs in Africa, Asia, and the Americas. [1, 2] Human infections arise through zoonotic transmission from infected rodents and their fleas or, in the case of pneumonic plague, by direct person-to-person spread, [3] including also the possibility of deliberate release [4]. Following the end of the third pandemic, the number of plague cases has decreased in the second half of the 20th century. [1, 2, 5] Following the end of the third pandemic, the number of plague cases has decreased in the second half of the 20th century. Today, plague occurs in mostly seasonal outbreaks in rural Madagascar and the Democratic Republic of the Congo (DRC), which account for over 90% of all cases worldwide, although periodic cases are reported across the world.

Despite centuries of epidemic and pandemic history, plague has been vastly under-researched, and substantial knowledge gaps impede the development and deployment of effective prevention, treatment, and control tools.

Plague manifests in three major clinical forms, each with distinct implications for detection and response [6]:

- **Bubonic plague**, the most common form, arises from flea-borne transmission and is characterised by painful, swollen lymph nodes (buboes) that are highly indicative of infection in endemic settings. Diagnosis is often made clinically because laboratory confirmation is not readily available in remote areas. Treatment is routinely given based on clinical suspicion.
- **Pneumonic plague** occurs less frequently than bubonic plague and can occur as a primary infection or secondary through progression from bubonic plague. Pneumonic plague is the only form capable of efficient human-to-human transmission. It progresses rapidly with respiratory symptoms and without timely antibiotic treatment, death can occur within 24–72 hours of the onset of severe, hypoxic pneumonia-like symptoms. This form requires rapid point-of-care diagnostic tools and immediate public health action.
- **Septicaemic plague** is rarely a fulminant bloodstream infection either through direct inoculation with *Y. pestis* and more commonly a complication of untreated or uncontrolled bubonic or pneumonic disease.

Plague is a highly fatal disease without prompt, effective treatment. CFR varies with the clinical form and the level of care. From historical data, in the absence of antibiotic treatment, the CFR

is >70% for bubonic and ~90% for pneumonic plague. Recent systematic reviews estimated overall CFRs of 15% for bubonic plague and 17% for pneumonic plague, with some variance between high-resource and low-resource settings [7, 8]. The CRFs for confirmed cases of bubonic and pneumonic plague during the 2017 urban outbreak in Madagascar were 24% and 25%, respectively [9]. In clinical trial conditions, the aggregated CRF of bubonic plague with a ten-day regimen of either aminoglycoside plus gentamicin or gentamicin alone in Madagascar was 4% [10].

Plague persists in long-standing enzootic foci, sustained by complex ecological interactions among rodents, fleas, and environmental drivers. [11] There are currently three main patterns of human plague: 1) recurrent seasonal plague in disease-endemic countries (rural Madagascar, DRC); 2) sporadic cases caused by occasional encounters with wildlife reservoir, and 3) larger outbreaks in either endemic or non-endemic settings (e.g. 2017 urban outbreak in Madagascar). While there is no evidence of antimicrobial resistance naturally emerging in plague-endemic areas, this should be monitored [12, 13], as strains resistant to streptomycin and doxycycline have been isolated. Most concerningly, multiple antibiotic-resistant plasmids have been identified, such as IP1202, which conferred resistance to 8 different antibiotics including multiple aminoglycosides, β-lactams, tetracyclines, sulfonamides, and chloramphenicol. Such conjugative plasmids have high rates of transfer from *E. coli* to *Y. pestis and* pan antibiotic resistance has already been observed in *E. coli* [14, 15].

Despite its historic and ongoing public health importance, plague lags behind other high-threat pathogens in product development and outbreak preparedness research as key R&D challenges differ across clinical forms of disease. In endemic settings where bubonic plague predominates, effective antimicrobial treatments exist, but improvements are needed in case management, including rapid and reliable diagnostics suitable for point-of-care use and simplified treatment regimens that are easier to administer and ensure adherence. Pneumonic plague presents a distinct set of challenges because of its high lethality, potential for human-to-human transmission, and relevance to biosecurity preparedness. Priorities therefore include improved diagnostics capable of early detection of pneumonic disease, therapeutic strategies that can be deployed rapidly during outbreaks, and development pathways for preventive and post-exposure interventions such as vaccines, monoclonal antibodies, or bacteriophage therapy [16]. Addressing these gaps will require a focused set of high-impact, achievable goals over the next 3–5 years.

Key scientific and operational challenges

Progress in plague prevention, detection, treatment, and control is limited by several persistent scientific and operational barriers. These challenges vary with the clinical form.

Bubonic plague

In endemic areas, available treatments are highly effective, with CFRs of ~4% in trial conditions [10]. The challenge is maintaining these levels of efficacy in routine conditions and ensuring adequate treatment compliance. Key challenges include:

- **Treatment complexity and adherence:** Although antimicrobial therapy is effective when delivered under controlled conditions, existing treatment regimens are relatively long and may be difficult to administer and complete in routine field settings, particularly in resource-limited or remote areas.
- **Limited diagnostics for case management:** The performance of available rapid diagnostic tools suitable for point-of-care use is inadequate for case-management decisions. Diagnostic strategies must prioritise extremely high sensitivity to avoid missed cases of a potentially fatal disease, which presents technical challenges for test development.

Pneumonic plague

Diagnostic test design should be tailored to pneumonic plague and are expected to be different from those for bubonic plague.

Key challenges include:

- **Operational complexity of treatment during outbreaks:** Current treatment regimens (e.g., gentamicin/fluoroquinolone-based combinations) can be difficult to deploy rapidly during outbreaks due to requirements for parenteral administration, monitoring, or logistics in emergency settings. All-oral regimens (e.g., ciprofloxacin, doxycycline) would be easier to deploy rapidly but should be accompanied by measures of ensuring adherence.
- **Limited options for rapid preventive interventions:** There are currently no widely available vaccines or rapidly deployable prophylactic agents suitable for outbreak response, leaving gaps in preparedness for pneumonic plague epidemics or deliberate release scenarios. Doxycycline is used for pre-exposure prophylaxis, although there is no formal evidence supporting this use case and/or post-exposure use. There are several vaccine candidates in late preclinical development, with some in phase I or II clinical trials, which require further evaluation [17].
- **Diagnostics for early detection:** Rapid identification of pneumonic plague remains challenging, particularly early in infection when symptoms may be nonspecific but timely intervention is critical to prevent transmission and improve patient's outcomes.

Additional research topics would include:

- **Rapid early pathogenesis**: *Y. pestis* suppresses innate immune responses during early infection, enabling bacterial proliferation before inflammatory responses become clinically significant, thereby narrowing the window for effective treatment, particularly for pneumonic plague [18-21].
- **Lack of validated immune correlates of protection**: The immunological mechanisms that provide protection against pneumonic or bubonic disease remain incompletely defined, impeding vaccine evaluation and regulatory pathways [17].

- **Complex One-Health transmission cycles**: Rodent reservoir diversity, flea vector competence, and climate-linked drivers remain poorly characterised, complicating outbreak forecasting.
- **Restricted laboratory and research capacity**: Local biosecurity regulation and biosafety Level 3 (BSL-3) requirements for culture and experimental work constrain global research capacity and complicate the development of reference materials and animal models [22, 23]. Also, case detection and surveillance currently limited by requiring advanced diagnostic confirmation procedures involving culture and PCR – with implications for outbreak response and containment,

These challenges collectively constrain product development, outbreak response, and global readiness for plague epidemics, compounded with the absence of commercial interest for medical innovation for plague and the practical challenges of conducting medical investigations in difficult field conditions and the low caseload in particular for pneumonic plague trials

Basic research priorities

Pathogenesis and host response

Y. pestis pathogenesis involves early immune modulation followed by progressive inflammatory activation. *Y. pestis* employs a type III secretion system to deliver effector proteins (including YopH, YopE, YopJ, and YopM) that inhibit phagocytosis and alter innate signalling, enabling early bacterial expansion [24, 25]. Bacterial loads increase, host inflammatory pathways become activated, contributing to clinical progression in bubonic, septicaemic, and pneumonic forms [26]. Animal models remain indispensable given the rarity of human cases. Non-human primate models, though costly, are essential for regulatory studies and have supported approvals under the FDA Animal Rule [23, 27]. Mouse and rat models are critical for discovery and mechanistic work [22].

*Broader challenges related to the development, validation, and translational relevance of pre-clinical infection models are addressed in the **Cross-cutting section on Product development science**.*

Over the next 3–5 years, harmonizing protocols and expanding capacity in animal models will provide the evidence base for vaccine and therapeutic licensure. Characterizing innate and adaptive immune responses—including why lung immune cells fail to contain infection—will identify biomarkers, therapeutic targets, and candidate vaccine antigens [19].

*The role of animal models in supporting countermeasure development for plague, particularly under regulatory pathways such as the FDA Animal Rule, is addressed further in **the Cross-cutting section on Regulatory pathways and policy alignment**.*

Key research needs include clarifying T3SS–host interactions, defining differences in early events across clinical forms, and identifying host determinants of susceptibility.

Immunity and correlates of protection

Protection against plague involves both humoral and cellular immunity, but the specific correlates of protection remain inadequately defined. The F1 capsular antigen (Caf1) and LcrV (a key type III secretion system protein) are widely used as vaccine antigens and contribute to protection in multiple animal models, [17, 28] although their relative importance differs between bubonic and pneumonic infection [28]. Evidence suggests that a balanced Th1/Th2 response correlates with protection in rodent models [17, 29, 30]. Recent evidence also suggests that lung resident memory T cells can play a significant role in protection against pneumonic plague [31, 32].

Priorities include establishing internationally standardised ELISAs for F1 and LcrV, developing T-cell functional assays, and creating an international reference serum to enable immunobridging approaches. Correlates of protection for pneumonic plague are particularly critical, given that traditional field efficacy trials are unlikely to be feasible [17].

Genomics, diversity, and evolution

Comparative genomics provides essential insight into the diversity, evolution, and antigenic profiles of *Y. pestis*. Although *Y. pestis* is often described as a clonal pathogen, recent genomic analyses reveal underappreciated strain diversity that may influence virulence and clinical outcomes [33]. Circulation of F1-negative strains, [34] for example, highlights the need for diagnostics and vaccines that do not rely solely on F1-based detection or immunity [32]. Reverse vaccinology has so far yielded limited breakthroughs, but genomic data remain valuable for confirming antigen conservation, identifying lineage-specific markers, and refining diagnostic targets.

Expansion of sequencing in under-represented regions, together with open-access genotyping schemes and standardised nomenclature, will improve outbreak attribution and global situational awareness.

Ecology, reservoirs, and vectors

Plague persists in a range of natural transmission systems involving wild rodents and their fleas. The specific reservoir and vector species vary by region, but common patterns include maintenance in small mammal populations and transmission by competent flea vectors such as *Xenopsylla cheopis* [35, 36]. These rodent–flea cycles form long-standing enzootic foci in parts of Africa, Asia, and the Americas, with occasional amplification leading to human cases.

Environmental factors including rainfall, temperature, and broader climate variability can influence rodent and flea abundance and may precede periods of increased transmission risk [37]. However, the ecological drivers that determine when and where spillover occurs remain incompletely characterised. Research priorities include improving understanding of vector competence across flea species, clarifying ecological drivers of increased transmission risk, and integrating ecological and climatic indicators into early-warning frameworks.

The role of animal models in supporting countermeasure development for plague, particularly under regulatory pathways such as the FDA Animal Rule, is addressed further in ***the Cross-cutting section on Product development science****.*

Surveillance priorities

Clinical and laboratory surveillance

Clinical recognition is an important first step, but confirmatory testing remains essential for accurate surveillance. Bubonic plague may be identified visually in endemic areas – still with the challenge of differential diagnosis with other causes of acute febrile lymphadenitis –, while pneumonic plague requires rapid point-of-care diagnostics due to non-specific symptoms and rapid progression. Sensitive blood-based assays would help detecting septicaemic plague [34].

Development and validation of lateral-flow antigen tests, isothermal amplification assays, and portable PCR platforms are priorities for the next 3–5 years. Because culture requires BSL-3 containment, expanding access to antigen- and molecular-based diagnostics is essential. Although AMR remains rare, routine genomic screening for resistance determinants is important for clinical management and preparedness for laboratory-modified or deliberate-release scenarios [14, 15].

Genomic surveillance

Genomic tools support outbreak investigation, mapping of transmission chains, and detection of rare antimicrobial-resistant strains. Recent studies have characterised regional and global diversity, [13, 38] but systematic implementation remains limited.

Omics approaches beyond genomics remain underutilized, despite recent efforts to make these data more accessible [39]. Systematic transcriptomic, proteomic, and metabolomic studies in laboratory models simulating flea, rodent, and human environments will help define how *Y. pestis* adapts to distinct host niches. Establishing standardized in vitro systems mimicking these environments is an achievable short-term milestone that will facilitate drug and vaccine discovery.

Priorities include developing regional sequencing hubs with harmonised workflows, establishing rational and transparent genotyping schemes, deploying portable sequencing technologies, and integrating genomic data with epidemiological and ecological indicators to improve early warning and response.

Cross-pathogen considerations related to surveillance platforms, laboratory capacity, data integration, and genomic systems are addressed in the ***Cross-cutting sections on Digital Infrastructure and Genomic Surveillance*** *and* ***Laboratory and Surveillance Capacity****.*

Environmental and reservoir surveillance

Environmental surveillance can provide early indicators of increased transmission risk. Key needs include practical field tools to detect *Y. pestis* in rodents or fleas, systematic monitoring of rodent mortality and flea indices, and frameworks that integrate ecological and climatic information into public health decision-making.

Product development priorities

Vaccines

Use cases for vaccines include prevention of recurrent seasonal outbreaks in endemic areas, response to outbreaks in previously non-endemic areas, preventive vaccination for high-risk groups, and biodefense applications. Advancement to readiness for regulatory submission could be achievable with support from immunobridging strategies once correlates of protection are established and Animal Rule pathways, which would still require follow up clinical trials. Live-attenuated vaccines may face additional regulatory and storage considerations.

Vaccine evaluation requires innovative trial strategies and must be adapted to the clinical form (bubonic or pneumonic). While Phase I/II studies are feasible [14], Phase III efficacy trials may be impractical given the rarity of cases and sporadic nature of the typical seasonal outbreaks [40]. Options include multi-outbreak adaptive protocols and potentially controlled human infection models (CHIMs) using attenuated strains. The recent discussion of CHIMs using attenuated vaccine strains as an approach to accelerate licensure [41], highlights both opportunities and ethical considerations.

Linking vaccine trials to clinical forms is essential: prevention of bubonic plague might be evaluated in endemic areas where populations are exposed to shared environmental or zoonotic sources, while pneumonic plague efficacy will likely rely on surrogate markers and outbreak-based evaluation frameworks given the potential for person-to-person transmission. Special attention should be paid to adapting vaccine candidate profiles, trial conduct and delivery strategies to the paediatric and immunocompromised populations.

High level challenges related to clinical trial feasibility for rare or outbreak-driven diseases, including the need for trial-ready platforms and standing protocols, are addressed in the ***Cross-cutting section on Clinical Trial Infrastructure and Research Readiness***

Therapeutics

Although *Y. pestis* remains treatable with commonly available antibiotics, [10, 27] early treatment is critical, and mortality can still be high where cases occur in remote areas with limited access to health services. Delayed presentation, delayed recognition, and delayed initiation of effective therapy, often driven by geographic remoteness, limited diagnostic capacity, and weak referral pathways, remain major contributors to poor outcomes. For bubonic plague, given recent evidence of efficacy of single-agent ciprofloxacin and

gentamycin/ciprofloxacin sequential combination regimens,[10] conducting additional clinical trials to gather more evidence of other available all-oral regimens (e.g., doxycycline) should be considered, along with pragmatic trials/operational research to optimise delivery to ensure the same levels of efficacy achieved in clinical trial conditions are maintained in routine clinical care. Additional priorities will be to identify regimens that are easier to adhere to, and that are adapted to children, who represent the majority of cases in plague-endemic areas occur.

For pneumonic plague, more effective and easier-to-use regimens are needed, but evidence generation will be problematic given the limited number of cases. Alternative study designs should be explored that are adapted to different epidemiological scenarios. Synergies with other diseases facing similar challenges should be explored to develop adapted study methodologies, clinical development plans, and regulatory pathways.

Diagnostics

Diagnostics should enable early detection, guide case management, and support surveillance and response.

Bubonic plague can often be identified clinically, when a subject living in or with history of recent travel to a plague-endemic area presents with recent onset of fever and enlarged, painful lymph nodes. Current diagnostics cannot provide definitive reliable results at point-of-care to guide case managements, so treatment decisions in routine practice in plague-endemic areas rely mostly on clinical judgement, resulting in significant overtreatment, especially in children. Confirmatory tests are available for surveillance but require advanced facilities with capacity for culture and PCR.

Pneumonic plague urgently requires rapid point-of-care tools, ideally based on sputum or blood samples, as clinical recognition alone is insufficient.

The immediate priority should be to develop the target product profile of diagnostics that are adapted to the different use cases (case management or surveillance, depending on clinical form and epidemiological context) to guide product development. Innovation should focus on novel targets for diagnostics, easily accessible biomarkers such as circulating microRNAs or metabolites as well as field-adapted diagnostic platforms

Research infrastructure, preparedness, and outbreak response

Preparedness for outbreak-related research remains limited. Pre-approved trial protocols, ethics and regulatory readiness, and frameworks for multi-outbreak data aggregation are essential for efficient vaccine and therapeutic evaluation. Adaptive designs can support evidence generation when case numbers are low.

Research, diagnostics, and surveillance for *Y. pestis* are constrained by biosafety and biosecurity requirements, including the need for appropriately equipped BSL-2 and BSL-3 laboratory facilities, trained personnel, and robust quality and safety systems. Limited availability of such facilities—particularly in endemic or high-risk regions—restricts routine diagnostic confirmation, genomic characterisation, and research on pathogen biology and countermeasure

evaluation. Strengthening and sustaining appropriate biosafety capacity is therefore a prerequisite for effective surveillance, outbreak preparedness, and product development for plague.

*System-level challenges related to laboratory biosafety infrastructure, workforce development, and long-term sustainability of high-containment capacity are addressed further in the **Cross-cutting section on Laboratory and Surveillance Capacity**.*

Unlike pathogens with sustained endemic transmission, demand for plague vaccines and therapeutics is episodic and driven primarily by preparedness and outbreak response rather than routine use. This creates limited commercial incentives for manufacturers and complicates traditional market-based development and procurement pathways. As a result, effective plague countermeasures are likely to depend on advance agreements, strategic stockpiling, and coordinated procurement mechanisms, rather than continuous production for routine programmes. Ensuring manufacturing readiness, shelf-life management, and equitable access during outbreaks will therefore require early engagement with manufacturers and clear governance arrangements for stockpiles, aligned with broader global preparedness strategies.

*Cross-pathogen issues related to episodic demand, manufacturing incentives, and strategic stockpiling are addressed in the **Cross-cutting section on Manufacturing, Supply Chain Readiness, and Stockpiling**.*

Strategic Priorities (3–5 Year Horizon)

1. Advance vaccine candidates and regulatory readiness

Objective:
By 2028, advance at least two *Y. pestis* vaccine candidates through early clinical development, including completion of Phase 1 trials for both candidates and progression of at least one candidate into Phase 2 evaluation, supported by validated immunogenicity assays and harmonised regulatory plans.

Key milestones:

- **2026:** International consensus established on immune correlates of protection for pneumonic plague, including validated ELISAs for F1/LcrV and standardised T-cell assays.
- **2026:** Harmonised protocols for immunobridging studies adopted by at least five reference laboratories.
- **2027:** Cross-laboratory proficiency panel completed to validate assay comparability across endemic and non-endemic regions.
- **2027:** Agreement reached on acceptable regulatory and ethical frameworks for attenuated CHIMs, adaptive platform trials, and Animal Rule evidence packages.
- **2028:** At least two candidates demonstrate immunogenicity meeting predefined benchmarks and have completed pivotal non-human primate studies.

2. Validate rapid diagnostics and expand genomic surveillance

Objective:
By 2027, implement and validate two rapid point-of-care diagnostic assays capable of detecting bubonic, pneumonic, and septicaemic plague, and establish genomic surveillance systems in at least three endemic regions.

Key milestones:

- **2026:** Analytical validation completed for two lateral-flow or isothermal amplification assays in BSL-2 laboratories.
- **2026:** Field validation initiated in at least four peripheral health facilities or district laboratories in endemic settings.
- **2027:** Integration of diagnostic and genomic data reduces the median time from suspected case to laboratory confirmation to under 48 hours.
- **2026:** Open-access *Y. pestis* genotyping framework published and adopted by regional partners.
- **2027:** At least three regional sequencing hubs established, each capable of processing a minimum of 50 isolates per outbreak and generating results within five days of sample receipt.
- **2027:** Genomic AMR screening incorporated into national surveillance workflows in at least three countries.

3. Operationalise outbreak-responsive research networks

Objective:
By 2028, establish functional research networks in at least three endemic regions capable of initiating adaptive vaccine or therapeutic trials within 14 days of outbreak declaration.

Key milestones:

- **2026:** Adaptive protocols for vaccine and therapeutic trials pre-approved by national ethics and regulatory authorities in at least three endemic countries.
- **2026:** At least three regional research hubs equipped with BSL-2/3 capacity, specimen repositories, GCP-trained investigators, and secure data systems.
- **2027:** Standardised training modules adopted for investigators, with at least 150 personnel trained in GCP, biosafety, and emergency research activation.
- **2027:** Harmonised data-management structures implemented and interoperable with WHO and national ministries.
- **2027:** At least one multi-country simulation exercise completed to validate network readiness.

4. Establish structures and governance frameworks for future stockpiles

Objective:
By 2028, establish a governance and operational framework for global and regional plague vaccine stockpiles, aligned with WHO mechanisms and enabling deployment within seven days once vaccines become available.

Key milestones:

- **2026:** Draft governance framework completed, including activation triggers, replenishment rules, and equitable-access principles.
- **2026:** At least two manufacturing partners commit to surge-capacity agreements covering subunit, viral vector-based, T4-based, mRNA-based, and/or live-attenuated vaccine platforms.
- **2028:** Financing options evaluated, including revolving funds and procurement mechanisms.
- **2029:** Operational stockpile blueprint published and harmonised with cholera, yellow fever, and meningococcal stockpile procedures.

These pathogen-specific priorities align with cross-cutting investments in diagnostics, surveillance, regulatory science, and research infrastructure described in the final section of this roadmap.

B. Vibrio cholerae

Introduction/Problem statement

Vibrio cholerae is a motile Gram-negative bacterium that causes cholera, an acute, dehydrating diarrhoeal disease characterized by profuse watery diarrhoea and rapid fluid loss. Although over 200 *V. cholerae* serogroups have been identified, only serogroups O1 and O139 have been responsible for large-scale outbreaks and epidemics [42]. Within this serogroup, the seventh pandemic El Tor lineage, known as 7PET, is responsible for the current global pandemic. Genomic studies have demonstrated that 7PET has spread across Asia, Africa, and the Caribbean in successive waves since the 1960s, with repeated cross-border introductions shaping contemporary transmission patterns [43, 44]. Transmission occurs via the faecal–oral route, typically through ingestion of faecally contaminated water or food. While most infections are asymptomatic or mild, a proportion of symptomatic cases progress to severe dehydration, hypovolaemic shock, and death if untreated [45].

Cholera remains endemic in more than 60 countries and continues to pose a major public health threat in settings with fragile water, sanitation, and hygiene (WASH) infrastructure, conflict, displacement, and climate vulnerability. Global estimates suggest between 1.3 and 4.0 million cholera cases and 21,000 to 143,000 deaths occur annually, [46] although the true burden is uncertain due to under-ascertainment and limited laboratory confirmation. In recent years, reported incidence has increased, [47] particularly in sub-Saharan Africa, where countries account for the majority of reported cases, [48] and in South Asia, where cholera remains highly endemic with large outbreaks that are often under-reported [49]. Case fatality rates can be kept below 1% with prompt treatment, but can exceed this threshold during large outbreaks or where access to care is delayed [50].

With prompt case management, including oral rehydration therapy and intravenous fluids for severe disease, mortality can be reduced substantially; this is more difficult to achieve among older adults, or people with comorbidities. Antibiotics are recommended for severe cases to shorten illness duration and reduce bacterial shedding, [51] but the emergence of antimicrobial resistance, driven largely by the acquisition of resistance genes carried on mobile genetic elements, underscores the need for strengthened surveillance and alternative therapies [43, 52].

Oral cholera vaccines (OCVs) are a critical tool for both preventive and reactive use. Although OCVs were licensed in the 1990s and WHO-prequalified in the 2000s [53], their programmatic use expanded following establishment of a global vaccine stockpile in 2013. Important limitations remain: protection is relatively short-lived, effectiveness wanes substantially within three years [54] single-dose regimens provide only modest short-term protection in reactive settings, [55, 56], and young children, who bear a disproportionate burden of cholera morbidity and mortality in endemic settings, experience markedly lower protection [57]. These age-specific differences are of particular concern given that children bear a disproportionate burden of severe cholera morbidity and mortality in endemic settings. Persistent global supply constraints continue to limit both preventive and reactive use of OCVs, challenging progress

toward global control targets, including the Global Task Force for Cholera Control (GTFCC) goal of reducing cholera deaths by 90% by 2030 [58].

Cholera transmission is closely linked to fragile water, sanitation, and hygiene (WASH) systems, and targeted improvements in sanitation, water access, and hygiene practices can substantially reduce cholera incidence, [59] although effectiveness depends on sustainability, cost, reliability, and cultural acceptability. Recent large outbreaks in sub-Saharan Africa, recurrent epidemics in South Asia, and resurgent transmission following infrastructure collapse and political instability in Haiti illustrate how quickly cholera can re-emerge when control systems fail [60, 61]. The increasing frequency and severity of extreme weather events associated with climate change are expected to further heighten these risks by disrupting water systems, displacing populations, and expanding conditions conducive to cholera transmission [62].

Environmental persistence of *V. cholerae* in aquatic environments in aquatic reservoirs further contributes to ongoing risk, with the relative contribution of environmentally mediated exposure and person-to-person transmission varying across settings and seasons [63-65]. The relative contributions of environmentally mediated exposure and person-to-person transmission vary across settings, but both are important drivers of outbreaks, particularly where water sources are unsafe or where sanitation systems are inadequate to prevent faecal contamination. Better understanding of these ecological and transmission dynamics is essential for improving risk prediction, designing context-appropriate preventive interventions, and developing new tools for cholera control.

The GTFCC, revitalised in 2014, provides the primary global coordination mechanism for cholera prevention and control, supporting national cholera control plans, hotspot identification, vaccine deployment, WASH interventions, and surveillance strengthening. The “Ending Cholera: A Global Roadmap to 2030” sets an ambitious target of reducing cholera deaths by 90% and eliminating cholera as a public health threat in 20 countries [58]. Despite this progress, rising incidence, climate instability, and persistent vaccine supply constraints underscore the need for continued, well-aligned research and development to support and extend GTFCC-led efforts over the next three to five years.

Key scientific and technical challenges

Cholera control and prevention efforts are impeded by several scientific and operational challenges that limit the effectiveness of current interventions and hinder the development of new tools. These challenges span basic biology, environmental and transmission dynamics, surveillance, product development, and health system infrastructure.

- **Incomplete understanding of cholera transmission dynamics -** Transmission arises from a complex interplay between human-to-human transmission, environmentally mediated exposures, and the capacity of *V. cholerae* to persist in aquatic ecosystems. The relative contributions of these pathways vary across settings and seasons. Uncertainty about how environmental reservoirs, population immunity, and climatic drivers interact limits the ability to predict outbreaks and target preventive interventions effectively.

- **Limited understanding of immunity and correlates of protection** - Protective immunity to cholera is multifaceted and varies by age, exposure history, and antigenic composition of circulating strains. Although oral cholera vaccines are widely used, the immunological mechanisms that underpin short-lived protection, reduced effectiveness in young children, and waning immunity after a single dose remain incompletely defined. The absence of validated immune correlates of protection hampers the development, evaluation, and licensure of new vaccine candidates.
- **Gaps in genomic and AMR surveillance** - The global spread and diversification of 7PET lineages, including the emergence of highly drug-resistant variants, underscore the need for robust genomic surveillance. Many high-burden countries lack routine sequencing capacity, standardised genotyping schemes, and systems for integrating genomic data into real-time surveillance. As a result, the movement of sublineages, the evolution of antimicrobial resistance, and the introduction of new variants often go undetected until large outbreaks are underway, and genomic data are rarely available in a time frame or format that directly informs vaccination strategy, outbreak classification, or district-level intervention decisions.
- **Constraints in environmental and climate-linked early warning** - Although environmental and climate signals are known to influence cholera risk, operational systems for environmental monitoring, climate-informed prediction, and water-quality surveillance remain limited. Tools for translating environmental signals into actionable early warning systems are underdeveloped and are not routinely integrated into national surveillance platforms.
- **Diagnostic delays and limited laboratory capacity** - Syndromic case definitions lack specificity, and many countries rely on clinical reporting without routine laboratory confirmation. Existing rapid diagnostic tests vary in accuracy and require confirmatory testing for outbreak declaration. Limited access to culture facilities, transport media, and molecular diagnostics reduces the timeliness and reliability of cholera detection, constraining rapid response and real-time monitoring during outbreaks.
- **Limitations of current vaccines and persistent supply shortages** - Current oral cholera vaccines provide only short- to medium-term protection, perform poorly in young children, and offer limited duration of immunity after a single dose. The global vaccine supply has been insufficient to meet rising demand, forcing adjustments to vaccination strategies and limiting preventive use. These limitations highlight the need for next-generation vaccines with improved durability, dose-sparing properties, and suitability for diverse age groups.
- **Limited therapeutic innovation and emerging antimicrobial resistance** - Treatment relies primarily on oral rehydration and a small number of antibiotics, but antimicrobial resistance is increasing. Novel therapeutics and supportive tools—including monoclonal antibodies, phage-based therapies, and adjunctive treatments to reduce stool output—remain at early stages of development. Preclinical models and regulatory pathways for evaluating new therapeutics are underdeveloped.
- **Operational challenges in outbreak settings** - Health systems facing cholera outbreaks often contend with overstretched facilities, shortages of trained staff, and disrupted supply chains. In addition, conducting research during outbreaks is

constrained by delayed ethics approvals, limited research coordination, and inadequate mechanisms for rapid activation of studies. These challenges hamper the ability to generate evidence needed for guiding interventions.

- **Persistent WASH vulnerabilities** - Many high-burden countries lack resilient water and sanitation infrastructure. Rapid urbanisation, informal settlements, financial constraints, and climate-related disruptions all contribute to sustained exposure risks. The development and evaluation of scalable, context-appropriate WASH interventions are hindered by insufficient implementation research and limited real-world performance data. There is also limited high-quality evidence on which combinations of WASH interventions are most effective during outbreaks, how they interact with vaccination strategies, and how they can be feasibly deployed at scale under emergency conditions.

Together, these challenges create a fragmented landscape for cholera control and impede progress toward global elimination goals. Addressing them will require coordinated investment in basic research, surveillance innovation, product development, and outbreak-ready research systems. The next section outlines priority areas for basic scientific research to address these gaps.

Basic research priorities

Transmission biology and host colonisation

Our understanding of cholera transmission continues to evolve, yet key uncertainties remain regarding how *V. cholerae* transitions from exposure to intestinal colonisation and onward transmission. While a fraction of symptomatic patients shed high concentrations of bacteria for 10–14 days, a much larger number of asymptomatic or mildly symptomatic individuals shed viable organisms for shorter periods (<7–10 days) and often do so without altering behaviour or seeking care [64]. These individuals may substantially contribute to population-level transmission because they are more numerous and less likely to be detected [64, 66]. Asymptomatic carriage has also been implicated in long-distance spread, including the introduction of *V. cholerae* into Haiti [67].

The biological mechanisms governing why some individuals develop severe disease while others remain asymptomatic remain poorly understood. Factors such as infectious dose, microbiome composition, underlying health, gastric acidity, nutritional status, prior exposure, and age likely play roles but are not well defined. For vaccinated individuals, colonisation and shedding may still occur, although the duration and infectiousness of such shedding remain unclear. A clearer understanding of early host–pathogen interactions and determinants of colonisation is essential for modelling transmission, refining vaccine evaluation, and designing interventions that interrupt chains of spread, including prophylactic treatment, household-based interventions, and ring vaccination strategies.

Immunity and correlates of protection

Although substantial progress has been made in defining immune responses to *V. cholerae*, fundamental gaps limit the development of next-generation vaccines. Antibodies targeting cholera toxin and O-specific polysaccharide (OSP) remain the best-established correlates of protection, and vibriocidal antibody titres are widely used endpoints in vaccine trials. However, vibriocidal titres serve as a non-mechanistic correlate of protection, reflecting immune status without directly mediating immunity [45, 53]. Serum antibody responses typically wane within a year and do not fully capture mucosal immune processes at the site of infection [68].

Immunological context varies between endemic and non-endemic settings. In endemic areas, repeated exposures may boost responses and extend the apparent duration of immunity, whereas individuals in non-endemic regions remain immunologically naïve [69, 70]. The mechanisms by which OSP and other antibodies confer protection—whether through inhibition of colonization, toxin neutralization, or other mucosal functions—are still being clarified. Similarly, while infection and vaccination appear to confer protection for several years, estimates of the true duration of immunity remain uncertain, as natural boosting in endemic regions may obscure underlying kinetics [53]. It also remains unclear whether immunity primarily protects against clinical disease or also limits colonization and transmission [64].

To refine correlates of protection, broader mucosal immune profiling and ultrasensitive immunological approaches (e.g., multiplex assays, single-cell analyses) are needed. These will be essential for evaluating candidate vaccines and tailoring strategies for populations with differing immunological backgrounds.

The role of the gut microbiome in susceptibility and vaccine performance

Emerging data indicate that gut microbiome composition influences susceptibility to *V. cholerae* infection as well as the effectiveness of OCVs. Individuals with more diverse or resilient microbial communities appear less likely to develop symptomatic disease, and specific taxa may modulate mucosal immunity or colonisation resistance [71]. Conversely, gut dysbiosis has been linked with weaker vaccine-induced responses and increased susceptibility to symptomatic infection [72]. Recent work suggests that sphingolipid-producing *Bacteroides* species may enhance development of protective immune responses following OCV administration [73].

Despite these insights, mechanistic pathways remain unclear, and microbiome-based interventions have not yet been developed. Research is needed to determine whether targeted modulation of the microbiome—through probiotics, prebiotics, or strain-specific adjuvants—can enhance OCV immunogenicity, particularly for young children and other high-risk populations.

Ecology, environmental persistence, and transmission

A deeper understanding of how *V. cholerae* persists in the environment and contributes to human transmission is essential for improving surveillance and forecasting. *V. cholerae* is a natural inhabitant of aquatic ecosystems, where it associates with biofilms, zooplankton, aquatic plants, and other environmental substrates [63, 74]. Many environmental isolates are non-

O1/non-O139 strains that do not cause cholera epidemics, underscoring the need to differentiate epidemic-capable lineages from the broader species diversity [43, 44].

Key adaptations—including formation of biofilms and entry into a viable but non-culturable (VBNC) state—may facilitate environmental persistence and enable epidemic lineages to re-enter human populations during favourable ecological conditions [75, 76]. However, major gaps remain in understanding the environmental conditions under which VBNC forms regain virulence, the contribution of biofilm-associated bacteria to epidemic waves, and how ecological factors such as salinity, plankton density, and temperature modulate transmission risk.

A detailed dissection of lineage-specific ecological strategies is required to understand how some strains persist for long periods in environmental reservoirs, cause only localised outbreaks, or contribute to the sustained global spread seen in the Seventh Pandemic lineage. Clarifying the environmental and ecological determinants of transmission is essential for designing targeted, ecosystem-specific WASH and surveillance interventions.

Climate and ecological drivers of cholera

Environmental and climatic factors—including temperature, rainfall, salinity, and plankton blooms—strongly influence *V. cholerae* abundance and cholera seasonality in many endemic settings [74, 77]. Remote sensing has been used to forecast outbreaks and transmission risk by tracking ecological indicators like sea surface temperature and chlorophyll [78, 79], although these models have not been rigorously validated across diverse settings.

Much of the existing evidence derives from coastal regions such as the Bay of Bengal, and the causal pathways linking environmental detection to human transmission remain uncertain [65]. Improving mechanistic understanding of climate-sensitive environmental reservoirs is particularly urgent in the context of climate change, which is expected to increase WASH system disruptions and expand ecological niches conducive to *V. cholerae* survival. Better integration of ecological modelling, hydrological data, and surveillance systems could enhance outbreak prediction and readiness.

The role of cholera bacteriophages in epidemic dynamics and evolution

Bacteriophages are increasingly recognised as important regulators of *V. cholerae* dynamics, influencing transmission, outbreak resolution, and bacterial evolution. In Bangladesh, amplification of lytic phages following peaks in clinical cases has been associated with declines in bacterial prevalence, suggesting phage predation contributes to the self-limiting nature of epidemics [80]. Mathematical models support the importance of phage–bacteria cycling in modulating epidemic patterns.

The lytic vibriophage ICP1 has been repeatedly isolated from cholera patients for more than a decade in Bangladesh, exerting strong selective pressure on circulating *V. cholerae* strains [81]. This has driven evolution of phage-resistance mechanisms and corresponding counter-adaptations in ICP1, including the emergence of phage-inducible chromosomal island–like

elements (PLEs) such as PLE11, reported to date only in Bangladesh [82]. These dynamics may help explain regional variation in *V. cholerae* evolution.

Phages may also shape genomic plasticity more broadly and influence virulence, antimicrobial resistance, and biofilm formation. Despite these roles, phage surveillance is rarely integrated into cholera control frameworks. A deeper understanding could enable use of phages as biomarkers of epidemic progression, components of predictive models, or even as preventive or therapeutic interventions. Experimental work shows that prophylactic phage administration can prevent *V. cholerae* infection in animal models [83].

Relevance of non-O1 or O139 V. cholerae serotypes

V. cholerae is a natural inhabitant of aquatic ecosystems [63], and non-O1/non-O139 strains of *V. cholerae* are widely distributed in aquatic environments and increasingly detected through environmental genomics [84]. Their growing visibility likely reflects improved surveillance rather than increased prevalence. Studies in Northern Cameroon have identified genetically diverse non-O1/non-O139 isolates related to both environmental and clinical strains from multiple continents, including some carrying virulence-associated genes such as *tcpA* [85]. Mobilome analyses further indicate that these strains can carry integrons and mobile genetic elements associated with virulence or antimicrobial resistance [86].

Although these strains do not cause epidemic cholera, they may act as reservoirs of genetic diversity and mobile elements for epidemic lineages. Clarifying their ecology, evolution, and interactions with toxigenic lineages is important for understanding long-term risks and for designing genomic surveillance systems that distinguish environmental background noise from signals relevant to outbreak prediction.

Surveillance priorities

Effective cholera control depends on early identification of cases, timely laboratory confirmation of *V. cholerae*, and integrated systems that combine epidemiological, laboratory, and environmental data. Surveillance capacity varies widely across endemic countries, and persistent gaps in diagnostics, sample transport, and data integration delay outbreak detection and limit the precision of targeted interventions.

Clinical surveillance and case detection

Most endemic countries rely on syndromic surveillance for acute watery diarrhoea, which is sensitive but not specific. Only a subset of suspected cases are confirmed as cholera, and limited laboratory access—particularly in peripheral or rural facilities—means outbreaks may be detected late or missed. Standardized strategies for selecting samples for diagnostic confirmation, with emphasis on known transmission hotspots and high-incidence districts, would improve situational awareness.

A large proportion of *V. cholerae* infections are asymptomatic or mildly symptomatic, and these individuals rarely seek care but may contribute substantially to community transmission.

Periodic cross-sectional or sero-epidemiological surveys could help estimate infection-to-case ratios and provide a more accurate understanding of underlying transmission dynamics.

Laboratory confirmation and diagnostic capacity

Culture remains the reference method for confirming cholera and is essential for antimicrobial susceptibility testing and isolate archiving. However, many health facilities lack appropriate media, biosafety conditions, or reliable sample transport systems. Where culture is available, delays in transport and suboptimal storage can reduce sensitivity.

Rapid diagnostic tests (RDTs) play an important role in early outbreak detection, especially in resource-limited settings. Available lateral-flow assays vary in sensitivity and specificity across brands, serogroups, and field conditions, and confirmatory testing remains necessary. PCR-based methods offer improved sensitivity but are typically restricted to national or regional laboratories. Expanding molecular testing capacity through regional hubs or portable platforms would improve confirmation, support AMR monitoring, and strengthen linkage between laboratory and field surveillance.

Cross-pathogen challenges related to laboratory infrastructure, diagnostic evaluation, biosafety requirements, and workforce development are addressed in the ***Cross-cutting section on Laboratory and Surveillance Capacity****.*

Environmental and One Health surveillance

Environmental surveillance for *V. cholerae* is increasingly used to complement clinical reporting, but interpretation requires caution. Both pathogenic and non-pathogenic strains may be detected in aquatic systems, and environmental presence does not always correlate with human risk. Standardized sampling approaches for water sources, sewage, and high-risk environmental interfaces are needed to enable meaningful comparisons across sites and seasons.

Environmental data should be integrated with genomic analyses to differentiate epidemic-capable lineages from background environmental diversity. Partnerships across public health, environmental, and meteorological sectors can support improved risk assessments, particularly in settings where climate-sensitive environmental reservoirs are believed to contribute to outbreak initiation.

Genomic surveillance and antimicrobial resistance

Genomic surveillance has transformed understanding of cholera transmission, enabling high-resolution tracking of the Seventh Pandemic El Tor (7PET) lineage, identification of introduction events across borders, and improved outbreak investigation. Genomic data also allow detection of plasmid-mediated AMR determinants, such as those reported in Yemen and Zimbabwe, supporting treatment guidance and risk assessment. However, the operational use of genomic data to distinguish sustained human transmission from environmental reintroduction, or to prioritise preventive vaccination and WASH interventions, remains limited.

Routine sequencing remains limited to a small number of international or national laboratories. Establishing regional sequencing hubs and standardized genotyping frameworks would allow more systematic characterization of circulating strains. Integration of genomic data, clinical metadata, and antimicrobial susceptibility data into national surveillance systems would strengthen early detection of emerging variants and inform public health decision-making.

General considerations related to genomic data integration, analytical pipelines, and operational use of genomic signals are addressed in the ***Cross-cutting section on Digital Infrastructure and Genomic Surveillance****.*

Linking surveillance to public health action

Surveillance systems must be closely connected to response mechanisms to ensure timely action. Clear, pre-defined thresholds for escalating investigations, rapid communication of laboratory results to district and national authorities, and integration of genomic findings into situational analyses are essential.

Strengthening these linkages will improve prioritization for oral cholera vaccine campaigns, targeting of WASH interventions, and identification of transmission hotspots. Embedding surveillance within national cholera control plans and aligning with GTFCC guidance will support coordinated, evidence-driven efforts and improve progress toward the 2030 global targets. Surveillance outputs should also be designed to trigger targeted household- or community-level responses, including case-area targeted interventions (CATI) such as focused vaccination, water treatment, or prophylactic strategies, rather than only large-scale reactive campaigns.

Product development priorities

Despite major progress in oral cholera vaccine (OCV) development and deployment, significant gaps remain in performance, supply, and suitability for different use cases. Therapeutic innovation beyond antibiotics is limited, although monoclonal antibodies, phage-based interventions, and microbiome-modulating strategies represent promising early-stage avenues. Strengthening manufacturing capacity and aligning product development with public health needs will be essential to achieving global cholera control goals.

Vaccines

Oral cholera vaccines (OCVs) are central to cholera control, but several well-documented limitations constrain their impact. Protection wanes substantially within several years, with effectiveness declining more rapidly in young children [57]. Single-dose regimens confer only modest short-term protection [55, 56], yet global supply shortages have necessitated their widespread use in reactive campaigns. These features underscore the need for next-generation vaccines with longer duration of protection, improved performance in high-risk groups, and operational flexibility for emergency use.

The 2017 WHO Position Paper on cholera vaccines highlights desirable attributes for future products, including improved immunogenicity in children under five, longer-lasting protection, suitability for both preventive and reactive contexts, and thermostability compatible with mass campaigns [87]. Approaches currently under development include:

- **Enhanced OSP-based formulations**, including optimized OSP conjugates and multivalent OSP presentations, aimed at improving the magnitude and durability of anti-OSP responses, particularly in young children.
- **Adjuvanted oral vaccine candidates**, such as killed whole-cell formulations combined with mucosal adjuvants like dmLT or other gut-targeted delivery systems, to strengthen mucosal immunity and extend duration of protection.
- **Live-attenuated vaccines**, including improved derivatives of the CVD 103-HgR strain, which are being evaluated for their potential to induce rapid and durable mucosal immunity. CVD 103-HgR is licensed for travelers but is not WHO-prequalified, and its programmatic suitability for endemic settings remains under assessment.

Although vibriocidal antibody titres and OSP-specific responses are widely used as immunological endpoints, no validated immunobridging framework exists for cholera vaccines. As mucosal correlates of protection become better defined, immunobridging approaches may eventually reduce reliance on large field trials, which are difficult to implement due to the unpredictable nature of cholera incidence.

Because cholera incidence is highly variable and often seasonal, vaccine efficacy trials require flexibility and readiness to activate studies rapidly in high-incidence districts. Opportunities for evaluating new vaccines may arise during large outbreaks, when high attack rates allow short recruitment windows. Vaccine supply constraints may also inform design feasibility, as during shortages, cluster- or ring-based allocation during reactive campaigns may provide pragmatic approaches for evaluating performance.

Evaluations of next-generation vaccines will benefit from:

- harmonised vibriocidal and OSP assay standards;
- standardised case definitions and dehydration assessments;
- the ability to embed studies within cholera treatment centres (CTCs), which often serve as focal points for rapid enrolment during outbreaks.

Broader challenges related to vaccine evaluation pathways, immunobridging, and trial design in fluctuating-incidence settings are addressed in the ***Cross-cutting sections on Product Development Science, Regulatory Pathways and Policy Alignment, and Clinical Trial Infrastructure and Research Readiness****.*

Therapeutics and adjunctive interventions

Rehydration therapy remains the cornerstone of cholera management. Prompt administration of oral rehydration therapy (ORT) or intravenous fluids can reduce case fatality rates to below 1% in otherwise healthy individuals [51]. Antibiotics shorten the duration of diarrhoea and reduce

bacterial shedding in severe cases, contributing to both clinical care and transmission control. However, *V. cholerae* has repeatedly acquired antimicrobial resistance through mobile genetic elements [43, 52], underscoring the need for alternative or adjunctive therapies.
Promising avenues for product development include:

- **Monoclonal antibodies** targeting cholera toxin or colonization factors, which may provide targeted prophylaxis for household contacts or adjunctive therapy for severe disease.
- **Bacteriophage-based interventions**, supported by animal-model evidence showing prophylactic efficacy against *V. cholerae* [83].
- **Microbiome-directed strategies**, motivated by evidence that gut microbial composition influences susceptibility to cholera and OCV immunogenicity [71-73], though these remain exploratory.
- **Adjunctive agents such as zinc**, which have shown benefit in reducing duration and severity of diarrhoea in children [88].

Large outbreaks and high patient volumes in cholera treatment centres create opportunities to evaluate adjunctive therapies within short timeframes, provided that studies do not disrupt the delivery of essential rehydration services.

Household- or contact-based designs are particularly well suited for testing prophylactic tools like monoclonal antibodies, post-exposure antibiotics, or phage therapy because secondary attack rates within households are high and well characterised.

Measuring treatment outcomes requires standardised dehydration scoring, rapid stool testing where feasible, and harmonised definitions of bacterial shedding to assess effects on transmission.

Cross-pathogen challenges related to preclinical model development, regulatory evaluation of novel therapeutics, and harmonised clinical endpoints are addressed in the ***Cross-cutting sections on Product Development Science and Regulatory Pathways and Policy Alignment****.*

Diagnostics

Despite their central role in outbreak detection, response, and evaluation of control strategies, cholera diagnostics remain constrained by limitations in performance, usability, and integration into decision-making workflows. Current approaches continue to rely heavily on syndromic case definitions, with laboratory confirmation often delayed or unavailable in outbreak-prone settings. Rapid diagnostic tests (RDTs) are increasingly deployed and are undergoing validation in multiple settings, offering important operational advantages; however, variability in sensitivity and specificity, differences in performance across epidemiological contexts, and uncertainty around optimal use cases continue to limit their effectiveness for routine surveillance and outbreak response. Culture and molecular diagnostics remain essential for confirmation and

characterisation but require laboratory infrastructure that is frequently inaccessible in high-burden or emergency contexts.

Product development priorities include the continued optimisation, validation, and standardisation of field-deployable diagnostic tools suitable for both outbreak response and endemic surveillance. Clear target product profiles should reflect realistic use cases, including requirements for speed, robustness, minimal infrastructure dependence, and compatibility with decentralised testing environments, as well as guidance on how diagnostics should be used in combination with clinical and epidemiological data. Improved diagnostic products would support earlier outbreak confirmation, more targeted deployment of oral cholera vaccines and other interventions, and more reliable assessment of disease burden and intervention impact. Strengthening diagnostic product pipelines is therefore an essential complement to investments in vaccines, therapeutics, and surveillance systems for cholera control.

Cross-pathogen challenges related to diagnostic target product profiles, evaluation pathways, regulatory considerations, and integration of diagnostic outputs into surveillance and decision-support systems are addressed further in the ***Cross-cutting sections on Product Development Science, Regulatory Pathways and Policy Alignment, and Digital Infrastructure and Genomic Surveillance.***

Strategic Priorities (3–5 Year Horizon)

1. Advance next-generation cholera vaccines and evaluation pathways

Objective:
By 2029, advance at least two next-generation oral or live-attenuated cholera vaccine candidates through early clinical development, supported by harmonised immunogenicity assays, standardised mucosal immune markers, and agreed evaluation pathways with national regulators and WHO PQ.

Key milestones:

- **2026**: Comparative immunogenicity data generated for enhanced OSP-based and adjuvanted candidates, including age-stratified analyses for children under five.
- **2026:** Standardised vibriocidal and OSP immunoassays adopted by three or more reference laboratories, with shared reagent panels distributed.
- **2027:** Priority mucosal immune markers identified and endorsed for exploratory use in candidate evaluations.
- **2027:** Regulatory and ethical pathways agreed with national authorities and WHO PQ for evaluating new oral and live-attenuated vaccines.
- **2028–2029:** Outbreak-readiness frameworks established for opportunistic evaluation in high-incidence districts, and at least one candidate demonstrates immunogenicity benchmarks and readiness for late-phase assessment.

2. Strengthen surveillance and genomic tools that support targeted interventions

Objective:
By 2029, establish integrated genomic–epidemiological surveillance in at least three endemic countries to guide targeted vaccination, AMR monitoring, and identification of high-risk transmission corridors.

Key milestones:

- **2025–2026:** Rapid confirmation tests for *V. cholerae* O1/O139 validated for district-level use during outbreaks.
- **2026:** National or regional sequencing workflows established for lineage identification and AMR gene detection in two endemic countries.
- **2027:** Integrated genomic–epidemiological analyses implemented to determine whether outbreaks arise from environmental reintroduction or sustained human transmission.
- **2028:** Genomic outputs used to guide targeted vaccination, AMR monitoring, and differentiation of transmission pathways relevant to intervention choice.
- **2029:** Genomic data fully linked with routine surveillance systems to support near–real time operational decision-making.

3. Advance adjunctive therapies and prophylactic tools to reduce disease severity and transmission

Objective:
By 2029, progress at least one adjunctive therapy or prophylactic tool (such as monoclonal antibodies or phage-based approaches) to readiness for early-phase clinical evaluation, supported by harmonised clinical and microbiological endpoints.

Key milestones:

- **2026:** Preclinical evaluation completed for priority monoclonal antibodies targeting colonisation factors or cholera toxin.
- **2026:** Controlled studies of bacteriophage-based approaches completed, including dosing, stability and delivery assessments.
- **2027:** Guidance finalised for evaluating adjunctive therapies during outbreaks without disrupting core rehydration services.
- **2028:** Harmonised clinical and microbiological endpoints adopted, including dehydration scoring and definitions of bacterial shedding.
- **2029:** At least one adjunctive or prophylactic candidate meets readiness criteria for Phase 1 evaluation pending feasibility.

4. Improve environmental and transmission-dynamics data to strengthen risk prediction

Objective:
By 2029, generate operationally useful ecological and transmission indicators that enable

national programmes to anticipate seasonal increases in risk and target interventions more efficiently.

Key milestones:

- **2026:** Integrated studies launched linking clinical surveillance, environmental sampling and genomics to identify drivers of persistence and re-emergence.
- **2026:** Quantitative estimates generated for the contribution of asymptomatic infections to transmission.
- **2027:** Simplified ecological indicators (e.g., rainfall, water quality, plankton or temperature anomalies) developed for use by national programmes.
- **2028:** Settings identified where environmental reservoirs meaningfully contribute to outbreak risk.
- **2029:** Guidance developed to support the integration of ecological and genomic indicators into preparedness and district-level planning.

5. Optimise oral cholera vaccine use for maximum impact under supply constraints

Objective:
By 2029, produce evidence to guide optimal use of limited OCV supply, including updated duration-of-protection estimates, district-level prioritisation tools, and evaluation of targeted reactive and preventive strategies, including household-focused and combined vaccination–WASH approaches.

Key milestones:

- **2026:** Updated estimates generated for protection duration after one- and two-dose regimens in endemic populations, including children under five.
- **2026:** Comparative evaluations of OCV allocation strategies completed, including targeted reactive campaigns, preventive district campaigns, and household or ring approaches.
- **2027:** Genomic, epidemiological and environmental data integrated to refine district-level vaccination strategies.
- **2028:** Modelling analyses completed to evaluate combined intervention packages, including WASH, vaccination and prophylaxis.
- **2029:** Evidence incorporated into national cholera plans and GTFCC guidance to support strategic use of OCV in high-risk settings.

C. Shigella spp.

Introduction/Problem statement

The genus *Shigella* comprises four human-adapted species: *Shigella flexneri*, *Shigella sonnei*, *Shigella dysenteriae*, and *Shigella boydii*, all of which cause diarrhoeal disease in humans. Although *Shigella* spp. are phylogenetically nested within *Escherichia coli*, they are distinguished by their ability to invade colonic epithelial cells and induce intense inflammatory diarrhoea, a phenotype associated with acquisition of a large virulence plasmid (pINV) encoding a type III secretion system (T3SS) and associated effectors [89-91]. Transmission occurs primarily via the faecal–oral route, facilitated by a very low infectious dose, and includes person-to-person spread, contaminated food or water, and in some settings, sexual transmission networks, particularly among men who have sex with men.

Shigella remains a leading cause of moderate to severe diarrhoea in young children in low- and middle-income countries (LMICs), particularly among children under five years of [92-95]. *S. flexneri* is the dominant cause of disease in LMICs, whereas *S. sonnei* accounts for most cases in high-income countries (HICs). In recent years, *S. flexneri* has re-emerged in several HIC settings, alongside continued global expansion of *S. sonnei* [96, 97]. Although *S. dysenteriae* type has historically caused large outbreaks with high fatality rates, it has rarely been detected in recent years [93, 98]. Its potential reemergence remains a concern [99].

Risk of infection is strongly influenced by contextual factors including poor water, sanitation and hygiene (WASH) conditions, malnutrition, and underlying enteropathy [93]. While young children experience the highest incidence and greatest vulnerability to severe outcomes, adults are also affected in specific high-risk settings, including large outbreaks, displaced or crisis-affected populations, and sexual transmission networks [100, 101].

Clinical manifestations range from watery diarrhoea to dysentery with blood and mucus, often accompanied by abdominal pain and fever. Severe disease can result in complications such as haemolytic uraemic syndrome, toxic megacolon, and, more rarely, neurological manifestations. Recurrent *Shigella* infections contribute to growth faltering and long-term adverse health outcomes in young children [93, 102].

Current management relies on rehydration and antimicrobial therapy. However, antimicrobial resistance has increased rapidly, with widespread resistance to ampicillin, trimethoprim–sulfamethoxazole, and increasing resistance to ciprofloxacin and azithromycin [97]. *Shigella spp*. are included on the WHO Bacterial Priority Pathogens List due to the global spread of multidrug-resistant and extensively drug-resistant (XDR) strains [103]. XDR *S. sonnei*, defined as resistance to all widely used oral treatment options including ciprofloxacin, azithromycin, and trimethoprim–sulfamethoxazole, has now been identified across multiple continents, complicating clinical management and outbreak response [97, 101, 104]. Multiple vaccine candidates are in development, but progress is constrained by antigenic diversity and limited understanding of correlates of protection [105, 106].

Key scientific and technical challenges

Progress in *Shigella* control, surveillance, and product development is constrained by several persistent scientific and operational challenges:

- **Incomplete understanding of immunity and correlates of protection.** Protective immunity to *Shigella* is only partially defined. Antibodies directed against O-antigen and invasion plasmid antigens are associated with protection, but no validated immune correlate exists across serotypes or age groups, limiting immunobridging strategies and slowing vaccine evaluation and licensure [105, 106].
- **Substantial serotype and antigenic diversity.** *S. flexneri* comprises multiple serotypes whose incidence varies across regions and over time, while *S. sonnei* continues to expand globally. The extent of cross-protection between serotypes remains uncertain, complicating vaccine design and strain coverage requirements [89, 107, 108].
- **Rapid emergence and spread of antimicrobial resistance.** Resistance to first-line and alternative oral antimicrobials is widespread, and XDR *S. sonnei* and *S. flexneri* have now been documented across multiple continents. Genomic data are not consistently integrated into routine surveillance for outbreak investigation, serotype tracking, or AMR monitoring, limiting early detection of high-risk lineages [97, 101, 104].
- **Limited diagnostic performance and laboratory capacity.** Culture-based diagnostics have low sensitivity for *Shigella* detection and substantially underestimate disease burden compared with molecular methods [90, 93]. A lack of standardised molecular endpoints complicates surveillance and vaccine trial design, and uneven access to PCR-based diagnostics in high-burden settings perpetuates reliance on syndromic case definitions [102, 109].
- **Challenges in conducting paediatric efficacy trials.** Low culture positivity, age-specific incidence patterns, heterogeneity in circulating serotypes, and ethical and logistical constraints associated with enrolling very young children complicate endpoint selection and trial feasibility for vaccines and therapeutics.

Together, these challenges limit progress in vaccine development, therapeutic innovation, and effective surveillance, underscoring the need for coordinated investment in basic research, diagnostics, genomics, and product development.

Basic research priorities

Pathogen biology, pathoadaptation, and host interaction

Shigella spp. are human-adapted intracellular pathogens that have undergone extensive pathoadaptation, characterised by genome reduction through gene loss and pseudogenisation mediated by insertion sequences [89, 110]. Gene loss events include antivirulence regulatory genes, flagellar components, and metabolic pathways, reflecting adaptation to the human host

niche [89]. These processes have contributed to the emergence of a highly specialised pathogen with enhanced invasive capacity and inflammatory potential.

Central to *Shigella* pathogenesis is the large virulence plasmid pINV, which encodes a ~30 kb pathogenicity island containing the type III secretion system (T3SS) and associated effector proteins [89, 111]. The T3SS mediates translocation of effector proteins into host epithelial cells, where they subvert host signalling pathways, facilitate cellular invasion, and promote intracellular survival. Although the T3SS and its effectors are well recognised as critical virulence determinants, important gaps remain in understanding how their expression is regulated in vivo and how variation across lineages influences disease severity and transmission.

Shiga toxin has historically been associated primarily with *S. dysenteriae* type 1; however, it has now been detected in other *Shigella* species, raising questions about its broader contribution to disease severity and outbreak potential [112-114]. Understanding how toxin expression, plasmid stability, and genomic plasticity interact across species and settings remains an important priority.

Key research needs include clarifying the regulation of virulence gene expression during infection, defining host determinants of susceptibility, and understanding how reductive evolution and mobile genetic elements shape pathogenic potential across circulating lineages.

Infection models and mechanisms of pathogenesis

Development of improved infection models is essential for advancing understanding of *Shigella* pathogenesis and accelerating preclinical evaluation of vaccines and therapeutics. Human-specific intestinal organoid models provide a platform to study epithelial invasion, inflammatory responses, and intracellular replication in a physiologically relevant context [115-119]. These models enable investigation of human-restricted aspects of *Shigella* biology that are not adequately captured by traditional animal systems.

Organoid-based platforms also offer opportunities to screen candidate therapeutics earlier in development and to model infection in key demographic contexts, including malnutrition, which is common in high-burden settings and may modify disease severity and immune responses [120]. Integrating host nutritional status into infection models will be important for improving the translational relevance of preclinical findings.

In vitro systems that incorporate gastrointestinal signals have enhanced understanding of virulence gene regulation and pathogenesis. Environmental cues such as bile salts, anaerobic conditions, iron availability, mucins, and fatty acids influence *Shigella* virulence expression and invasion capacity [121-126]. Continued refinement of these models, including incorporation of microbiome components and host immune factors, will support more accurate dissection of infection dynamics.

Priorities over the next three to five years include harmonising infection model approaches, improving their reproducibility across laboratories, and linking model outputs to clinically meaningful endpoints relevant for vaccine and therapeutic development.

Cross-pathogen challenges related to the standardisation, validation, and regulatory relevance of pre-clinical infection models are addressed further in the ***Cross-cutting section on Product Development Science.***

Immunity and correlates of protection

Protective immunity to *Shigella* remains incompletely defined, representing a major barrier to vaccine development. Antibodies directed against O-antigen and invasion plasmid antigens are associated with protection, yet no validated immune correlate exists to support licensure or immunobridging across serotypes or age groups [105, 106]. Systemic immune responses may not fully reflect mucosal immunity at the site of infection, and the relative contributions of mucosal IgA, serum antibodies, cell-mediated immunity, and innate responses remain unclear.

Immunological context varies substantially between endemic and non-endemic settings, where repeated exposure may boost immunity and alter apparent duration of protection [93]. Protection following natural infection or vaccination appears to be serotype-specific and of uncertain durability, particularly in young children who experience the highest disease burden and weakest vaccine responses [127].

Priority research areas include comprehensive mucosal immune profiling using advanced immunological approaches, identification of immune signatures associated with reduced disease severity or protection from reinfection, and development of standardised assays suitable for use in vaccine trials.

Shared challenges related to defining immune correlates of protection, harmonising immunological assays, and enabling immunobridging across bacterial pathogens are addressed further in the ***Cross-cutting section on Product Development Science.***

Asymptomatic and subclinical infection and transmission

Asymptomatic and paucisymptomatic *Shigella* infections are common in high-prevalence settings and are frequently missed by surveillance systems that rely on culture or symptom-based case definitions [93, 102]. Individuals with mild or no symptoms may shed low concentrations of bacteria and contribute to household and community transmission, although the duration and infectiousness of shedding are not well defined.

These knowledge gaps limit understanding of transmission dynamics and hinder the design of interventions aimed at interrupting spread. Longitudinal population-based studies using sensitive molecular diagnostics are needed to define shedding patterns, quantify infection-to-case ratios, and clarify the epidemiological significance of subclinical infections.

Genomic plasticity, evolution, and antimicrobial resistance

Shigella spp. exhibits substantial genomic plasticity driven by horizontal gene transfer, mobile genetic elements, and recombination. These processes underlie serotype switching, antigenic diversity, and the rapid emergence of multidrug-resistant and XDR lineages [89, 96, 107]. Comparative genomics provides critical insight into lineage evolution, transmission, and the identification of conserved antigenic targets for multivalent or cross-protective vaccines.

Despite the growing availability of genomic data, harmonised genotyping frameworks and standardised analytical pipelines remain limited. Strengthening genomic analyses and integrating them into routine surveillance will enable earlier detection of emergent high-risk clones and improve understanding of the evolutionary forces shaping pathogen diversity.

Surveillance priorities

Effective *Shigella* control depends on surveillance systems that accurately capture disease burden, detect changes in species and serotype distribution, and identify emerging antimicrobial resistance. In many HICs, national *Shigella* surveillance relies predominantly on passive reporting of laboratory-confirmed human infections, typically through culture- or PCR-based diagnostics [128, 129]. These systems have enabled detection of changing epidemiological patterns, including increasing transmission within sexual networks, spillover into homeless populations in North America, and rising reports of invasive *Shigella* infections in recent years in the United Kingdom and elsewhere [112, 130-132]. In contrast, surveillance in LMICs remains under-resourced and underfunded, with limited routine microbiological testing and antibiotic susceptibility testing, particularly among children presenting with diarrhoeal disease. Strengthening surveillance in LMICs is essential not only to improve burden estimates but also to enhance detection of *S. dysenteriae*, which is likely under-recognised in current datasets. Improved surveillance across settings is needed to characterise disease burden by species, serotype, and antimicrobial resistance profile in order to guide public health interventions, including vaccine development, deployment strategies, and antimicrobial stewardship.

Clinical and laboratory surveillance

Most *Shigella* surveillance systems rely on syndromic definitions of diarrhoeal disease, which lack specificity and substantially underestimate true *Shigella* incidence, particularly in LMICs. Culture-based diagnosis has limited sensitivity and is increasingly supplanted by molecular diagnostics, yet access to PCR remains uneven and standardised molecular case definitions suitable for surveillance and clinical trials are lacking [90, 93, 109]. As a result, *Shigella* burden, age-specific incidence patterns, and serotype distribution are incompletely characterised, complicating prioritisation of vaccine strategies and evaluation of intervention impact.

Routine microbiological testing and antibiotic susceptibility testing (AST) of stool samples from children with diarrhoea is rare in many endemic settings, further limiting understanding of circulating strains and resistance patterns. This gap is particularly pronounced in LMICs, where surveillance systems remain under-resourced and underfunded.

Antimicrobial resistance and genomic surveillance

Rising antimicrobial resistance represents a major threat to *Shigella* control. Multi-drug resistant (MDR; defined as resistance to ≥3 antimicrobial classes) strains have been detected in all four *Shigella* species, and extensively drug-resistant (XDR) *S. sonnei* and *S. flexneri* have been reported across multiple regions [97, 133-141]. XDR strains are defined by resistance to all widely used oral treatment options, including ciprofloxacin, azithromycin, and trimethoprim-sulfamethoxazole, severely constraining outpatient management.

The recently concluded Enterics for Global Health (EFGH) *Shigella* Surveillance Study documented high levels of resistance to WHO-recommended antibiotics across multiple settings.

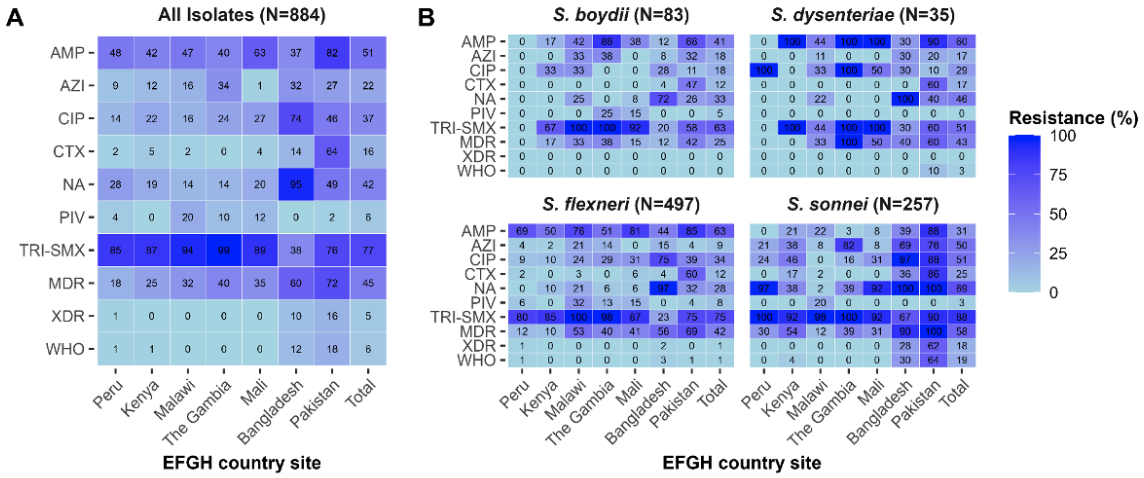

Figure 1. Antimicrobial resistance among *Shigella* isolates overall and by **Shigella** serogroup. The percentage of *Shigella* culture-positive isolates resistant to antibiotics (AMP: ampicillin, AZI: azithromycin, CIP: ciprofloxacin, CTX: ceftriaxone, NA: nalidixic acid, PIV: pivmicellinam, and TRISMX: trimethroprim-sulfamethoxazole) or combined indicators of interest (MDR: multi-drug resistant; XDR: extensively drug resistant; and WHO: resistant to WHO-recommended antibiotics) was determined overall, by EFGH country site, and stratified by *Shigella* serogroup. Resistance was defined as non-susceptibility (resistant or intermediate zone size classifications) per CLSI guidelines, multidrug resistant was defined as resistance to three or more antibiotics, extensively drug resistant was defined as resistance to all of the following: azithromycin, ciprofloxacin, ceftriaxone, trimethoprim-sulfamethoxazole, and ampicillin, and resistant to all WHO-recommended antibiotics was defined as resistance to azithromycin, ciprofloxacin, and ceftriaxone. Source: Yousafzai et al 2025, available: https://ssrn.com/abstract=5386776

Genomic surveillance is critical for tracking *Shigella* species distribution, serotype dynamics, and the emergence and spread of antimicrobial resistance. To date, implementation has been inconsistent, with sequencing concentrated in a small number of reference laboratories and limited routine integration into national surveillance systems.

Plasmids play a central role in the dissemination of antimicrobial resistance determinants in *Shigella*. However, tracking plasmid movement, diversity, and evolution remains challenging using current computational and surveillance approaches, limiting our ability to understand how resistance emerges and propagates across *Shigella* lineages and settings [97, 139-141]. Resistance genes are frequently shared with other gut bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, organisms that also have the capacity for intestinal translocation and invasive disease [142, 143]. These shared reservoirs underscore the need for integrated genomic surveillance across enteric pathogens.

Novel computational methods and improved parameterisation of existing models are needed to better estimate *Shigella* disease burden and transmission across regions and settings [144]. Priority areas include modelling the interaction between climate variability and *Shigella* incidence, as well as distinct transmission dynamics such as the spread of MDR and XDR *Shigella* within sexual transmission networks in high-income countries.

There is also substantial potential for metabolic modelling as a high-throughput approach to examine *Shigella* growth under different environmental and host conditions, facilitating identification of factors that enable persistence and highlighting essential metabolic pathways that could be targeted by novel therapeutics [110].

Higher-level needs related to harmonised genomic surveillance frameworks, plasmid tracking, and integration of AMR data across enteric pathogens are addressed further in the ***Cross-cutting section on Digital Infrastructure and Genomic Surveillance.***

Environmental and wastewater surveillance

Wastewater surveillance may provide a valuable complementary approach for detecting *Shigella* circulation and addressing under-reporting, particularly in urban settings [92, 145]. Environmental detection can help identify transmission trends that are missed by clinical surveillance alone and may support earlier outbreak detection.

While environmental surveillance is not yet widely implemented for *Shigella*, expanding these approaches could enhance understanding of community-level transmission and support more accurate burden estimation.

Shigella dysenteriae type 1

Shigella dysenteriae type 1 (Sd1) has been identified as a pathogen of pandemic potential, despite being rarely detected in contemporary surveillance. Multiple public health laboratories in high-income countries with routine whole genome sequencing (WGS) of *Shigella* report infrequent or no detection of *S. dysenteriae*. Similarly, no Sd1 has been identified in recent large-scale studies conducted in low- and middle-income countries, including GEMS, VIDA, and the Enterics for Global Health (EFGH) study [93, 146, 147].

Historically, Sd1 has played a major role in global shigellosis epidemiology. It was first isolated in Japan in 1897 [148] and caused multiple large-scale transmission waves throughout the twentieth century [117]. The last documented detection of Sd1 occurred in 2011 in Niger [149].

The apparent disappearance of Sd1 from routine surveillance may reflect true epidemiological decline, limitations of current surveillance systems, or both. Given its historical association with severe disease and high mortality, continued surveillance capacity to detect re-emergence remains important, particularly in under-resourced settings where diagnostic coverage is limited.

Product development priorities

Despite the substantial global burden of shigellosis and the rapid emergence of antimicrobial resistance, there is currently no licensed vaccine for *Shigella*, and therapeutic options are increasingly constrained. Product development priorities over the next three to five years span vaccines, therapeutics, diagnostics, and the clinical and translational frameworks needed to evaluate them.

Vaccines

A first-generation *S. sonnei* O-antigen conjugate vaccine demonstrated protective efficacy in adults and children older than three years; however, declining efficacy over time was associated with waning serum O-antigen–specific IgG concentrations [150]. These findings provided early evidence supporting the hypothesis that serum IgG directed against O-antigen correlates with protection against shigellosis.

Several *Shigella* vaccine candidates currently in development are therefore O-antigen–based, reflecting the central role of O-antigen–specific immune responses in protection [105, 151, 152]. Data from the Global Enteric Multicenter Study (GEMS) suggest that a multivalent vaccine targeting *S. sonnei* and *S. flexneri* serotypes 2a, 3a, and 6 (or alternatively 1b) could provide direct protection against at least 72% of circulating *Shigella* strains, with potential cross-protection covering up to 89% of strains globally [90, 153].

Multiple next-generation vaccine platforms are under active development, including:

- Recombinant bioconjugate vaccines produced in genetically engineered *Escherichia coli*, proposed by LimmaTech Biologics [154-156].
- A bivalent conjugate vaccine against *S. sonnei* and *S. flexneri* 2a is currently under evaluation in a Phase 3 trial in infants and young children at icddr,b in Bangladesh [157].
- Synthetic glycoconjugate vaccines, consisting of chemically synthesised oligosaccharides conjugated to a tetanus toxoid carrier protein, developed at the Institut Pasteur [158-160].

- Generalized Modules for Membrane Antigens (GMMA) as an O-antigen delivery platform, proposed by the GSK Vaccines Institute for Global Health [161-163].
- Whole-cell inactivated oral vaccines, including ShigOraVax, which targets *S. sonnei* and *S. flexneri* serotypes 2a, 3a, and 6 and is being developed by Hilleman Laboratories in partnership with EVI and EDCTP.

Despite these advances, major challenges remain, including antigenic diversity, uncertainty around correlates of protection, and the difficulty of conducting efficacy trials in young children who bear the highest disease burden. Beyond immunogenicity and efficacy, candidate *Shigella* vaccines must meet programmatic requirements for use in endemic settings, including suitability for young children, feasibility of multivalent formulations, and compatibility with routine immunisation or outbreak-response delivery. Efforts should be made to assess the impact of candidate vaccines on antimicrobial use/consumption and resistance patterns, including reductions in empiric antibiotic treatment for diarrhoeal disease, in licensure trials as well as in post-introduction impact assessments.

Therapeutics and adjunctive interventions

Current treatment of shigellosis relies on rehydration and antimicrobial therapy. International guideline-recommended empiric treatment for *Shigella*, indicated by visible blood in stool, includes ciprofloxacin as first-line therapy for *Shigella*, with azithromycin as an alternative, and ceftriaxone as second-line. These guidelines strongly encourage that treatment decisions be driven by local susceptibility data, but such data rarely exist for *Shigella*.

Increasing resistance to recommended antibiotics has renewed interest in alternative and adjunctive therapeutic approaches. Hyperimmune bovine colostrum (HBC) has demonstrated bioactivity and efficacy against shigellosis in a non-human primate model, with broad cross-reactivity observed across all four *Shigella* species [164]. Although further preclinical work is required, HBC represents a promising candidate for development as an alternative therapeutic. Bacteriophage-based therapies, including both naturally occurring and engineered phages, have also shown potential as treatments for *Shigella* infection [165-169]. Additional research is needed to demonstrate clinical efficacy, define optimal delivery strategies, and mitigate the risk of phage resistance.

New antibiotics targeting Gram-negative pathogens may offer improved activity against MDR and XDR *Shigella* while potentially preserving the gut microbiome [170]. Lessons from the COVID-19 pandemic have also prompted renewed interest in passive immunotherapy, supported by in vitro evidence demonstrating activity of IgA and IgM antibodies against Shigella [171, 172].

Diagnostics

Accurate and timely diagnosis of *Shigella* infection remains a critical bottleneck for both clinical management and surveillance. Diagnostic limitations contribute to substantial under-

ascertainment of disease burden, delayed outbreak detection, and inappropriate antibiotic use, particularly in low-resource settings.

Culture remains the reference standard for *Shigella* diagnosis and is essential for isolate archiving, serotyping, and antimicrobial susceptibility testing (AST). However, culture has low sensitivity, particularly in children, individuals with prior antibiotic exposure, and asymptomatic or paucisymptomatic infections, and substantially underestimates true disease burden compared with molecular methods [90, 93]. Culture also requires laboratory infrastructure that is often unavailable in peripheral facilities in LMICs, and delays associated with sample transport and processing further reduce yield.

PCR-based diagnostics provide substantially higher sensitivity and have reshaped understanding of *Shigella* epidemiology in research settings. However, lack of standardised PCR targets, cycle threshold cut-offs, and case definitions complicates comparability across studies and limits integration into routine surveillance or vaccine trial endpoints [109]. Molecular detection also does not distinguish viable from non-viable organisms and does not provide antimicrobial susceptibility profiles.

Point-of-care and rapid diagnostics

There is a pressing need for rapid point-of-care diagnostics that can identify *Shigella*-attributable diarrhoea and support appropriate antibiotic use. The WHO-led Antibiotics for Children with Severe Diarrhea (ABCD) trial demonstrated that children with *Shigella*-attributed watery diarrhoea benefit from targeted antibiotic treatment, including improved linear growth outcomes [112, 146, 173]. However, identifying these children at the point of care remains challenging, highlighting the need for deployable diagnostics that can distinguish *Shigella* infection from other causes of diarrhoea.

A rapid nucleic acid amplification-based assay (LAMP) has been evaluated in several settings with promising diagnostic performance [174-176]. While such assays offer improved sensitivity and faster turnaround times, nucleic acid-based diagnostics may face feasibility constraints in routine care, including cost, supply chains, training requirements, and lack of embedded antimicrobial susceptibility information.

Diagnostics to support treatment decisions

Diagnostic gaps directly affect antibiotic stewardship. Routine microbiological testing and AST for children presenting with diarrhoea are rare in most LMICs, contributing to empiric antibiotic use and selection pressure for resistance (see Surveillance priorities and AMR sections). Improved diagnostics could enable targeted therapy, particularly in settings where resistance to ciprofloxacin, azithromycin, and other first-line oral agents is increasing.

The identification of effective oral treatment options such as Pivmecillinam in Bangladesh, where resistance remains low, underscores the need for diagnostics that can guide antibiotic

choice [177]. Similarly, evaluation of tebipenem-pivoxil as an oral carbapenem for drug-resistant *Shigella* infections highlights the importance of diagnostics in preserving last-line therapies and targeting their use appropriately.

Role of diagnostics in research and product development

Diagnostics are also central to vaccine and therapeutic development. Sensitive and specific detection methods are required to define trial endpoints, distinguish vaccine failure from non-*Shigella* diarrhoea, and characterise serotype- and resistance-specific outcomes. Lack of harmonised diagnostic standards complicates cross-trial comparisons and slows evaluation of candidate vaccines and adjunctive therapies.

Further development and validation of diagnostics that are sensitive, affordable, operationally feasible, and aligned with programmatic needs will be essential to improve clinical care, strengthen surveillance, and support evaluation of emerging medical countermeasures.

Accurate diagnosis of *Shigella* infection remains a major challenge for surveillance, burden estimation, and clinical trial design. Conventional culture has low sensitivity and substantially underestimates disease burden compared with molecular methods, particularly in young children and in settings with delayed sample transport or prior antibiotic exposure [90, 93, 102]. As a result, many *Shigella* infections are missed by routine surveillance systems that rely on culture-confirmed cases.

PCR-based diagnostics offer markedly improved sensitivity and have revealed a substantially higher burden of *Shigella*-attributable diarrhoea. However, access to molecular testing remains uneven across endemic settings, and the absence of standardised PCR thresholds or endpoints complicates comparisons across studies and limits their use in regulatory contexts [109]. Continued reliance on syndromic case definitions further obscures species-specific burden and transmission dynamics, particularly in paediatric populations [93, 102].

The lack of harmonised diagnostic endpoints has important implications for vaccine and therapeutic trials, where inconsistent case definitions and low culture positivity reduce statistical power and complicate efficacy assessments. Priority needs include development of standardised, culture-independent diagnostic criteria suitable for surveillance and clinical research, improved access to molecular diagnostics in high-burden settings, and integration of diagnostic outputs with genomic surveillance to support real-time monitoring of circulating species, serotypes, and resistance patterns.

Cross-pathogen challenges related to diagnostic target product profiles, evaluation pathways, regulatory alignment, and integration of diagnostic outputs into surveillance and decision-support systems are addressed further in the ***Cross-cutting sections on Product Development Science, Regulatory Pathways and Policy Alignment, and Digital Infrastructure and Genomic Surveillance.***

Strategic Priorities for *Shigella* (3–5 Year Horizon)

1. Strengthen and standardise *Shigella* diagnostics to guide treatment, surveillance, and trials

Objective:
By 2027–2029, improve detection of *Shigella* infections across clinical, surveillance, and research settings through validated culture-free diagnostics, harmonised immunological assays, and strengthened laboratory capacity, particularly in LMICs.
Key milestones:

- **2025–2026:** Advance and validate improved culture-free diagnostic approaches, including PCR- and metagenomics-based methods, with accompanying analytical pipelines to support molecular epidemiology during outbreaks and routine surveillance.
- **2026–2027:** Evaluate and refine point-of-care diagnostic strategies capable of identifying children and adults who would benefit from antibiotic therapy, informed by findings from the ABCD trial showing benefit of azithromycin in *Shigella*-attributable watery diarrhoea [146, 173].
- **2026–2027:** Further field evaluation of rapid nucleic-acid amplification assays (including LAMP) across multiple settings to define performance characteristics, feasibility, and limitations, acknowledging lack of AST information [174-176].
- **2027–2028:** Develop and standardise immunoassays for measuring serum IgG binding to O-antigen and other candidate antigens, calibrated to WHO reference reagents, to support vaccine evaluation and immunobridging.
- **2028–2029:** Integrate diagnostic advances into vaccine trials and surveillance systems through agreed endpoints and standardised case definitions.

2. Advance development and evaluation of *Shigella* vaccines and alternative therapeutics

Objective:
By 2028–2030, accelerate evaluation of leading *Shigella* vaccine candidates and advance alternative preventive and therapeutic approaches to address MDR and XDR Shigella.
Key milestones:

- **2025–2026:** Continue evaluation of advanced O-antigen-based vaccine candidates, building on evidence of protection associated with serum O-antigen IgG in Phase II trials [105, 150-152].
- **2026–2027:** Progress multivalent vaccine strategies targeting *S. sonnei* and *S. flexneri* (2a, 3a, 6 or 1b), informed by GEMS-derived estimates of strain coverage [90, 153].
- **2026–2028:** Support comparative evaluation of vaccine platforms, including recombinant glycoconjugates (LimmaTech) [154-156], synthetic glycoconjugates (Institut Pasteur) [158-160], GMMA-based approaches (GSK) [161-163], and whole-cell oral vaccines (ShigOraVax).

- **2026–2028:** Advance pre-clinical and translational research on alternative therapeutics, including:
    - Hyperimmune bovine colostrum [164],
    - Natural and engineered bacteriophages [165-169],
    - New oral antibiotics with microbiome-sparing potential [170],
    - Passive immunotherapy approaches supported by in vitro evidence for IgA, IgG, and IgM efficacy [171, 172, 178].
- **2028–2030:** Establish clearer regulatory and evaluation pathways for vaccines and alternative therapeutics, including correlates of protection to enable immunobridging [105, 106].

3. Expand genomic, AMR, environmental, and epidemiological surveillance for *Shigella*

Objective:
By 2027–2029, establish integrated *Shigella* surveillance systems that combine genomics, AMR, environmental data, and epidemiology to better estimate burden, detect emerging threats, and guide interventions.
Key milestones:

- **2025–2026:** Expand routine genomic surveillance of *Shigella*, particularly in LMICs where WGS capacity remains limited, to improve tracking of species, serotypes, AMR profiles, and transmission dynamics [97, 139].
- **2026–2027:** Strengthen AMR surveillance to monitor MDR and XDR Shigella across all four species, including plasmid-mediated resistance and gene sharing with other *Enterobacterales* [133-138, 140, 142, 143].
- **2026–2027:** Integrate wastewater and environmental surveillance to address under-ascertainment and improve burden estimates [92, 145].
- **2026–2028:** Improve surveillance in LMICs to better characterise disease burden, including rare but high-impact pathogens such as *S. dysenteriae* type 1 [93, 98, 147, 149].
- **2027–2029:** Monitor changing epidemiology in HICs, including outbreaks in homeless populations, sexual transmission networks, and increases in invasive disease [112, 130-132].
- **2027–2029:** Develop standardised metadata frameworks capturing species, serotype, Shiga toxin presence, AMR determinants, epidemiological risk factors, and geography.

4. Advance computational and modelling approaches to inform burden estimates and intervention design

Objective:
By 2028–2030, strengthen computational tools to better quantify *Shigella* burden, transmission dynamics, and intervention impact across diverse epidemiological contexts.
Key milestones:

- **2025–2026:** Develop and parameterise models to improve burden estimation across settings, accounting for under-reporting, diagnostic gaps, and age-specific disease patterns [144].
- **2026–2027:** Incorporate climate variability, seasonality, and environmental drivers into transmission models where relevant.

- **2026–2028:** Develop models addressing specific transmission contexts, including MDR/XDR *Shigella* in sexual networks in HICs.
- **2027–2029:** Apply metabolic modelling to identify essential pathways for Shigella growth and persistence, supporting prioritisation of novel therapeutic targets [110].

5. Strengthen digital infrastructure, coordination, and community engagement for *Shigella* preparedness

Objective:
By 2027–2029, enhance global coordination, data sharing, and stakeholder engagement to support *Shigella* R&D, surveillance, and future implementation of vaccines and therapeutics.
Key milestones:

- **2025–2026:** Build consensus on shared digital platforms for secure data sharing, including genomic, AMR, and surveillance datasets.
- **2026–2027:** Establish and maintain online repositories for SOPs, analytical pipelines, and clinical trial protocols.
- **2026–2028:** Convene regular *Shigella*-focused meetings bringing together researchers, public health practitioners, regulators, and funders.
- **2027–2029:** Develop guidance for community engagement, vaccine acceptance, and communication strategies tailored to high-risk populations.
- **2027–2029:** Strengthen mechanisms for incorporating country and regional stakeholder perspectives into *Shigella* R&D prioritisation and policy development.

D.Klebsiella pneumoniae

Introduction/Problem statement

Klebsiella pneumoniae is a Gram-negative opportunistic bacterial pathogen and a leading cause of severe healthcare-associated and community-acquired infections worldwide, including pneumonia, urinary tract infections, and sepsis. The burden of disease is highest among hospitalised patients, neonates, older adults, and individuals with underlying comorbidities, particularly in low- and middle-income countries where access to diagnostics, infection prevention and control (IPC), and effective antimicrobial therapy may be limited.

The species is a major contributor to the global antimicrobial resistance (AMR) crisis. *K. pneumoniae* is naturally resistant to aminopenicillins and frequently resistant to other antimicrobials – a growing proportion of bacteraemic isolates are extensively drug resistant – making it a priority pathogen for development of new antibiotics. Carbapenem-resistant *K. pneumoniae* (CRKP) has spread rapidly across all world regions and is associated with high mortality, prolonged hospitalisation, and frequent outbreaks, particularly in intensive care and neonatal units. Resistance is largely mediated by mobile genetic elements encoding carbapenemases and other resistance determinants, facilitating rapid dissemination within and between healthcare settings.

In parallel, so-called hypervirulent *K. pneumoniae* (hvKp) lineages have emerged and expanded beyond their originally described geographic range. These strains are associated with invasive community-acquired infections, including liver abscess, meningitis, and endophthalmitis, and can cause severe disease in otherwise healthy individuals. Historically, hvKp strains were largely antimicrobial-susceptible; however, recent reports describe increasing convergence of hypervirulence and multidrug resistance within the same genetic backgrounds. The emergence of strains that combine extensive drug resistance with enhanced virulence represents a particularly serious threat, with limited therapeutic options and high epidemic potential.

Despite its substantial and growing global impact, important gaps remain in understanding *K. pneumoniae* transmission dynamics, risk factors for acquisition and invasive disease, and the relative contributions of hospital, community, and One Health reservoirs. Surveillance remains uneven across regions, with limited integration of genomic, epidemiological, and clinical data in many high-burden settings. There is no licensed vaccine, diagnostic tools for rapid risk stratification remain limited, and the antibiotic development pipeline for Gram-negative pathogens remains fragile.

Together, the expanding burden of drug-resistant infections, the emergence of convergent hypervirulent and resistant lineages, and persistent gaps in prevention, diagnostics, and treatment underscore the urgent need for coordinated research and development efforts. Addressing these challenges will require integrated investment across basic science,

surveillance, product development, and outbreak-ready research infrastructure over the next three to five years.

Key scientific and technical challenges

Progress in the prevention, detection, and control of *Klebsiella pneumoniae* infections is constrained by a set of interrelated scientific, technical, and operational challenges that span pathogen biology, transmission, surveillance, and product development.

- **Poorly defined risk factors and disease stratification**
  K. pneumoniae causes a wide spectrum of disease ranging from asymptomatic colonisation to severe invasive infection, yet the host, microbial, and environmental determinants that drive progression to disease remain incompletely understood. Risk factors differ substantially between populations (e.g. neonates, hospitalised adults, community cases), and there is limited ability to prospectively identify individuals at highest risk of invasive disease, transmission, or poor outcomes. In addition, the relative contribution of *K. pneumoniae* to different clinical syndromes—particularly pneumonia in comparison with bloodstream infection, urinary tract infection, and meningitis in at-risk populations—remains incompletely characterised across settings, limiting accurate burden estimation and prioritisation of preventive strategies.
- **Incomplete definition and operationalisation of hypervirulent *K. pneumoniae***
  Although hypervirulent *K. pneumoniae* (hvKp) lineages are increasingly recognised, there is no universally agreed definition that integrates genomic markers, virulence phenotypes, and clinical outcomes. The absence of standardised criteria complicates surveillance, comparison across studies, and prioritisation of interventions. In addition, the clinical relevance of individual virulence determinants and their interaction with host susceptibility remains poorly characterised.
- **Convergence of antimicrobial resistance and hypervirulence**
  Historically distinct populations of multidrug-resistant and hypervirulent *K. pneumoniae* are increasingly converging. The emergence of strains that combine extensive drug resistance with enhanced virulence poses a major threat, yet the evolutionary pathways, transmission dynamics, and fitness costs associated with this convergence are not well understood. This limits the ability to anticipate and mitigate the spread of highest-risk clones.
- **Complex transmission pathways across healthcare, community, and One Health reservoirs**
  K. pneumoniae circulates across hospitals, communities, animals, food systems, and the environment. The relative contributions of these reservoirs to human colonisation and infection vary by setting and remain poorly quantified. In particular, the role of asymptomatic intestinal carriage in sustaining transmission and seeding invasive disease is insufficiently defined, limiting the design of effective infection prevention and control (IPC) strategies.

- **Fragmented and uneven surveillance capacity**
  Surveillance for *K. pneumoniae* infections and AMR is highly variable across regions. Many high-burden settings lack routine microbiological testing, antimicrobial susceptibility testing, and genomic sequencing capacity. Where sequencing is performed, heterogeneity in analytical pipelines, metadata standards, and reporting frameworks limits comparability and real-time use for public health decision-making.
- **Limited integration of genomics with clinical and epidemiological data**
  Although whole-genome sequencing has transformed understanding of *K. pneumoniae* population structure and resistance mechanisms, genomic data are not consistently linked with clinical severity, outcomes, or transmission context. This hampers the ability to identify high-risk clones, predict phenotype from genotype, and translate genomic findings into actionable interventions.
- **Absence of validated correlates and models to support vaccine and therapeutic development**
  There is currently no licensed vaccine against *K. pneumoniae*, and progress is hindered by incomplete understanding of protective immune mechanisms, antigenic diversity, and the relative importance of humoral versus cellular immunity. Preclinical models do not fully recapitulate human disease, particularly in neonates, limiting confidence in candidate selection and evaluation.
- **Limited antibiotic development pipeline for Gram-negative pathogens**
  Despite the need for new treatments, development of novel antibiotics active against *K. pneumoniae* remains slow, with substantial scientific, regulatory, and economic barriers. The rapid evolution and dissemination of resistance further complicate evaluation of new agents and underscore the need for coordinated stewardship, surveillance, and R&D strategies.

Basic research priorities

Despite the major global burden of *Klebsiella pneumoniae* (Kpn) infections, substantial gaps remain in understanding its ecology, pathogenesis, immunity, and genetic determinants of clinical risk. Addressing these gaps is essential to support the rational development of diagnostics, therapeutics, vaccines, and surveillance tools.

Ecology, reservoirs, colonisation, and transmission

K. pneumoniae inhabits a wide range of ecological niches, including humans, animals, and the environment. While gastrointestinal colonisation in humans is common and is generally considered a prerequisite for invasive disease, there is limited understanding of colonisation dynamics, persistence, and factors governing progression from colonisation to infection. In particular, major knowledge gaps remain regarding reservoirs outside the clinical setting, including environmental and non-human sources, and the relative contribution of different sources and transmission routes across settings, which may vary substantially between regions and epidemiological contexts.

Improved understanding is needed of transmission dynamics within and beyond healthcare environments, including the role of food, water, contaminated medical equipment, and other environmental interfaces. Integrated One Health studies are required to clarify the importance of these pathways in different epidemiological contexts and geographic settings and to inform targeted interventions such as infection prevention and control (IPC), water, sanitation and hygiene (WASH), and other countermeasures.

Pathogenesis and host–pathogen interactions

K. pneumoniae is associated with a diverse spectrum of clinical syndromes, yet comparatively little is known about the mechanisms underpinning pathogenesis across these diseases. Critical gaps remain in understanding how Kpn transitions from colonisation to invasive infection, including determinants of epithelial invasion, translocation from the gut, and dissemination to sterile sites. The timing of infection following colonisation, and factors influencing this interval in different contexts (e.g. healthcare-associated versus community-acquired infection, neonates versus adults), remain poorly defined.

Host–pathogen interactions during this transition, including the role of host immunity, microbiota-mediated colonisation resistance, and bacterial virulence determinants, require further investigation. Particular attention is needed for neonatal infections, including the sources and transmission pathways leading to early-life colonisation and sepsis, and the influence of maternal and perinatal risk factors.

Immunity and correlates of protection

There are substantial gaps in knowledge regarding innate and adaptive immune responses to *K. pneumoniae*. Correlates of immune protection have not been defined, and little is known about the durability of immunity following infection, the degree of protection against reinfection, or the extent of cross-protection between strains. The role of mucosal immunity in susceptibility to colonisation and infection remains especially poorly characterised. There are also questions about which immunological targets on the bacteria are the most important. These gaps complicate the rational development and evaluation of vaccines and immunotherapies.

Genetic diversity, hypervirulence, and clinical risk

K. pneumoniae is genetically and functionally diverse, comprising thousands of deep-branching lineages that vary extensively in antimicrobial resistance (AMR), virulence determinants, serotype, and metabolic capacity. This diversity presents major challenges for experimental design, standardisation of infection models, and development of medical countermeasures, as findings derived from individual strains may not generalise across the species.

The relationship between genetic variation and clinical risk remains unclear, particularly for hypervirulent *K. pneumoniae* (hvKpn). Although hvKpn strains have been associated with specific features such as hypermucoidy, acquired siderophores, colibactin, and particular capsule types, there is no consensus definition of hvKpn, nor a coherent framework for detection and tracking. Moreover, virulence determinants in non-hypervirulent lineages remain

poorly understood, and the convergence of virulence and AMR traits poses a growing public health concern.

Antimicrobial resistance, heteroresistance, and within-host evolution

Understanding resistance mechanisms in *K. pneumoniae* remains a critical research priority. Key gaps include the prevalence, mechanisms, and clinical impact of resistance to last-line and novel therapies, including cefiderocol, gepotidacin, and β-lactam/β-lactamase inhibitor combinations. Heteroresistance and heterotolerance to antimicrobials are increasingly recognised but remain poorly detected and interpreted in routine clinical practice, limiting both patient management and surveillance for complex AMR phenotypes.

Within-host evolution of *K. pneumoniae* during colonisation and infection also requires further study, as it may influence treatment failure, promote resistance emergence, and generate phenotypically diverse bacterial populations.

Experimental models and strain resources

Standardisation of experimental models is needed to address gaps in understanding of *K. pneumoniae* pathogenesis and immunity and to support countermeasure development. This includes the development and harmonisation of animal models representing different disease syndromes, such as neonatal sepsis, pneumonia, and urinary tract infection. Where feasible, non-mammalian models should be employed to reduce animal use and lower barriers to participation across diverse research settings.

Broadly accessible and well-characterised strain diversity panels are also needed, including canonical representatives of major AMR lineages and hypervirulent strains. Such resources would support reproducible experimentation, comparative studies, and evaluation of medical countermeasures.

Cross-pathogen challenges related to standardisation, validation, and sharing of infection models and strain resources are addressed further in the ***Cross-cutting section on Product Development Science.***

Molecular tools and functional genomics

Although numerous molecular tools exist for genetic manipulation of *K. pneumoniae*, their performance varies substantially by strain background. Improved and standardised tools are needed to enable clean construction, curation, and complementation of mutants across diverse lineages, allowing rigorous attribution of gene function.

Surveillance priorities

Effective surveillance of *K.pneumoniae* is essential for understanding disease burden, identifying high-risk lineages, tracking antimicrobial resistance, and informing the deployment of infection prevention and control measures. Surveillance challenges arise from the organism’s

ecological breadth, its capacity for asymptomatic colonisation, extensive genetic diversity and geographic variation, and the increasing convergence of antimicrobial resistance and virulence. Addressing these challenges requires integrated clinical, genomic, and One Health surveillance approaches that are feasible across diverse settings, including low-income countries.

Clinical and laboratory surveillance

Clinical surveillance for *K. pneumoniae* infection is largely based on passive reporting of invasive disease, particularly bloodstream infections, pneumonia, and neonatal sepsis. However, this approach underestimates the broader burden of disease, as it does not capture colonisation, non-bacteraemic infections, or progression from carriage to disease, including the potentially substantial but poorly quantified burden of non-bacteraemic pneumonia in at-risk populations. Surveillance systems also vary widely in case definitions, laboratory capacity, and antimicrobial susceptibility testing practices, limiting comparability across sites and regions.

Routine microbiological testing and antimicrobial susceptibility testing are essential for guiding treatment and detecting emerging resistance, yet remain inconsistently implemented, particularly in low- and middle-income countries. Surveillance systems frequently fail to capture heteroresistance and complex resistance phenotypes, further obscuring clinical risk. Strengthening routine diagnostic capacity and standardising reporting practices are therefore critical to improving situational awareness and informing empirical treatment guidelines.

System-level challenges related to sustainable laboratory capacity, workforce development, and quality systems are addressed further in the ***Cross-cutting section on Laboratory and Surveillance Capacity****.*

Genomic surveillance and antimicrobial resistance monitoring

Whole genome sequencing has become a primary tool for characterising *K. pneumoniae* diversity, enabling identification of lineages, resistance determinants, virulence factors, and plasmid content. Although analytical frameworks and tools exist, there are no agreed standards for sequence quality control, reporting of genomic results, or contextual metadata. The absence of harmonised approaches reduces interpretability at the individual laboratory level and limits opportunities for data aggregation and meta-analysis.

Genomic surveillance is particularly important for tracking the emergence and spread of extensively drug-resistant lineages, plasmid-mediated resistance, and the convergence of antimicrobial resistance with virulence-associated traits. Improved frameworks are needed to identify and track plasmids, mobile genetic elements, and within-host evolution. Integrating genomic data with clinical outcomes and epidemiological context will be essential for translating sequence data into actionable public health insights.

While analytical frameworks exist for extracting clinically relevant features from genome sequences, there are no agreed standards for sequence quality control, reporting of results, or contextual metadata. In particular, robust frameworks for identifying, reconstructing, and tracking plasmids, which play a central role in AMR dissemination, are lacking.

These limitations reduce interpretability of genomic data at the laboratory level and constrain opportunities for data aggregation and meta-analysis, which are essential for epidemiological studies, surveillance, and risk assessment. Development of standard protocols and best practices spanning laboratory methods, bioinformatics, and reporting is therefore a key research priority.

Common issues related to genomic data standards, metadata harmonisation, plasmid tracking, and integration of genomic outputs into public health decision-making are addressed further in the ***Cross-cutting sections on Digital Infrastructure and Genomic Surveillance****.*

Neonatal surveillance

Neonates represent the highest-burden and highest-risk population for *K. pneumoniae* infection, yet neonatal surveillance remains fragmented and underdeveloped. Surveillance systems often fail to distinguish neonatal infections from those occurring later in infancy or childhood, obscuring transmission pathways and risk factors specific to this group. There is limited understanding of neonatal colonisation dynamics, sources of acquisition, and progression to invasive disease, including the roles of maternal colonisation, delivery mode, hospital environment, and antimicrobial exposure.

Dedicated neonatal surveillance is required to characterise disease burden, transmission routes, and strain diversity in this population. Linking neonatal clinical data with genomic and phenotypic characterisation of isolates will support improved risk stratification, inform infection prevention and control strategies in maternity and neonatal units, and guide prioritisation of countermeasures.

Environmental, One Health, and colonisation surveillance

K. pneumoniae occupies a wide range of ecological niches, including humans, animals, food, water, and the built environment. Despite this, reservoirs, sources, and modes of transmission outside the clinical setting remain poorly characterised. Surveillance efforts have largely focused on hospital-associated infection, with limited integration of community, environmental, or animal sampling.

Expanded One Health surveillance is needed to clarify the relative importance of different reservoirs and transmission pathways in diverse settings. This includes surveillance of human colonisation in the gut, respiratory tract, and skin, as well as targeted sampling of environmental and non-human niches. Understanding colonisation prevalence, duration, strain diversity, and factors associated with progression to infection will be essential for designing interventions such as infection prevention and control measures, WASH interventions, and targeted prophylaxis.

Data integration, modelling, and early-warning frameworks

The complexity of *K. pneumoniae* epidemiology necessitates improved integration of clinical, genomic, and ecological data. At present, data streams are fragmented, use heterogeneous metadata standards, and are rarely analysed jointly. This limits the ability to assess transmission dynamics, predict risk, or evaluate the impact of interventions.

There is a need for enhanced computational approaches and modelling frameworks to estimate disease burden, explore transmission in different contexts, and assess the spread of multidrug-resistant and extensively drug-resistant lineages. This includes modelling colonisation-to-infection transitions, hospital and community transmission, and the interaction between antimicrobial use, resistance evolution, and clinical outcomes. Improved data integration will support early warning systems, prioritisation of surveillance resources, and evidence-based decision-making for prevention and control.

Cross-pathogen challenges related to data integration, modelling frameworks, and early-warning systems are addressed further in the ***Cross-cutting section on Digital Infrastructure and Genomic Surveillance****.*

Product development priorities

The development of effective medical countermeasures against *K. pneumoniae* is challenged by extensive genetic and phenotypic diversity, high levels of antimicrobial resistance, incomplete understanding of correlates of protection, and the absence of agreed frameworks for evaluating candidate products. Product development must therefore proceed in parallel with advances in basic research, surveillance, and translational infrastructure to ensure that new tools are both scientifically robust and operationally relevant.

Vaccines

There is currently no licensed vaccine for *K. pneumoniae*, despite its substantial contribution to morbidity and mortality from healthcare-associated infections, community-acquired disease, and neonatal sepsis. Vaccine development is complicated by extensive capsular (K-antigen) and O-antigen diversity, uncertainty around correlates of protection, and limited understanding of immune mechanisms that protect against colonisation versus invasive disease.

Priority areas over the next three to five years include identification of antigen combinations that provide broad coverage across clinically relevant lineages, including both classical and hypervirulent strains; development of standardised immunological assays and reference reagents to support comparative evaluation of candidates; and establishment of feasible clinical development pathways. Particular attention is required for neonatal disease, where direct infant vaccination may be challenging and maternal immunisation or passive protection strategies may offer alternative routes to protection. Efforts should be made to assess the impact of candidate vaccines on antimicrobial use/consumption and resistance patterns associated with *K. pneumoniae* infections, including potential reductions in treatment for multidrug-resistant and carbapenem-resistant infections, in licensure trials as well as in future post-introduction impact assessments.

Milestones:

- By 2026: Prioritise capsular and/or O-antigen targets with potential for broad strain coverage using comparative genomic and immunological data.
- By 2026–2027: Establish standardised immunological assays and shared reference reagents to support evaluation and comparison of vaccine candidates.
- By 2027: Generate preclinical immunogenicity and protection data in harmonised infection models, including neonatal-relevant models where feasible.
- By 2028–2029: Define feasible clinical development and regulatory pathways for candidate vaccines, including consideration of maternal immunisation and passive protection strategies.
- By 2029–2030: Advance at least one vaccine candidate to readiness for late-phase clinical evaluation, pending epidemiological feasibility.

Broader challenges related to immune correlates, immunobridging, regulatory expectations, and target product profile development for bacterial vaccines are addressed further in the ***Cross-cutting sections on Product Development Science and Regulatory Pathways and Policy Alignment****.*

Therapeutics and alternative interventions

Antibiotic therapy remains the cornerstone of treatment for *K. pneumoniae* infection, yet intrinsic resistance and widespread acquisition of additional resistance determinants severely limit available options. Development of new antibiotics active against multidrug-resistant and extensively drug-resistant *K. pneumoniae* remains slow and faces substantial scientific, regulatory, and economic barriers.

Alternative and adjunctive approaches, including bacteriophages, monoclonal antibodies, and other immune-based interventions, offer potential routes to mitigate disease severity, reduce bacterial burden, or prevent progression from colonisation to infection, but remain at early stages of development.

Milestones:

- By 2026: Complete preclinical evaluation of at least two novel or repurposed antibiotics or combinations active against MDR/XDR *K. pneumoniae*, including assessment of resistance emergence and heteroresistance.
- By 2026–2027: Advance at least one bacteriophage-based or immune-based adjunctive therapy through proof-of-concept efficacy studies in relevant infection models
- By 2027–2028: Define pharmacokinetic/pharmacodynamic targets and harmonised efficacy endpoints across major disease syndromes, including pneumonia, sepsis, urinary tract infection, and neonatal disease.
- By 2028–2030: Establish evaluation pathways for adjunctive or prophylactic interventions aimed at reducing colonisation or preventing progression to invasive disease.

Cross-pathogen issues related to evaluation pathways, regulatory considerations, and incentives for development of therapeutics targeting multidrug-resistant bacteria are addressed further in the ***Cross-cutting sections on Product Development Science and Manufacturing, Supply Chain Readiness, and Stockpiling****.*

Diagnostics

Diagnostic tools are central to effective control of *K. pneumoniae* infection, informing clinical management, surveillance, and evaluation of medical countermeasures. Current approaches rely largely on culture-based diagnostics, which are slow and may fail to detect colonisation, mixed populations, or heteroresistance. Molecular diagnostics offer improved sensitivity but do not routinely provide antimicrobial susceptibility information and remain unevenly accessible.

Priority diagnostic needs include rapid detection of *K. pneumoniae* and key resistance determinants, improved methods for identifying heteroresistance and complex resistance phenotypes, and diagnostics capable of supporting early risk stratification for severe disease or outbreak potential. Alignment of diagnostic development with surveillance and clinical workflows will be essential to ensure feasibility and uptake.

Milestones:

- By 2026: Validate molecular or rapid diagnostics capable of detecting *K. pneumoniae* and priority resistance determinants within clinically actionable timeframes.
- By 2026–2027: Develop and evaluate methods for detecting heteroresistance and mixed populations suitable for routine clinical and surveillance use.
- By 2027–2028: Integrate diagnostic outputs with genomic and clinical data to support early identification of high-risk strains.
- By 2028–2029: Pilot diagnostic-guided intervention strategies in high-risk settings such as neonatal units and intensive care units.

Generalised challenges related to diagnostic target product profiles, regulatory pathways, and integration of diagnostic outputs into surveillance and decision-support systems are addressed further in the ***Cross-cutting sections on Product Development Science, Regulatory Pathways and Policy Alignment, and Digital Infrastructure and Genomic Surveillance****.*

Translational infrastructure and evaluation pathways

Progress in product development is constrained by limited translational infrastructure and fragmented evaluation pathways. There is a need for:

- Standardised animal models across key disease syndromes to support preclinical evaluation of vaccines, therapeutics, and adjunctive interventions.

- Access to diverse, well-characterised strain panels representing clinically relevant lineages for testing countermeasures.
- Coordinated clinical trial networks capable of evaluating products across different settings, populations, and disease manifestations.
- Harmonised endpoints and outcome measures that enable comparison across studies and products.

Strengthening these enabling systems will accelerate the translation of basic research findings into viable medical countermeasures and improve preparedness for emerging threats posed by *K. pneumoniae*.

System-level challenges related to trial-ready networks, shared evaluation platforms, and sustained clinical research infrastructure are addressed further in the ***Cross-cutting sections on Clinical Trial Infrastructure and Research Readiness****.*

Strategic priorities (3–5-year time horizon)

1) Identify and quantify bacterial and host risk factors, and develop frameworks for assessing clinical and public health risk associated with diverse *K. pneumoniae* strains

Objective:

Develop an integrated risk framework linking bacterial genetic features, host factors, and epidemiological context to clinical outcomes, transmission potential, and public health impact, including agreement on definitions and detection of hypervirulent *K. pneumoniae* (hvKpn).

Key milestones:

- **By 2026:** Map available clinical cohorts (healthcare-associated and community-acquired) with linked patient metadata and matched *K. pneumoniae* isolate genomic and/or phenotypic data ("Kpn risk datasets").
- **By 2027:** Establish an agreed working definition of hvKpn based on molecular markers and/or phenotypes, informed by synthesis of epidemiological, genomic, and mechanistic evidence.
- **By 2027:** Establish a multinational consortium to support collaborative meta-analyses of Kpn risk datasets to:
  - Characterise differences in disease manifestation (classical vs hypervirulent infection) by geography, demographics, comorbidities, and setting.
  - Quantify the impact of known virulence, pathogenicity, and AMR determinants—alone and in combination—across diverse strain backgrounds.
  - Identify novel virulence determinants associated with severe disease or poor outcomes.
- **By 2028:** Complete at least one consortium-led meta-analysis addressing these aims, prioritising neonatal disease where possible.

- **By 2030**: Produce a validated risk framework linking host and bacterial factors to disease syndrome, transmission risk (inside and outside hospitals), AMR evolution, and adverse patient outcomes, to inform surveillance and countermeasure prioritisation.

2) Identify key routes of acquisition and transmission of *K. pneumoniae* to guide interventions inside and outside healthcare settings

Objective:
Clarify reservoirs, sources, and transmission pathways of *K. pneumoniae* across human, environmental, and animal niches to inform effective infection prevention, control, and community-level interventions.

Key milestones:

- **By 2026:** Identify and/or establish cohorts to investigate human colonisation dynamics in diverse community settings (high/low resource, high/low AMR burden), including cross-sectional, longitudinal, and household studies.
- **By 2026:** Map existing One Health surveillance programmes that could be expanded to include *K. pneumoniae*.
- **By 2027:** Engage stakeholders across One Health platforms to develop and deploy protocols for systematic inclusion of *K. pneumoniae*.
- **By 2027:** Develop a best-practice framework for integrated investigations of colonisation and transmission across human and non-human niches, including:
  - Sampling strategies and niche prioritisation.
  - Analytical approaches integrating genomic, epidemiological, and ecological data.
  - Explicit modelling of AMR transmission between *K. pneumoniae* and other organisms.
- **By 2028:** Establish integrated human–environment–animal studies in diverse settings to identify actionable transmission pathways.
- **By 2030:** Evaluate the impact of at least two targeted interventions aimed at disrupting *K. pneumoniae* transmission in community and healthcare settings.

3) Build shared community resources to accelerate *K. pneumoniae* research and countermeasure development

Objective:

Create accessible, well-characterised resources that enable reproducible research, accelerate countermeasure development, and promote global equity in *K. pneumoniae* research capacity.

Key milestones:

- **By 2026:** Establish a community platform to share information on molecular tools (e.g. mutagenesis methods), including strain-specific performance and limitations.

- **By 2026:** Review existing public strain panels (e.g. BEI Resources, Institut Pasteur collections, neonatal sepsis panels) to identify gaps in genotype, phenotype, serotype, and geographic representation.
- **By 2027:** Develop an online strain information hub providing curated information on publicly available strains, including:
    - Key genomic, phenotypic, and epidemiological features.
    - Links to matched multi-omics data and experimental datasets.
    - Access conditions for strains and derived mutant libraries.
- **By 2027:** Establish new strain diversity panels and propagate existing panels into regionally distributed repositories or hubs (e.g. WHO BioHub).
- **By 2028:** Develop mechanisms to facilitate LMIC researcher access to advanced testing facilities (e.g. animal models, high-throughput screening) through centralised services, consortia, or collaborative networks.

4) Develop and standardise infection models to support pathogenesis research and evaluation of medical countermeasures

Objective:
Establish robust, standardised infection models that reflect the diversity of *K. pneumoniae* disease syndromes and support investigation of pathogenesis, immunity, and therapeutic efficacy.

Key milestones:

- **By 2026:** Establish standardised mouse models for major *K. pneumoniae* disease syndromes (e.g. neonatal sepsis, pneumonia, urinary tract infection), and collate existing virulence data for clinically prevalent lineages.
- **By 2027:** Share virulence and model performance data through the strain information hub, and prioritise testing of strains lacking data but of high clinical relevance.
- **By 2027:** Refine animal models using immune signatures (innate, adaptive, and mucosal responses) to better reflect human disease.
- **By 2028:** Evaluate refined models for their ability to prioritise and test candidate countermeasures, including pharmacokinetic/pharmacodynamic (PK/PD) assessment.
- **Across milestones:** Where feasible, incorporate non-mammalian models to reduce animal use and lower barriers to model deployment in diverse research settings, and investigate bacteriophage biology and bacterial anti-phage defence mechanisms relevant to therapeutic development. In parallel, evaluate the scientific, ethical, and operational feasibility of controlled human infection models for *K. pneumoniae*, including ongoing exploratory efforts, to determine whether such approaches could support vaccine or therapeutic development in the future.

5) Develop standards for genomic epidemiology and data integration in *K. pneumoniae* research and surveillance

Objective:
Improve the interpretability, comparability, and public health utility of *K. pneumoniae* genomic data through harmonised standards for sequencing, analysis, and reporting.

Key milestones:

- **By 2026:** Define standard parameters and thresholds for *K. pneumoniae* genome sequence quality, including detection of inter- and intra-species contamination.
- **By 2026:** Agree on contextual metadata standards that accommodate different sampling frameworks and settings to enable data integration.
- **By 2027:** Establish standards and formats for reporting *K. pneumoniae* genomic data across hospital, community, AMR, and vaccine-related surveillance, including:
    - Standardised nomenclature for lineages and high-risk clones.
    - Explicit communication of data quality and uncertainty.
    - Integration with risk frameworks to flag clinically and epidemiologically important strains.
- **Across milestones:** Improve frameworks for identifying, tracking, and interpreting plasmids, heteroresistance, and heterotolerance to support surveillance for complex AMR phenotypes and prediction of treatment failure.

E. Invasive Non-Typhoidal Salmonella (iNTS)

Introduction/Problem statement

Non-typhoidal *Salmonella* (NTS) refers to the diverse group of *Salmonella enterica* serovars excluding the typhoidal serovars Typhi and Paratyphi. The largest burden of disease attributable to non-typhoidal *Salmonella* is *Salmonella* gastroenteritis, which is estimated to cause 93.8 million cases annually (61.8- 231.6 million) and 155,000 deaths (39,000 - 303,000). Of these, roughly 80 million are attributed to foodborne transmission [179].

A subset of infection with non-typhoidal *Salmonella* serovars causes invasive disease characterised by bloodstream infection and a sepsis syndrome. Invasive non-typhoidal *Salmonella* (iNTS) disease is a significant but under-recognised cause of bloodstream infection globally, with an estimated 535,000 cases and 77,500 deaths in 2017 [180]. Approximately 85% of iNTS-related deaths occur in Sub-Saharan Africa, where the disease burden is highest [180]. In many African countries, iNTS has emerged as a leading cause of bloodstream infection in young children, second only to pneumococcal disease [181].

Invasive non-typhoidal *Salmonella* disease occurs predominantly in the context of impaired immune function. Malaria is perhaps the most well-documented risk factors for invasive nontyphoidal Salmonella disease, particularly in sub-Saharan Africa [182-185]. Sickle cell disease and acute malnutrition are also associated with a significant increased risk of invasive disease in children [186]. In adults, immune defects predisposing patients to iNTS have been studied most extensively in advanced HIV disease and other defects of cellular immunity.

The majority (~75%) of iNTS cases worldwide are attributable to *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium; serogroup O:4) and *Salmonella enterica* serovar Enteritidis (*S*. Enteritidis; serogroup O:9).

Invasive non-typhoidal *Salmonella enterica*, particularly serovars Enteritidis and Typhimurium, is highly fatal and disproportionately affects infants, young children, and immunocompromised individuals in sub-Saharan Africa. Current burden estimates are informed by limited primary data, which may have slowed prioritisation and investment in vaccine development. Major epidemiological knowledge gaps persist, including the relationship with co-morbidities and co-infections, limited geographic coverage of surveillance, and differential serovar distribution by setting.

There is a need for combination vaccine approaches. Optimal combinations will depend on age and the geographic *distribution* of disease. The epidemiology suggests an earlier peak of iNTS disease than observed for Salmonella Typhi, and multivalent strategies (including combinations with typhoidal Salmonella serovars) are already in advanced clinical development. These dynamics have market implications, including uncertain demand and potential for product switching.

Diagnostics remain challenging. Blood culture is the primary tool, yet bacteraemia is often low, pretreatment with antibiotics is common, and there are strict limits to blood volumes obtainable

from infants and young children. In parallel, increasing antimicrobial resistance threatens effective treatment and heightens urgency for prevention.

Fundamental knowledge gaps include transmission reservoirs, sources and pathways of infection, colonisation dynamics, the relation between diarrhoeal and invasive disease, and host factors that modulate susceptibility and outcomes. Addressing these gaps is essential to inform product design, deployment, and equitable access.

Reproducible models (animal and cellular) are required to capture intracellular proliferation, effector biology, and host–pathogen interactions; validation against contemporary clinical isolates is critical to ensure relevance. Priorities highlighted by recent expert consultations include: models that better recapitulate human disease; identification of genetic determinants of invasiveness and their ecological drivers; improved understanding of host factors and underlying health conditions that increase susceptibility in low-income settings; links to chronic sequelae (e.g., inflammatory bowel disease); and the influence of food safety and climate on transmission dynamics. Access to patient-derived samples remains a foundational enabler.

Despite its substantial mortality burden and concentration in vulnerable populations, iNTS disease has historically received less attention than other invasive bacterial infections. There is no licensed vaccine for iNTS, diagnostic capacity remains limited in endemic regions, and surveillance systems are fragmented. Recognising this gap, iNTS has been prioritised by global stakeholders, including the World Health Organization, as a pathogen requiring accelerated research and development to inform prevention, treatment, and control strategies.

Addressing iNTS disease will require coordinated advances in basic biology, epidemiology, surveillance, diagnostics, therapeutics, and vaccine development. A clear articulation of the key scientific and operational challenges is essential to guide investment and align efforts over the next three to five years.

Key scientific and technical challenges

Progress in preventing and controlling invasive non-typhoidal *Salmonella* (iNTS) disease is limited by persistent gaps in understanding of transmission, host susceptibility, pathogen diversity, and by weaknesses in surveillance, diagnostics, and clinical trial infrastructure.

- **Uncertain epidemiology and transmission patterns across age groups and settings**. The incidence, age distribution, and geographic patterns of iNTS disease vary substantially across regions, with particularly high burden in young children and neonates in sub-Saharan Africa and lower incidence in many other settings. The relative contributions of foodborne, zoonotic, environmental, and household transmission remain poorly defined and may differ by age group, geography, and epidemiological context. These uncertainties limit accurate burden estimation and hinder the design of targeted prevention strategies.
- **Strong host susceptibility gradients with poorly defined immune mechanisms.** iNTS disproportionately affects young children, neonates, people living with HIV,

individuals with malaria, and those with malnutrition and sickle cell disease. While these risk factors are well described epidemiologically, the immune mechanisms underlying susceptibility and protection remain unclear. There are no validated correlates of protection to support vaccine development or immunobridging.

- **Limited understanding of pathogen determinants of invasiveness**. Invasive disease is dominated by a small number of lineages, notably *Salmonella* Typhimurium ST313 and invasive *S.* Enteritidis, yet the bacterial features driving invasiveness and their interaction with host factors are incompletely understood. This limits risk stratification and the rational design of vaccines and diagnostics.
- **Increasing prevalence of antimicrobial resistance.** Multidrug-resistance is increasingly prevalent in high-burden regions, particularly in sub-Saharan Africa, and are associated with increased morbidity and mortality [187, 188]. Resistance to first-line agents and emerging resistance to third-generation cephalosporins threaten effective case management, while routine antimicrobial susceptibility testing remains uncommon in many endemic settings.
- **Diagnostic and surveillance limitations**. Diagnosis relies largely on blood culture, which has limited sensitivity and requires laboratory capacity often unavailable in peripheral settings. The lack of rapid diagnostics leads to under-detection, misclassification of febrile illness, and delayed treatment. Surveillance systems are fragmented, with limited integration of clinical, microbiological, and genomic data.
- **Constraints on clinical trials and product evaluation.** Heterogeneous incidence, ethical challenges in high-risk populations, and limited trial infrastructure complicate evaluation of vaccines and therapeutics. The absence of validated endpoints beyond invasive disease further slows product development.

Basic research priorities

A coordinated basic research agenda is required to address persistent gaps in understanding the biology, epidemiology, and pathogenesis of invasive non-typhoidal *Salmonella* (iNTS), and to provide a robust foundation for product development, surveillance, and policy.

Ecology, reservoirs, and transmission

A fundamental gap remains in understanding the reservoirs, sources, and transmission pathways of iNTS within and beyond clinical settings. While *Salmonella* is known to circulate in human, animal, food, and environmental reservoirs, the relative contribution of these reservoirs to invasive disease varies by geography and remains poorly defined. Improved understanding is needed of transmission pathways in households, healthcare settings, food and water systems, and the wider environment, including their role in sustaining antimicrobial resistance. These data are essential to inform One Health interventions, including infection prevention and control, WASH strategies, and vaccine deployment.

Colonisation dynamics and progression to invasive disease

Gastrointestinal colonisation is common and is generally considered a prerequisite for invasive disease, yet colonisation dynamics remain poorly characterised. Key gaps include the

prevalence, duration, and diversity of colonising strains; determinants of colonisation resistance; and factors governing progression from colonisation to bloodstream infection. Particular emphasis is required on neonatal colonisation, including maternal risk factors, modes of delivery, and early-life exposures, given the high burden of neonatal sepsis. Understanding the interval between colonisation and infection, and how this varies by host age, immune status, and setting, is critical for risk stratification and intervention design.

Host–pathogen interactions and immunity

Despite the high fatality associated with iNTS, relatively little is known about host immune responses that confer susceptibility or protection. There are substantial gaps in understanding innate and adaptive immune mechanisms, mucosal immunity, and the duration and breadth of protection following infection. Immune defects associated with malaria, HIV, malnutrition, and other comorbidities strongly modify disease risk, yet their mechanistic contribution remains incompletely defined. Improved understanding of these pathways is essential to identify correlates of protection, inform vaccine design, and guide immunisation strategies in high-risk populations.

Pathogen diversity, genomics, and evolution

iNTS is characterised by substantial genetic and functional diversity, with deep-branching lineages displaying variation in virulence, metabolic capacity, and antimicrobial resistance. Although *S. Typhimurium* and *S. Enteritidis* account for approximately 75% of global iNTS cases, other serovars (e.g. Dublin, Panama, Concord) may be under-recognised contributors to invasive disease. The relationship between genetic variation and clinical risk remains unclear, including the determinants of invasiveness and epidemic potential. Improved integration of epidemiological studies with genomic surveillance, using harmonised metadata and quality standards, is needed to clarify lineage-specific risks, AMR evolution, and carriage-disease relationships.

Experimental models and biological systems

Reproducible experimental models are required to capture intracellular proliferation, effector biology, and host–pathogen interactions relevant to invasive disease. Existing animal and cellular models must be refined and validated against contemporary clinical isolates to ensure relevance, particularly for neonatal sepsis and African disease contexts. Exploratory work to assess the feasibility and ethical considerations of controlled human infection models may also provide insights into colonisation dynamics, early host responses, and evaluation of candidate interventions, although such approaches remain at an early stage for iNTS. There is a need for models that better recapitulate human disease, including host immune status and co-morbidities. Access to patient-derived samples remains a foundational enabler for these efforts.

Broader challenges related to model standardisation, validation, and regulatory relevance across pathogens are discussed in the ***Cross-cutting section on Product Development Science****.*

Microbiome and metabolic context

The metabolic environment and gut microbiome play a critical role in colonisation resistance, virulence modulation, and chronic carriage, yet remain poorly understood in the context of iNTS. Longitudinal metagenomic and metabolomic studies, controlling for antibiotics, diet, and co-infections, are required to define microbiome-mediated susceptibility and persistence. These insights may inform both prevention strategies and vaccine performance.

Research tools, platforms, and standards

Scalable research platforms, including barcoded mutant libraries, single-cell and spatial imaging approaches, minimal-element reconstructions, and engineered human gut tissues or organoids, should be deployed to interrogate host–pathogen interactions across geographies and age groups. Basic research efforts should be underpinned by harmonised reagents, reference materials, SOPs, and data standards, and should prioritise the inclusion of contemporary iNTS isolates alongside legacy laboratory strains.

Surveillance priorities

Effective control of invasive non-typhoidal *Salmonella* (iNTS) requires strengthened surveillance systems capable of capturing disease burden, transmission dynamics, and antimicrobial resistance across diverse epidemiological settings. Current surveillance remains fragmented, geographically limited, and biased toward severe or hospital-presenting disease, a limitation common to bloodstream infection surveillance for many invasive bacterial pathogens, constraining both burden estimation and evaluation of medical countermeasures.

Clinical and epidemiological surveillance

Estimates of iNTS incidence and mortality remain uncertain in many high-burden regions, particularly in sub-Saharan Africa, due to limited access to blood culture diagnostics, variable clinical case definitions, and inconsistent case ascertainment. iNTS disease is frequently under-detected in children and adults presenting with febrile illness or sepsis, and age- and setting-specific burden estimates vary widely across studies.

Strengthening surveillance requires expanded use of harmonised clinical case definitions, improved access to blood culture and microbiological diagnostics, and systematic collection of age-stratified data. Surveillance platforms should capture key clinical syndromes, including bloodstream infection and meningitis, and support linkage to outcome data to better characterise disease severity and risk factors.

Genomic surveillance and antimicrobial resistance

Genomic epidemiology frameworks for *Salmonella* Typhimurium and *S.* Enteritidis are needed to support routine surveillance, outbreak detection, and product-policy decision-making. Whole-genome sequencing enables high-resolution tracking of circulating lineages, identification of clonal expansion, and characterisation of antimicrobial resistance determinants relevant to invasive disease.

Surveillance systems should incorporate software tools capable of detecting mutations associated with virulence and invasiveness, alongside antimicrobial resistance genotyping where genotype–phenotype relationships are well defined. Existing genomic frameworks developed for typhoidal *Salmonella*, including nomenclature systems and accessible web-based platforms, provide useful models for usability, governance, and global coordination.

Quality-control guidelines are required to define inclusion criteria for genomes and associated metadata in population-level analyses. Standardised metadata fields capturing sampling purpose, age, clinical syndrome, geography, and resistance profiles will enable interoperability, data aggregation, and re-use. Reporting standards should specify which genome-derived outputs are required for different audiences, including clinicians, public-health practitioners, and policy makers.

Operational guidance is also needed for interpreting genomic signals suggestive of local outbreaks, such as SNP-distance thresholds, and for translating resistance genotypes into actionable phenotypic predictions. User-facing dashboards and structured reviews of surveillance gaps may further improve situational awareness and guide strategic investment.

Genomic epidemiology frameworks should be designed to support integrated One Health surveillance, enabling comparison of *Salmonella* Typhimurium and Enteritidis isolates across human, animal, food, and environmental sources to inform outbreak detection, transmission inference, and product policy.

These needs align with priorities for digital infrastructure, data standards, and genomic analytics described in the ***Cross-cutting section on Digital Infrastructure and Genomic Surveillance****.*

Environmental reservoirs, carriage, and transmission

The environmental reservoirs and transmission pathways that sustain iNTS differ from those of typhoidal *Salmonella* and remain incompletely understood. Community-based studies are needed to quantify asymptomatic non-typhoidal *Salmonella* carriage in healthy populations, particularly among children under five years of age, and to characterise household-level transmission via food, water, and close contact.

Asymptomatic carriage, including among household contacts and convalescing individuals, should be quantified and linked to onward transmission, invasive disease risk, and persistence

of antimicrobial resistance. The duration of shedding, the contribution of convalescent carriers, and the role of repeated exposure in sustaining transmission remain key knowledge gaps.

Integrated One Health studies combining human, animal, food, and environmental sampling are required to clarify the relative contribution of different reservoirs and transmission routes in diverse settings. Such studies will inform prioritisation of preventive interventions, including vaccination strategies, food-safety measures, and antimicrobial-stewardship policies.

Linking surveillance to public health action

Surveillance systems should be designed to directly inform public-health decision-making. Clear thresholds for action, timely reporting of microbiological and genomic results, and mechanisms to translate surveillance findings into policy are essential. Strengthened linkage between surveillance data and national immunisation, antimicrobial-use, and outbreak-response planning will be critical to maximising the impact of future iNTS vaccines and therapeutics.

Product development priorities

Product development for invasive non-typhoidal *Salmonella* (iNTS) remains a critical gap. There are currently no licensed vaccines, diagnostics remain suboptimal for clinical decision-making in high-burden settings, and therapeutic options are increasingly limited by antimicrobial resistance. Progress over the next three to five years will require coordinated advancement of vaccines, diagnostics, and adjunctive therapies, alongside clearer regulatory pathways to enable evaluation and policy adoption.

Vaccines

Vaccination represents the most promising long-term strategy for reducing iNTS morbidity and mortality, particularly among infants and young children in sub-Saharan Africa and other high-incidence settings. Vaccine development efforts have focused primarily on *Salmonella* serovars most commonly associated with invasive disease, *S.* Typhimurium and *S.* Enteritidis. There is also interest in combination vaccine approaches that include antigens from both iNTS serovars and *S.* Typhi, or other enteric pathogens, to maximise public health impact and facilitate integration with existing immunisation programmes.

Vaccine development strategies include O-antigen-based glycoconjugate vaccines as well as approaches that present multiple *Salmonella* membrane antigens to the immune system, with the aim of inducing both humoral and cellular immune responses. These strategies are informed by evidence that antibodies to O-antigen contribute to protection, while recognising that immunity to iNTS is likely multifactorial and incompletely understood, and that further work is needed to identify and prioritise key bacterial antigens and virulence-associated targets that may confer broad and durable protection across invasive *Salmonella* lineages.

Key challenges include limited understanding of immune correlates of protection, uncertainty regarding the durability and breadth of immunity following natural infection or vaccination, and

the need to define optimal target populations. Infants and young children experience the highest disease burden but may respond differently to vaccination due to immune immaturity, maternal antibodies, malnutrition, and co-infections. Older children and adults with HIV infection also represent important target populations.

Priorities include advancing at least one bivalent or multivalent iNTS vaccine candidate through early clinical development, with careful evaluation of safety and immunogenicity across relevant age groups and risk strata. Harmonised immunological assays and agreed exploratory correlates will be essential to support comparison across candidates and to inform eventual immunobridging strategies.

From a regulatory perspective, early engagement with national regulators and WHO will be required to clarify acceptable clinical and immunological endpoints, particularly given the episodic nature of iNTS incidence and the challenges of conducting large efficacy trials. Alignment with existing paediatric immunisation schedules and consideration of co-administration will be important for downstream policy adoption. Efforts should be made to assess the impact of candidate vaccines on antimicrobial use/consumption and resistance patterns in invasive *Salmonella* infections, including potential reductions in treatment with third-generation cephalosporins and other critically important antimicrobials, in licensure trials as well as in post-introduction impact assessments.

Diagnostics

Improved diagnostics are essential to support iNTS case management, surveillance, and product evaluation. Blood culture remains the reference standard but has limited sensitivity, requires laboratory infrastructure, and is often unavailable or delayed in high-burden settings. This constrains timely treatment decisions and limits the ability to identify cases for inclusion in vaccine or therapeutic studies.

Product development priorities include evaluation of culture-independent diagnostic tools capable of detecting iNTS in febrile patients, particularly in paediatric and neonatal populations. Diagnostics that can be deployed at or near the point of care and integrated into routine clinical workflows would substantially improve case detection. However, nucleic acid-based assays may pose feasibility challenges in peripheral settings and do not directly provide antimicrobial susceptibility information, highlighting the need for complementary strategies.

Clear regulatory pathways for validation and approval of new diagnostics, including definition of appropriate reference standards and performance criteria in endemic settings, will be essential to accelerate uptake.

System-level challenges related to diagnostic development, validation, and integration with surveillance and clinical decision-making are addressed further in the ***Cross-cutting sections on Diagnostics, Laboratory Capacity, and Surveillance Systems****.*

Therapeutics and adjunctive interventions

Antimicrobial therapy remains the cornerstone of iNTS treatment, but increasing prevalence of antimicrobial resistance threatens the effectiveness of commonly used regimens. Multidrug-resistant iNTS strains are widely reported, and resistance to third-generation cephalosporins and other critical antibiotics has been documented in multiple regions [188-190].

Product development priorities include evaluation of novel or repurposed antimicrobials with activity against invasive *Salmonella*, particularly oral agents suitable for use in children. There is also interest in adjunctive approaches that may improve outcomes or reduce bacterial burden, although evidence remains limited and largely preclinical.

Regulatory clarity is needed regarding acceptable endpoints for therapeutic evaluation, especially in settings where mortality is high but case numbers fluctuate. Guidance on how new agents can be assessed ethically and efficiently in endemic settings will be critical to sustaining innovation in this space.

Regulatory and policy pathways

Across vaccines, diagnostics, and therapeutics, clearer regulatory and policy pathways are required to enable timely evaluation and adoption of new tools. Early and sustained engagement with WHO, national regulatory authorities, and normative bodies will be essential to define acceptable evidence packages, including the role of immunogenicity data, surrogate endpoints, and post-licensure effectiveness studies.

Alignment of product development strategies with anticipated WHO policy processes will help ensure that promising tools can move efficiently from development to implementation in high-burden countries.

*Cross-pathogen considerations related to regulatory pathways, evidence requirements, and policy alignment are addressed in the **Cross-cutting section on Regulatory Pathways and Policy Alignment**.*

Strategic Priorities (3–5 Year Horizon)

1. Strengthen regional burden estimation and genomic surveillance capacity

Objective:
By 2028, improve the precision and policy utility of iNTS burden estimates and genomic surveillance in high-burden settings.

Key milestones:

- **2026:** Expand harmonised iNTS surveillance in at least three high-burden African countries, using shared case definitions and age-stratified analyses.

- **2026–2027:** Integrate genomic sequencing to refine subnational burden estimates and track *S.* Typhimurium and *S.* Enteritidis lineages.
- **2028:** Establish a prototype open genomic database linking sequence types, AMR profiles, and core metadata to support vaccine target selection and policy modelling.

2. Characterise host and environmental drivers of invasiveness through exemplar studies

Objective:
Clarify how host, pathogen, and environmental factors interact to drive invasive disease in different epidemiological contexts.

Key milestones:

- **2026:** Leverage existing longitudinal cohorts (CHAIN, GEMS, MAL-ED) and selected One Health sites to initiate two to three integrated human–animal–environment studies.
- **2026–2027:** Quantify the contribution of malaria, HIV, malnutrition, and foodborne reservoirs to iNTS risk across study settings.
- **2027:** Generate comparative analyses to inform vaccine target populations and prevention strategies.

3. Advance at least one vaccine candidate and enable evaluation of novel therapeutics

Objective:
Accelerate translational progress for vaccines and alternative interventions against iNTS.

Key milestones:

- **2026–2027:** Support progression of one O-antigen–based or multivalent iNTS vaccine candidate to mid-stage clinical testing (Phase 2).
- **2026–2027:** Develop and harmonise immunoassays and exploratory correlates-of-protection data to support candidate evaluation.
- **2027–2028:** Conduct proof-of-concept preclinical studies of monoclonal antibody and/or bacteriophage therapies to establish feasibility for later translational investment.

4. Develop rapid diagnostic tools and consolidate trial-ready infrastructure

Objective:
Improve detection of iNTS and readiness to evaluate interventions efficiently.

Key milestones:

- **2026:** Validate one rapid, field-deployable diagnostic achieving ≥85% sensitivity and <60-minute turnaround time.
- **2026–2027:** Expand two existing typhoid-vaccine trial platforms to include iNTS endpoints.

- **2027:** Ensure microbiology capacity, data standards, and ethical approvals are in place to support efficient vaccine efficacy evaluation.

5. Pilot community-level carriage and transmission studies to inform implementation

Objective:
Generate evidence to support equitable vaccine introduction and antimicrobial stewardship.

Key milestones:

- **2026–2027:** Conduct pilot community carriage and household transmission studies in one to two high-burden countries.
- **2027:** Integrate findings with national AMR and genomic surveillance platforms.
- **2027**: Produce evidence-based recommendations for vaccine introduction and stewardship interventions.

III. Cross-Cutting R&D Themes

While the preceding sections outline pathogen-specific research and development priorities, many of the scientific, regulatory, and operational challenges identified are shared across pathogens. Historically, investments in surveillance, laboratory capacity, and clinical research have been organised around individual pathogens or products, resulting in fragmented and time-limited capacity that is difficult to sustain once project funding ends.

The cross-cutting priorities described in this section aim to address these systemic challenges by strengthening shared platforms, harmonising methodologies, and enabling efficient reuse of capacity across pathogens and product classes. These priorities are not intended to replace pathogen-specific investments, but rather to ensure that advances in product development, surveillance, and preparedness are cumulative, sustainable, and aligned with country needs.

In several cases, pathogen-specific sections intentionally reference, rather than repeat, detail that is addressed here. This approach reflects the view that progress against high-threat bacterial pathogens will depend not only on advances in individual countermeasures, but also on coordinated investment in enabling systems that support multiple diseases and product pipelines.

While each pathogen included in this roadmap presents distinct epidemiological, biological, and operational challenges, the preceding sections reveal a set of common constraints that repeatedly limit progress across pathogens and product areas. These cross-cutting issues affect the pace, efficiency, and sustainability of research and development for vaccines, diagnostics, and therapeutics, as well as their translation into public-health impact.

Experience across plague, cholera, *Shigella spp.*, *Klebsiella pneumoniae*, and invasive non-typhoidal Salmonella highlights that many of the most persistent bottlenecks are not pathogen specific. These include gaps in foundational product development science, uncertainty in regulatory and policy pathways, insufficiently sustained clinical trial and laboratory infrastructure, fragmented digital and genomic systems, and limited coordination of funding and implementation efforts. In parallel, inadequate early engagement with communities, policymakers, and end users has constrained uptake and effectiveness of otherwise promising interventions.

Addressing these challenges requires deliberate cross-pathogen coordination and investment in shared enablers. Strengthening these systems will not only accelerate development of pathogen-specific countermeasures but also ensure that capacity built through individual programmes is retained, reused, and adapted over time. The cross-cutting priorities outlined below are therefore intended to complement rather than replace pathogen-specific strategies, and to support a more integrated, resilient, and efficient global R&D ecosystem for bacterial preparedness.

1. Cross-cutting product development science

Foundational scientific challenges continue to constrain development of vaccines, diagnostics, and therapeutics across multiple bacterial pathogens. These challenges influence the interpretation of early stage research findings, the design of clinical trials, and the evidentiary pathways required for regulatory and policy decisions.

A major barrier is the absence of validated immune correlates of protection for many bacterial pathogens. Without reliable correlates, vaccine evaluation frequently depends on large efficacy trials, which may be difficult to conduct for pathogens with sporadic outbreaks or geographically heterogeneous incidence. Identification and validation of correlates of protection will require coordinated efforts to harmonise immunological assays, establish shared reference standards, and enable comparability of data across studies and pathogens. Such work has been critical in accelerating vaccine development in other infectious diseases and remains a priority for bacterial pathogens with complex immune responses [191-193].

For several bacterial pathogens, substantial antigenic diversity and geographic variation in circulating strains may further complicate vaccine development and evaluation. Understanding the breadth of immune responses and identifying antigens capable of eliciting cross protective immunity across strains will therefore remain an important area of research [194].

Preclinical infection models also vary widely in their translational relevance. In many cases, existing animal models do not fully reproduce human disease syndromes or transmission dynamics, limiting their predictive value for evaluating candidate vaccines or therapeutics. Differences between human and animal immune responses further complicate interpretation of experimental findings [195]. Improving the reproducibility and standardisation of infection models, strengthening their linkage to clinically meaningful endpoints, and expanding complementary experimental systems such as organoids or ex vivo infection models may improve the efficiency of early product evaluation.

Controlled Human Infection Models (CHIMs) have emerged as a potentially valuable tool for accelerating evaluation of candidate vaccines and improving understanding of host–pathogen interactions. However, their use raises important ethical, safety, and generalisability considerations, particularly when translating findings to populations in high-burden settings. Clear guidance on the appropriate evidentiary role of CHIMs and their integration into regulatory and policy pathways will therefore be important [196, 197]. Addressing these cross cutting scientific challenges will help strengthen the evidence base needed for clinical trials, regulatory evaluation, and the development of effective vaccines, diagnostics, and therapeutics across priority bacterial pathogens.

2. Regulatory pathways and policy alignment

Efficient translation of new vaccines, diagnostics, and therapeutics into public health use depends on clear and predictable regulatory pathways. For many countermeasures targeting epidemic and high burden bacterial pathogens, uncertainty remains regarding the types of evidence required for regulatory approval, policy recommendation, and programmatic implementation. Early engagement between product developers, national regulatory authorities, and WHO can help clarify acceptable evidence packages and reduce delays between product development and policy adoption.

For some epidemic pathogens, traditional efficacy trials may be difficult to conduct because disease incidence is unpredictable or occurs primarily during outbreaks. In such situations, regulatory pathways may need to consider alternative evidence approaches that combine immunogenicity data, observational effectiveness studies, and post introduction evaluations. Mechanisms such as the WHO Emergency Use Listing procedure have been developed to support regulatory decision making in public health emergencies while additional evidence is generated [198]. Clear alignment between regulatory expectations and clinical trial design will therefore be important to ensure that development programmes generate evidence that is relevant for both regulatory review and public health decision making. Regulatory reliance mechanisms, joint review procedures, and collaborative registration pathways, including WHO prequalification and regional regulatory harmonisation initiatives, may help accelerate evaluation while maintaining rigorous regulatory standards.

Regulatory capacity remains uneven across regions. WHO benchmarking assessments indicate that many national regulatory authorities operate below maturity levels required to independently evaluate complex biological products and novel diagnostics. Strengthening regulatory workforce capacity, institutional frameworks, and reliance mechanisms will therefore be essential to support timely evaluation and approval of new countermeasures [199].

Regional regulatory collaboration and harmonisation initiatives may also play an important role. Efforts such as the African Medicines Regulatory Harmonisation programme and the establishment of the African Medicines Agency aim to strengthen regulatory systems while facilitating more efficient review of vaccines and medicines across multiple countries [200]. Regulatory pathways for diagnostics may involve additional considerations, including analytical validation, clinical performance evaluation, and integration with surveillance and laboratory systems. Ensuring that regulatory frameworks are able to evaluate novel diagnostic technologies will be important for supporting early detection of outbreaks and monitoring antimicrobial resistance [201]. Regulatory reliance mechanisms, joint review procedures, and collaborative registration pathways, including WHO prequalification and regional regulatory harmonisation initiatives, may help accelerate evaluation while maintaining rigorous regulatory standards.

Clear articulation of product use cases and Target Product Profiles can support alignment between developers, regulators, and policy makers by clarifying intended product characteristics and evidence needs. Continued dialogue among regulators, public health agencies,

researchers, and product developers will therefore be critical to ensuring that new tools can move efficiently from development to deployment. Alignment between regulatory review, WHO normative guidance, and global policy recommendation processes such as SAGE can help ensure that evidence generated during product development supports both regulatory approval and subsequent policy adoption.

Post licensure studies and pharmacovigilance systems will also be important to monitor the safety, effectiveness, and real-world performance of new tools following introduction. WHO normative guidance and global coordination mechanisms can help support regulatory convergence and facilitate timely evaluation of countermeasures across countries. Strengthened coordination between regulatory agencies, normative bodies, procurement partners, and implementing programmes may also help reduce delays between regulatory authorisation, policy recommendation, and large-scale public health implementation.

3. Clinical trial infrastructure and research readiness

Efficient evaluation of vaccines, therapeutics, and diagnostics requires sustained clinical research infrastructure in settings where disease burden is highest. However, many clinical trial platforms remain project-specific and time-limited, leading to repeated cycles of capacity creation and loss once individual studies conclude.

Strengthening trial-ready research networks in endemic regions could enable more rapid and efficient evaluation of candidate countermeasures. Such networks would benefit from standing governance structures, harmonised protocols, pre-approved ethical review mechanisms, and interoperable data systems. Standardised endpoints and laboratory procedures would also facilitate comparison of results across trials and pathogens.

Preparedness for outbreak-driven studies is particularly important for epidemic bacterial pathogens, where disease incidence may fluctuate substantially over time. Maintaining clinical research sites capable of rapidly activating studies during outbreaks or seasonal peaks will therefore be essential. Coordinated use of shared sites and infrastructure across pathogens may also improve efficiency and help sustain capacity between studies [202, 203].

Clinical trial platforms should support evaluation of the impact of vaccines and other preventive interventions, including monoclonal antibodies, on antimicrobial use and resistance patterns. This may include incorporation of antimicrobial use endpoints, microbiological sampling, and genomic analyses into trial designs to assess downstream effects on antimicrobial prescribing and resistance dynamics.

4. Manufacturing, supply chain readiness, and stockpiling

Even when effective countermeasures are developed, their public health impact ultimately depends on the ability to manufacture, procure, and deploy them at scale. For many epidemic bacterial pathogens, uncertain and episodic demand, particularly for outbreak-focused vaccines, creates challenges for sustainable manufacturing and supply. Such demand uncertainty can

discourage long-term manufacturing investment and complicate production planning for manufacturers [204].

Strengthening surveillance systems can improve forecasting of health needs and support more reliable demand projections. Improved epidemiological intelligence and early detection of outbreaks can help guide production planning, procurement strategies, and stockpile management. Close coordination between public health authorities, manufacturers, procurement agencies, and global partners will therefore be essential to align production capacity with anticipated needs and reduce supply uncertainty [205].

Early engagement with manufacturers can help address issues related to scalability, cost of goods, and technology transfer. This may include planning for secondary manufacturing steps such as fill–finish capacity, which can become a bottleneck during large-scale vaccine deployment. Strengthening regional manufacturing hubs and technology transfer partnerships may improve resilience of supply chains and reduce delays in access during outbreaks [204, 206].

Global procurement mechanisms will also play an important role in ensuring sustainable demand and equitable access. Pooled procurement approaches used by organisations including Gavi, the Vaccine Alliance, and UNICEF Supply Division have helped stabilise vaccine markets, reduce price volatility, and expand access in lower-income settings. Similar market-shaping mechanisms may be needed for outbreak-related countermeasures with uncertain demand [207, 208].

Strategic stockpiling mechanisms may also be necessary for products primarily used during outbreaks. Experience with global vaccine stockpiles, particularly for oral cholera vaccine, illustrates the importance of clear governance structures, sustainable financing, and transparent allocation mechanisms to ensure timely deployment during public health emergencies [209, 210].

5. Community engagement and social & behavioural science

The success of new medical countermeasures depends not only on their technical performance but also on their acceptability and effective use within affected communities. Social, cultural, and behavioural factors strongly influence the uptake of vaccines, diagnostics, and therapeutics, particularly during outbreaks where trust in health authorities may be fragile.

Early and sustained engagement with communities can help identify priorities, address concerns, and ensure that interventions are aligned with local needs and expectations. Integrating community perspectives into product development, clinical trial design, and implementation strategies may improve both participation in research and eventual uptake of new tools.

Insights from social and behavioural science can also inform programmatic characteristics that maximise impact, including delivery strategies, dosing schedules, and communication

approaches. Lessons from previous outbreak responses highlight that community engagement is essential to building trust and improving the effectiveness of public-health interventions [211].

6. Target Product Profiles (TPPs) and use case development

Target Product Profiles (TPPs) provide a structured framework for translating scientific advances into products that can be effectively evaluated, regulated, and implemented in public health programmes. By defining minimum and preferred characteristics for vaccines, diagnostics, and therapeutics, TPPs help guide developers, funders, regulators, and policy makers toward products that are feasible to deploy in real world settings. In this way, TPPs serve as an important bridge between scientific discovery, clinical development, regulatory evaluation, and public health implementation [201, 212].

For vaccines, WHO also develops Preferred Product Characteristics (PPCs) through the Product Development for Vaccines Advisory Committee (PDVAC). PPCs describe the optimal characteristics of vaccines from a public health perspective and are intended to guide early-stage research and development priorities. TPPs build on this guidance by translating public health needs into more detailed product specifications that can inform product development, clinical evaluation, and regulatory assessment [213, 214].

A key step in developing a TPP is defining the intended use case for the product. Structured approaches such as the 7Ws framework can help define product use cases by specifying who will use the product, what the product is, when and where it will be used, why it is needed, which level of the health system will deliver it, and how it will be implemented. Explicit definition of these parameters early in development can help ensure that candidate products are designed with realistic delivery contexts and programmatic constraints in mind [215].

Programmatic considerations including dosing schedules, cold chain requirements, diagnostic turnaround time, affordability, and compatibility with existing health systems should therefore be incorporated early in product development. Clear articulation of use cases and product characteristics can also support alignment between developers, regulators, and policy makers regarding the evidence required for product evaluation and eventual implementation [212].

Development of TPPs typically involves consultation with a broad set of stakeholders including researchers, clinicians, product developers, regulators, public health authorities, and affected communities. This process helps align expectations across stakeholders and can guide research investments toward products most likely to meet public health needs [216].

Within the BacPREP initiative, efforts will focus on facilitating the development, refinement, and periodic review of relevant TPPs across priority bacterial pathogens, drawing on insights from the pathogen specific sections of this roadmap and from ongoing advances in epidemiology, microbiology, and clinical research.

TPPs should be considered living documents that evolve as new scientific evidence emerges, epidemiological patterns change, and implementation experience accumulates. Maintaining this iterative process will help ensure that product development remains closely aligned with evolving public health priorities and can support coordination between research priorities, clinical development programmes, regulatory evaluation, and manufacturing readiness.

7. Digital infrastructure and genomic surveillance

Advances in pathogen genomics and digital technologies are transforming the ability to detect outbreaks, monitor antimicrobial resistance and virulence, and guide public health responses. Whole-genome sequencing can provide high-resolution insights into pathogen evolution, transmission dynamics, and the emergence of antimicrobial resistance, supporting timelier and more targeted public-health interventions [217, 218]. Despite these advances, sequencing capacity and digital infrastructure remain unevenly distributed across regions, and genomic data are often not fully integrated with epidemiological and clinical surveillance systems.

Developing interoperable platforms that link genomic, phenotypic, epidemiological, and clinical data will be critical for improving situational awareness and supporting rapid response to emerging threats. Integration of pathogen genome sequences with epidemiological data substantially improves the ability to infer transmission pathways, track the geographic spread of bacterial lineages, and identify the emergence and international dissemination of antimicrobial-resistant clones [219, 220]. Standardised metadata requirements, including documentation of the purpose of sampling, patient and population context, and laboratory methods, are essential to ensure comparability, generalisability, and interoperability of genomic surveillance data across laboratories and countries.

Analytical approaches including phylogenetic reconstruction enable identification of transmission clusters, geographic spread, and the emergence of high-risk lineages. Several digital platforms illustrate the potential of integrated genomic surveillance approaches. Platforms such as Pathogenwatch provide automated interpretation of pathogen genomes for antimicrobial resistance prediction and lineage assignment, while visualisation tools such as Microreact enable interactive exploration of phylogenetic relationships alongside epidemiological and geographic metadata, facilitating interpretation of genomic epidemiology data by public-health laboratories and researchers [221-223]. Pathogen-specific platforms are also emerging to support integrated genomic surveillance and policy-relevant analyses. TyphiNET integrates genomic data with epidemiological and antimicrobial resistance information to track the global spread of *Salmonella* Typhi lineages and support analyses of antimicrobial resistance trends and vaccine impact [224].

Global antimicrobial resistance surveillance systems also play a complementary role in linking genomic and phenotypic data. The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) provides a global framework for collecting standardised antimicrobial resistance data from participating countries. While GLASS currently focuses primarily on phenotypic surveillance, ongoing efforts aim to strengthen the integration of genomic

approaches within national antimicrobial resistance surveillance programmes and broader genomic surveillance initiatives [225].

Expanding genomic surveillance capacity will require sustained investment in sequencing infrastructure, bioinformatics expertise, and data management systems. Analytical tools that translate genomic signals such as antimicrobial resistance determinants, virulence markers, or transmission clusters into actionable public health insights will be essential. User-facing dashboards and decision-support tools can help public health authorities interpret genomic data and guide responses in near real time. Strengthening digital infrastructure that supports secure data sharing, integrated analysis, and timely interpretation will therefore be critical for improving outbreak detection, monitoring antimicrobial resistance, and informing public health interventions across priority bacterial pathogens.

Within BacPREP, strengthening digital infrastructure and genomic surveillance will support earlier detection of emerging bacterial threats, improved monitoring of antimicrobial resistance, and stronger integration of laboratory, epidemiological, and clinical data to inform public health decision making.

8. Laboratory and surveillance capacity

Laboratory and surveillance capacity should be developed as a permanent component of national public health systems, not as a temporary activity tied to individual projects or emergency funding cycles. This means investing in country-owned laboratory networks, routine surveillance pathways, and national decision-making systems that remain functional between outbreaks and can be rapidly mobilized during emergencies. WHO guidance has consistently emphasized that laboratory-based surveillance should be embedded within national public-health infrastructure and existing surveillance mechanisms, with national commitment, designated reference functions, standardized methods, quality systems, and established channels for data sharing and action [219, 226].

Surveillance investments are most effective when they strengthen integrated national systems rather than parallel, externally driven projects. Laboratory findings have the greatest public health value when they are linked to clinical and population data and reported through routine national structures. By contrast, fragmented research initiatives or isolated institutional efforts often generate data that are difficult to use for stewardship, outbreak detection, or policy [227, 228]. Integrated surveillance systems that connect laboratory networks with epidemiology and health information systems should be prioritised, enabling preparedness data to guide action from local to global levels.

Access to bacteriology and antimicrobial susceptibility testing remains highly uneven across many settings. In a 14-country assessment of tiered laboratory networks in sub-Saharan Africa, only about 1% of listed laboratories were formally assigned to bacterial testing, and laboratories with antimicrobial susceptibility testing capacity collectively served less than half of the

population in seven countries. Major gaps were also identified in accreditation, laboratory information systems, standardization, and quality assurance [229]. Strengthening laboratory capacity across the network, including expanded bacteriology and susceptibility testing, sentinel-site designation, and stronger quality systems at lower tiers and in underserved settings, should therefore be prioritised.

Effective surveillance systems depend not only on equipment and assays, but also on governance, policy alignment, stakeholder engagement, data infrastructure, representativeness, and sustainable financing. Although many systems begin with external support, long-term viability requires transition to domestic institutional and governmental funding [219, 228]. BacPREP should therefore promote models designed from the beginning for integration into routine national public-health functions, with clear national ownership and financing, rather than short project cycles that generate temporary outputs without lasting capacity.

Investing in trained staff and strong quality systems is essential for preparedness because laboratory capacity only matters if the results are accurate and trustworthy. WHO guidance emphasises that laboratory-based surveillance requires trained personnel, standard operating procedures, recognized testing methods, external quality assessment, and quality management systems. The aim is not simply to increase testing volume, but to produce data that are reliable, comparable, and trusted over time. This workforce agenda should also extend beyond bench microbiology, since preparedness increasingly depends on personnel able to manage data systems, interpret microbiological and genomic findings in an epidemiological context, and translate laboratory outputs into public-health action [226, 227]. WHO should therefore support multidisciplinary capacity building that includes laboratorians, epidemiologists, informatics specialists, data managers, and end users in public health programs.

Digital and informatics capacity is also essential for sustainability. In many resource-limited settings, microbiology data systems are still too weak to ensure that laboratory results can be fully used for surveillance and public health action. Without information systems that enable efficient workflows, result validation, analysis, and reporting, investments in laboratory capacity cannot be fully translated into surveillance and action. At the same time, digital tools cannot compensate for weak infrastructure, workforce shortages, or fragmented governance [230, 231]. BacPREP should therefore treat laboratory informatics as a core part of preparedness, while ensuring that investments in data systems accompany broader strengthening of laboratories, workforce, and governance.

Data-sharing governance should likewise be established before acute events occur. Agreed data and metadata standards, shared access principles, harmonized methods, and pre-existing collaboration arrangements are essential if microbiological, genomic, clinical, and epidemiological data are to be linked and shared rapidly during outbreaks. Without this groundwork, even technically capable laboratories may fail to provide timely situational awareness [219, 227].

Preparedness also depends on operational resilience. Recent emergencies showed that shortages of reagents and consumables can quickly become major bottlenecks, and that weak stock management, sample transport, metadata handling, and inter-laboratory coordination can sharply reduce surveillance effectiveness [219, 227]. Systems should also be designed to function routinely and expand during emergencies, with surge procedures, technical and material reserves, simulation exercises, and stepwise strengthening through in-country capacity and referral networks.

Reducing disparities between high-income settings and low- and middle-income settings should be an explicit objective of laboratory investments. Persistent gaps in access to culture and susceptibility testing, quality assurance, digital systems, trained personnel, and financing continue to limit both national surveillance capacity and participation in global platforms [229, 231]. Investments should therefore support realistic, phased, and country-led strengthening of laboratory networks, including referral and regional support models where appropriate, so that countries bearing the highest bacterial burden can generate and use preparedness data on more equal terms.

Finally, laboratory preparedness requires governance that is both rigorous and enabling. Poorly defined or excessive review requirements can create administrative bottlenecks, delay time-sensitive surveillance and pathogen characterization work, and disproportionately burden less-resourced institutions [232].

Surveillance systems should support evaluation of the impact of vaccines and other preventive interventions, including monoclonal antibodies, on antimicrobial use and resistance patterns. This includes integrating antimicrobial consumption data, AMR phenotyping, and genomic surveillance into clinical trials and post-introduction studies to assess downstream effects on antimicrobial prescribing and resistance dynamics.

9. Funding, coordination, and sustainability

Implementation of the research priorities outlined in this roadmap will require sustained coordination across governments, international organisations, research institutions, product developers, and funders. However, financing for countermeasures targeting epidemic bacterial pathogens remains limited compared with other global health threats, reflecting uncertain demand and limited commercial incentives.

Coordinated investment across the full product lifecycle—from surveillance and basic research through product development, clinical trials, manufacturing, and deployment—will therefore be essential. Public–private partnerships, pooled financing mechanisms, and coordinated procurement strategies may help reduce financial risk for developers while ensuring equitable access to products once they become available.

Global coordination platforms can also help align research priorities, facilitate data sharing, and avoid duplication of effort across initiatives. Strengthening collaboration between international

organisations, regional public-health bodies, and national research institutions will be important for sustaining preparedness and accelerating the translation of research into policy and practice.

IV. The Way Forward

Measuring Progress

To ensure accountability and sustained momentum, BacPREP will establish a monitoring framework with clear performance indicators and measurable outcomes. Key metrics will include:

- Number of validated correlates of protection (CoPs) and reference standards established across pathogens;
- Licensed or prequalified vaccines, diagnostics, and therapeutics meeting BacPREP Target Product Profiles;
- Functional genomic and epidemiological surveillance systems operating in at least three regions;
- Expansion of regional manufacturing, stockpile, and clinical-trial capacity; and
- Evidence of improved community engagement, equitable access, and integration of social-science research.
- Progress will be reviewed annually and benchmarked against defined three- to five-year strategic milestones.

Tracking Reach

A dedicated BacPREP dashboard will be developed to visualise progress across scientific, operational, and policy domains. The dashboard will capture indicators such as number of active country partnerships, research outputs, product pipeline status, and training activities. An annual review mechanism—led jointly by WHO and consortium partners—will assess achievements, identify bottlenecks, and align priorities for the next implementation cycle. Regular publication of these reports will enhance transparency and foster cross-consortium learning.

Alignment with Global Frameworks

BacPREP will operate in full alignment with existing WHO and global preparedness frameworks. This includes the WHO R&D Blueprint, the Global Action Plan on Antimicrobial Resistance (AMR-GAP), and the Health Emergency Preparedness, Response and Resilience (HEPR) framework. Synergies will also be pursued with CEPI, Gavi, Wellcome, and other partners to ensure coherence between bacterial-pathogen research, AMR initiatives, and broader health-security agendas. Continuous engagement with WHO regional offices and national ministries of

health will support translation of roadmap priorities into country-level implementation plans, ensuring that BacPREP contributes meaningfully to global pandemic-preparedness goals by 2030.

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