Protection Against Invasive NTS Disease (PAiNTS)

Identifying correlates of protection and thresholds of protective immunity using a *Salmonella* Typhimurium challenge-rechallenge model

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

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This protocol describes the PAiNTS study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AR	Adverse reaction
ASC	Antibody secreting cell
AST	Aspartate transaminase
BD	bis in die (Latin: twice a day; prescription medicines)
BCR	B cell receptor
BMI	Body Mass Index
CHANTS	Challenge Non-Typhoidal Salmonella study
CHIM	Controlled human infection model
CH1/CH2	Challenge 1/Challenge 2
CI	Chief investigator or confidence interval
CFU	Colony forming unit
CoP	Correlates of Protection
COPS	Core and O-polysaccharides conjugate vaccines
CRF	Case Report Form
CRP	C-reactive protein
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CyTOF	Cytometry time of flight
DALY	Disability-adjusted life year
DNA	Deoxyribonucleic acid
DSMC	Data Safety and Monitoring Committee
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme linked immunosorbent spot assay
FBC	Full blood count
FFQ	Food Frequency Questionnaire
GAD-7	General Anxiety Disorder-7
GCP	Good Clinical Practice
GMMA	Generalised modules for membrane antigens
GP	General Practitioner
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HPU	Health Protection Unit
HRA	Health Research Authority
ICF	Informed Consent Form
ICHT	Imperial College Healthcare NHS trust
Imperial	Imperial College London
ICRF	NIHR/Wellcome Trust Imperial Clinical Research Facility
IFN	Interferon
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IL	Interleukin
iNTS	Invasive non-typhoidal *Salmonella*
ISF	Investigator site file
LFT	Liver function tests
LPS	Lipopolysaccharide
MAPS	Multiple-antigen presenting system
MAX	Maximum (prescription medication)

NIHR	National Institute for Health and Care Research
NRES	National Research Ethics Service
NTS	Non-typhoidal *Salmonella*
NWLP	North West London Pathology Laboratory (Charing Cross Hospital, Imperial Healthcare NHS Trust)
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PE	Protective Effect
PHQ-9	Patient Health Questionnaire-9
PIS	Participant information sheet
PO	Per oral (by mouth)
PRN	pro re nata (latin: as required; prescription medicines)
QDS	quater die sumendus (Latin: four times a day; prescription medicines)
RDAR	Negative red, dry and rough
REC	Research Ethics Committee
REC ref.	Research Ethics Committee reference
RNA	Ribonucleic acid
SAE	Serious adverse event
SBA	Serum bactericidal assay
SCR	Summary Care Record
SD	*Salmonella* Diagnosis
SMP(C)	Summary of Medicinal Product (Characteristics)
SOP	Standard Operating Procedure
Spp.	Species (plural)
ST	Sequence type
SUSAR	Suspected unexpected serious adverse reactions
TCR	T cell receptor
TDS	ter die sumendum, (Latin: three times a day, prescription medications)
TMF	Trial Master File
TOPS	The Over volunteering Prevention System (see: http://www.tops.org.uk)
TSB	Tryptone soya broth
TSC	Trial Steering Committee
ULN	Upper limit of normal
U&E	Urea & electrolytes
WBC	White blood cell/count
WGS	Whole genome sequencing
WHO	World Health Organisation
WRAIR	Walter Reed Army Institute of Research
XLD	Xylose lysine deoxycholate
95%CI	95% Confidence Interval

KEYWORDS
Salmonella
Salmonella Typhimurium
Non-typhoidal *Salmonella*
Human challenge model
Controlled human infection model
Correlates of protection
D23580

STUDY SUMMARY

Study Title	Protection Against Invasive NTS Disease (PAiNTS) - correlates of protection towards vaccine licensure	
Short title (internal ref.)	PAiNTS study	
Clinical Phase	Phase 1 exploratory controlled human infection study	
Study Design	Human infection challenge and re-challenge study	
Study Participants	Healthy adults aged 18 to 50 years inclusive	
Planned Sample Size	30-50	
Follow up duration	6 to 9 months	
Planned Trial Period	This will align with grant dates, once grant is activated	
Challenge Strain	*Salmonella* Typhimurium (Strain D23580)	
Challenge Dose	1-5 x 10^5 Colony Forming units (CFU)	
Formulation	Oral challenge suspended in 0.9% saline given for oral ingestion, with sodium bicarbonate administered prior to ingestion.	
Objectives and Endpoints		
	Objective(s)	**Endpoint(s)**
Primary endpoints	To determine the attack rate of systemic *Salmonellosis* after challenge and re-challenge with *Salmonella* Typhimurium.	The proportion of participants who develop either: Fever ≥38°C on ≥2 occasions ≥12 hours apart **AND/OR** *Salmonella* Typhimurium bacteraemia
Secondary	To describe the *Salmonella* colonisation rate following oral challenge and re-challenge with *Salmonella* Typhimurium	The proportion from whom *Salmonella* Typhimurium is isolated from stool on ≥2 occasions ≥48 hours from challenge OR re-challenge.
	To describe the rate of gastroenteritis following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants at different doses at each strain developing any of: Any diarrhoea **and/or** Severe diarrhoea **and/or** Moderate diarrhoea **and/or** Mild Diarrhoea
	To determine the fever rate following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants who develop any fever ≥38°C

To describe the *Salmonella* bacteraemia rate following oral challenge or re-challenge with *Salmonella* Typhimurium	The proportion of participants developing *Salmonella* Typhimurium bacteraemia at any timepoint following challenge or re-challenge.
To describe the rate of systemic Salmonellosis according to **an alternative composite diagnostic criteria** following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants meeting the criteria for a composite diagnosis of *Salmonellosis* defined as any of 1) *Salmonella* Typhimurium is isolated from stool on ≥2 occasions ≥48 hours from challenge and/or 2) *Salmonella* gastroenteritis and/or 3) Any fever ≥38°C 4) *Salmonella* Typhimurium bacteraemia detected by blood culture and/or novel molecular methods
To describe the safety of challenge and re-challenge with *Salmonella* Typhimurium	The proportion of participants at different doses of each strain reporting Adverse events (AE), Adverse events of special interest (AESI), Serious Adverse Events (SAE). SUSARs. Concomitant medication usage as outlined in the study protocol
To compare **clinical** features following oral challenge or re-challenge with *Salmonella* Typhimurium	A comparison of the clinical features of *Salmonella* infection after challenge and re-challenge with D23580 strain, with specific reference to: • The proportion of participants in each group developing any diarrhoea. • The severity of diarrhoea in each group, as measured by: • Frequency of bowel motions/24hrs • Total gastrointestinal symptom severity score calculated by summing numerical values assigned to the severity of all solicited symptoms between Day 0 to Day 14 • Total systemic symptom severity score calculated by summing numerical values assigned to the severity of all solicited and unsolicited gastrointestinal and systemic symptoms between Day 0 to Day 14 (graded 0-4). • Area under the curve for gastrointestinal and systemic symptom severity score between Day 0 to Day 14 • Time to onset of diarrhoea in each group, measured in hours since challenge to the first grade 6/7 stool.

	• The proportion of participants in each group developing any fever ≥38°C • Fever clearance time of febrile participants in each group measured by time in hours to first temperature <38°C lasting ≥12hrs The proportion of participants in each group reporting the following solicited symptoms at any time: • headache, • malaise • diarrhoea • abdominal pain • nausea • vomiting • myalgia • arthralgia • cough • tenesmus Duration of solicited symptoms measured in days from first symptom onset to complete resolution of symptoms Severity of solicited and unsolicited symptoms measured by: The proportion of participants with maximum symptom severity score graded 0-4 as defined in The proportion of participants meeting the criteria for severe *Salmonellosis* Symptom severity scores for individual solicited and unsolicited symptoms calculated by summing numerical values assigned to the severity of symptoms between Day 0 to Day 14 (graded 0-4).
To compare **microbiological** features of **gastrointestinal infection** following oral challenge or re-challenge with *Salmonella* Typhimurium	Gastrointestinal *Salmonella* infection after challenge or re-challenge, with specific reference to: Time to onset in days in each group from challenge to first stool sample positive for *Salmonella* Typhimurium by culture and/or PCR Duration of stool shedding in each group measured in days from first stool sample positive for *Salmonella* The duration of stool shedding in each group measured in days from first stool sample positive by culture and/or for *Salmonella* Typhimurium to first persistently negative stool sample (defined as three consecutive samples negative)

		The magnitude of stool shedding measured in CFU/ml in quantitative stool culture analysis
	To compare **microbiological** features of **bloodstream infection** following oral challenge and re-challenge with *S.* Typhimurium	*Salmonella* bloodstream infection after challenge or re-challenge, with specific reference to The proportion of participants in each group developing any bacteraemia The severity of bacteraemia in each group as measured by: The duration of bacteraemia measured by time in hours from the first positive blood culture to first persistently negative blood culture Blood culture clearance time measured by time in hours from initiation of antibiotics treatment to first persistently negative blood culture Magnitude of bacteraemia measured in CFU/ml in quantitative blood culture samples collected immediately prior to the initiation of treatment The time to onset of bacteraemia in each group, measured by hours since challenge
	To compare biochemical and haematological laboratory parameters following oral challenge or re-challenge with *S.* Typhimurium	Absolute values of laboratory parameters from time of challenge to Day 28 and/or Day 90, with specific reference to Total haemoglobin (d/L) Haemoglobin change from baseline (Hb g/l D0 – nadir Hb g/l) Total white cell count ($x10^9$/l) Neutrophil count ($x10^9$/l) Lymphocyte count ($x10^9$/l) Eosinophil count ($x10^9$/l) Monocyte/lymphocyte ratio Urea and electrolytes (Na+,K+, Urea, Creatinine – mmol/l) C-reactive protein (mg/l) Liver function tests (Bilirubin [umol/l], aspartate transaminase (AST IU/l), alkaline phosphatase (ALP IU/l), alanine transaminase (ALT IU/l), Albumin (g/l)
	To describe and compare the serum antibody response following oral challenge and re-challenge with *S.* Typhimurium	Absolute values of laboratory assays on serum samples measured at baseline and post challenge time points, including but not limited to *S.* Typhimurium O-specific polysaccharide serum IgG and IgA concentration measured by enzyme-linked immunosorbent assay (ELISA) *S.* Typhimurium specific serum IgG and IgA concentration against other S. Typhimurium antigens measured by ELISA

		Serum bactericidal antibody titres against *Salmonella* Typhimurium Other binding and functional antibody activity measurements including (but not limited to) antigen arrays, systems serology platforms etc.
Exploratory	To investigate how the human microbiota interacts with oral challenge of *S.* Typhimurium	Pathogen specific and metagenomic analysis of stool and/or saliva to measure constituent microbiological flora (and their metabolome, transcriptome and anti-microbial resistance profile) at baseline and post-challenge time points
	To investigate the impact of diet on the outcome of *Salmonella* challenge.	Rate of challenge outcomes stratified by dietary composition, as assessed by a food diary.
	To describe and compare cell-mediated immune response following oral challenge with *S.* Typhimurium	To describe and compare cell-mediated immune response following oral challenge with S. Typhimurium Absolute values and inter-group comparison of laboratory assays performed on peripheral blood mononuclear cells measured at baseline and post challenge time points including (but not limited to) Description of lymphocyte populations at baseline and following challenge as measured by flow cytometry and/or Cytometry by time of flight (CyTOF) Frequency and magnitude of S. Typhimurium specific cell-mediated immune responses as measured by ELISPOT and flow cytometry and/or CyTOF
	To describe and compare the mucosal antibody response following oral challenge and re-challenge with *S.* Typhimurium	Absolute values of laboratory assays on saliva (and/or stool copro-antibodies) samples measured at baseline and post challenge time points using laboratory assays including (but not limited to) S. Typhimurium specific IgG and IgA concentration measured by ELISA Bactericidal antibody titres Other functional antibody activity measurements including systems serology platforms
	To investigate novel diagnostic methods for detecting *S.* Typhimurium infection	Exploratory analysis of blood and faecal samples, including novel molecular techniques
	To describe and compare additional host immune responses following oral challenge and re-challenge of *S.* Typhimurium D23580 strains.	Analysis of B-cell repertoire, T-cell repertoire, plasma cytokine profile and kinetics, additional exploratory immunology

	To evaluate host-pathogen interactions following *Salmonella* infection including the presence and mechanisms of *Salmonella* persister infected macrophages	Exploratory analysis of infected immune cells from blood including, but not limited to, single-cell RNA-seq.
	To describe participant experience following oral challenge or re-challenge with S. Typhimurium	Descriptive statistics following administration of a questionnaire post-challenge
	To described epigenetic markers associated with susceptibility to *Salmonella* infection.	Identification and quantification of epigenetic modifications (e.g., DNA methylation, histone modifications) in whole blood and specific leukocyte sub-populations that correlate with susceptibility to *Salmonella* infection.

REFERENCE DIAGRAM

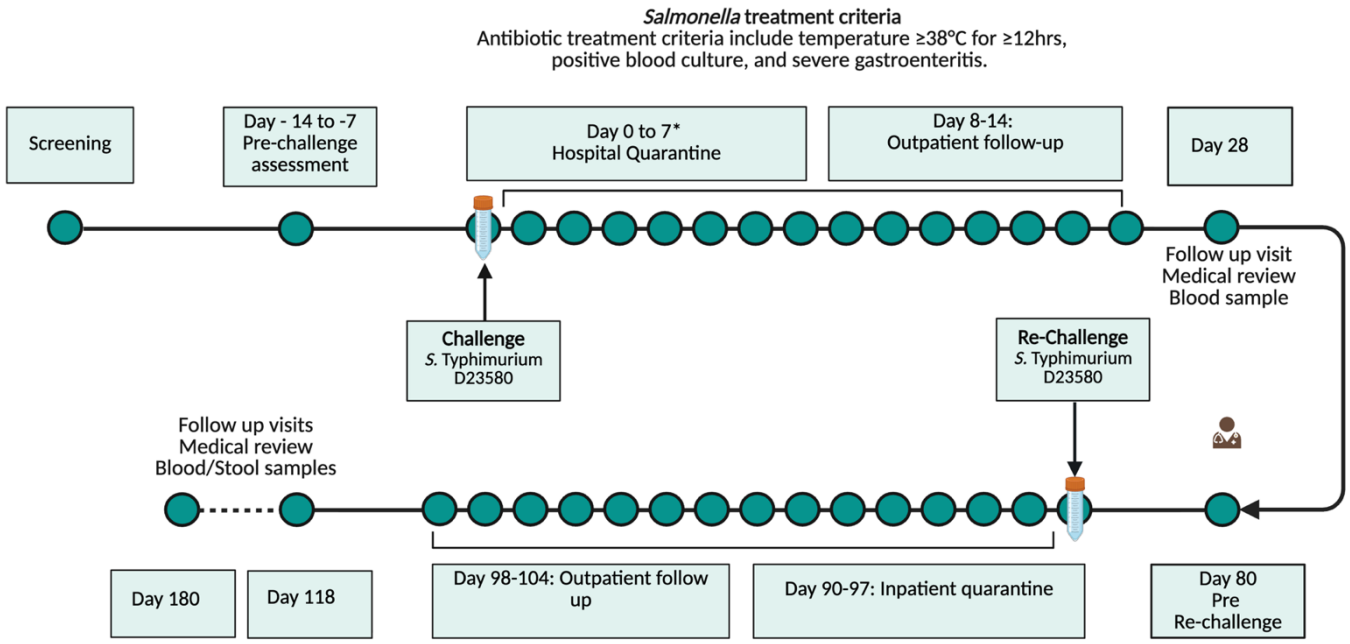

Figure 1 – ***Study reference diagram. Re-challenge can occur 3-6 months after primary challenge.***

1. LAY SUMMARY

This document outlines the protocol for a human infection challenge and re-challenge study investigating Salmonella Typhimurium, a member of the non-typhoidal Salmonella (NTS) subgroup.

NTS typically causes gastroenteritis, with symptoms such as diarrhoea, abdominal pain, and fever that resolve within a few days. In vulnerable groups, however, infection can progress to invasive non-typhoidal Salmonella (iNTS), where bacteria spread to the bloodstream or other sites. iNTS is most common in people with weakened immunity, including those with HIV, young children, the elderly, and individuals with risk factors such as malaria or sickle cell disease.

Globally, iNTS is a major health problem, particularly in sub-Saharan Africa, where it affects an estimated half a million people each year. The disease disproportionately affects children under five, many of whom are malnourished or have coexisting conditions. Preventive strategies, including vaccines, are urgently needed.

This study, a “challenge and re-challenge” model, will expose participants to S. Typhimurium under controlled conditions. Participants will ingest a defined dose of the bacteria during a closely monitored inpatient stay, and then 3-6 months later will be re-challenged with the same strain. The aim is to understand how the immune system responds to repeated exposure, providing insights that can guide vaccine development.

Study Procedures and Visits

- Screening: Participants first attend an outpatient clinic for eligibility assessment.
- Challenge One: Seven days before the challenge, participants attend a clinic visit. On the challenge day, they are admitted to hospital quarantine for seven days. After discharge, they will receive daily telephone follow-up for six days, followed by an outpatient visit seven days later. Further visits occur on day 14 and day 28 post-challenge.
- Challenge Two: Conducted 3–6 months later, following the same schedule. Outpatient visits will also be held on day 14, day 28, and day 90 post-challenge.

Across both challenge periods, participants will be carefully monitored, with additional in-person assessments if symptoms occur or if the study team deems it necessary.
Our recent first-in-human NTS challenge study confirmed safety and defined the optimal dose that induces gastroenteritis in about 60% of participants. This follow-up study will determine whether initial infection provides protection against re-exposure.

The study lasts approximately 6–9 months. Each participant will:

- Attend eight scheduled outpatient visits.

- Spend a total of 14 days in hospital quarantine.
- Receive 12 daily telephone calls after discharge.
- Additional visits may be arranged for safety reasons.

2. INTRODUCTION

2.1. BACKGROUND

This protocol describes the Protection Against invasive Non-Typhoidal Salmonella (PAiNTS) study. This is a challenge and re-challenge human infection study, conducted in healthy volunteers aged 18 to 50 years.

The overall aim of this study is to build upon the data generated from the first-in-human non-Typhoidal Salmonella (NTS) Controlled Human Infection challenge Model (CHIM) carried out at Imperial College London and explore the precise nature and mechanisms of protective immunity to NTS infection.

We will challenge healthy volunteers with a Salmonella Typhimurium strain D23580. Participants will be admitted to an inpatient quarantine facility after challenge and treated with antibiotics if they meet defined criteria.

The primary objective of the study is to determine the rates of systemic Salmonellosis following challenge and a re-challenge, to identify the degree of and characterise protective immunity. The secondary objectives of the study are to compare the clinical and laboratory features of challenge versus re-challenge with Salmonella Typhimurium, with the goal of defining correlates of protection. It is hoped that using the NTS human challenge model to dissect mechanisms of protective immunity can be used in the future to support vaccine development and optimisation for NTS disease.

Salmonella enterica are amongst the most frequently isolated pathogens in patients with community onset bloodstream infections in Africa and Asia. In particular, invasive disease caused by non-typhoidal *Salmonella* serovars (iNTS) is common across Africa, where the most recent data estimate that iNTS is responsible for 535 000 cases, 77 500 deaths and 4·26 million Disability-Adjusted Life Year (DALYs) lost in 2017. The precise burden of disease is, however, relatively poorly understood and estimates vary depending on case-definitions and methods of surveillance. The World Health Organisation (WHO) has recently listed *Salmonella* enterica as a priority pathogen, identified as one of 12 families of bacteria that pose the greatest risk to human health through rising antimicrobial resistance. (Tacconelli et al. 2017; Marchello et al. 2020; Ao TT, Feasey NA, Gordon MA, Keddy KH 2015; Crump et al. 2015)

iNTS disease typically presents as a febrile illness with clinical signs of sepsis, often without gastrointestinal symptoms. The case-fatality rate is estimated at between 15-20%. The high mortality of iNTS is explained by its strong association with human immunodeficiency. Recognised risk factors include malnutrition, recent malaria and

chronic anaemia (including sickle cell disease) in children, and human immunodeficiency virus (HIV) infection and malignancy in adults. Primary NTS bacteraemia is also observed in patients with defects in neutrophil function (e.g. chronic granulomatous disease) and those with defects in IL-12/23 pathway. Immune defects predisposing patients to iNTS have been studied most extensively in HIV disease, involving depletion of mucosal CD4+ T-cells, systemic cytokine dysregulation and dysregulated humoral immunity, coupled with hypergammaglobulinaemia and aberrant response to O-specific polysaccharides. The incidence of iNTS initially rises in HIV-infected patients starting antiretroviral therapy and then falls, but remains much higher than in HIV-uninfected controls. (Feasey et al. 2012; MacLennan et al. 2010; Crump et al. 2015; Klemm et al. 2016)

The NTS serovars commonly isolated from bacteraemic patients vary by location, but usually include *Salmonella* enterica subspecies enterica serovars Typhimurium and Enteritidis. S. Typhimurium accounts for approximately two-thirds of iNTS in Africa. Sequencing of isolates from across Africa has identified *Salmonella* Typhimurium multilocus sequence type 313 (ST313) as the major cause of invasive disease, which is distinct from the sequence types 19 and 34 that are more frequently associated with gastrointestinal disease in high-income settings such as the UK.

ST313 appears to have undergone sequential evolutionary changes that converge towards a genotype associated with invasive disease, in the same way as reported for *Salmonella* Typhi and Paratyphi serovars. The key changes are characterized by stepwise genomic degradation typified by multiple gene deletions and pseudogene formation, often in genes associated with gastrointestinal survival. The data suggests that ST313 has adapted to a human niche and can evade mucosal immunity. Similar findings have been observed in invasive S. Enteritidis ST11 isolates from Africa. Transmission of invasive isolates is complex and likely involves a combination of zoonotic reservoirs (particularly for S. Enteritidis) as well as human-to-human transmission. Invasive NTS isolates are increasingly associated with multi-drug resistance, significantly limiting treatment options.

2.2. Vaccines for iNTS disease

There is increasing interest in the development of broadly protective vaccines for invasive *Salmonella* disease. Vaccines for NTS would represent a valuable public-health tool in Sub-Saharan Africa, in part because other effective methods for disease control are slow and cost prohibitive in many endemic countries.

Several candidate vaccines are in development, including conjugate vaccines, live-attenuated oral vaccines and those using the Generalised Modules for Membrane Antigens (GMMA) platform, typically in multivalent formulations.(MacLennan et al. 2014; Baliban et al. 2020)

2.2.1. Conjugate vaccines

The University of Maryland and Bharat Biotech are developing a conjugate vaccine comprising core and O-polysaccharides conjugated to serovar specific phase 1 flagellin protein FliC (COPS conjugate vaccines). S. Enteritidis and S. Typhimurium COPS vaccines are immunogenic and able to protect against homologous intra-peritoneal challenge with S. enteritidis and S. Typhimurium in a CD-1 mouse model. This vaccine is being developed as a trivalent formulation comprising S. Typhi Vi-tetanus toxoid conjugate (Vi-TT), S. typhimurium COPS FliC and S. enteritidis COPS FliC conjugates. Rabbits immunised with this formulation mount a strong anti-O-antigen IgG and bactericidal antibody response, which was able to protect mice from fatal challenge following passive transfer. (Simon et al. 2011)

Preliminary data from a Phase 1 first-in-human study (n=22) using vaccine doses of 6.25ug and 12.5ug of COPS/Vi demonstrated an excellent safety profile, with 100% seroconversion of anti-STm-COPS and anti-SEn-COPS IgG and IgA. A notable finding was persistently elevated antibody titres 16 months after a single dose of the half-strength vaccine (12.5ug). Robust immunogenicity with a single dose has been attributed ot high levels of prior immune priming to *Salmonella*. Comparable findings were demonstrated in a phase 1/2a bridging study using new Good Manufacturing Practise (GMP) lots in healthy US adults, confirming the immunogenicity of the 12.5ug COPS dose (half of that originally predicted from pre-clinical studies). This vaccine has since progressed to a Phase 2 age-de-escalation study in healthy adults and infants in partnership with Centre pour le Développement des Vaccins du Mali (CVD-Mali); Centro de Investigaçao em Saude (CISM), Manhiça, Mozambique and Kenya Medical Research Institute Center for Global Health (KEMRICGH). This study includes assessment of a prime-boost schedule in children aged 12-18 weeks.(Chen et al. 2025; Baliban et al. 2018)

2.2.2. Generalised Modules for Membrane Antigens

GSK vaccines for Global health are developing a bivalent S. enteritidis and S. typhimurium vaccine using the generalised modules for membrane antigens (GMMA) platform. GMMA vaccines are derived from wild-type parent strains engineered with tolR mutations to induce continuous over-blebbing. GMMA vaccines allow multiple antigens to be presented in their natural confirmational state, potentially increasing the breadth of vaccine induced immune responses. In pre-clinical studies, GMMA vaccines were at least as immunogenic as O-antigen CRM197 conjugate comparators at inducing anti-O IgG. They also generated greater serum bactericidal antibody responses than glycoconjugates and were associated with reduced bacterial burden in animal models.30 Phase 1 trials of a bivalent S. typhimurium/S. enteriditis have been completed and are scheduled to report in early 2025. Phase 1 studies will follow in Kenya, through the Vacc-iNTS consortium.(Hanumunthadu et al. 2025)

2.3. Other vaccines in Development

In addition, Boston Children's hospital are developing a bivalent vaccine against S. Typhimurium and S. Enteritidis using the Multiple-Antigen Presenting System (MAPS). MAPS is a novel conjugation system that leverages biotin-rhizavidin pairing

to link polysaccharides and proteins. The International Vaccine Institute (IVI) are also collaborating with SK Biosciences and Biofarma on the development of O-antigen conjugates, and new live-attenuated oral vaccines are also in pre-clinical development.(MacLennan et al. 2014)

2.4. Challenges for NTS vaccine development

Vaccine development for iNTS is hampered, in part, by an incomplete understanding of mechanisms and determinants of immunity during natural infection, as well as a lack of commercial incentives. Non-typhoidal *Salmonella* are facultative intracellular pathogens adapted for survival with the intracellular niche of immune cells, and also capable of cell-free survival. Much is known about the complex life-cycle and pathogenesis of *Salmonella*, consistent with a role for cellular immunity to clear disease and antibody to prevent fatal bacteraemia.

2.5. Correlates of protection (CoP)

Several lines of evidence indicate a role for antibodies in conferring a degree of protection to iNTS disease. In particular, acquisition of bactericidal antibodies inversely corresponds to the age at which African children are susceptible to iNTS disease. Nevertheless, inhibition of killing of S. Typhimurium occurs in the presence of high-level of antibodies against O-antigen in HIV infected adults, thought to be due to aberrant response to O-specific polysaccharides. Important unknowns include the antigenic targets of bactericidal antibodies beyond O-specific polysaccharides and the role of other antibody effector mechanisms, which remain to be fully elucidated. (Mastroeni and Rossi 2020; MacLennan et al. 2008; Nyirenda et al. 2018)

Data from animal models, coupled with the strong association of iNTS with advanced HIV infection, indicates that robust T-cell immunity is required for full elimination of *Salmonella*. (Dougan et al. 2011)

The development of effective NTS vaccines can be improved by better understanding the basis of protective immunity against iNTS disease in humans. The main goal of this study is to identify putative CoP.

2.6. Application of CHIM studies for vaccine development

There is growing intertest and regulatory awareness of the utility of human infection challenge studies in vaccine development, particularly for diseases which occur sporadically, or which are difficult to conduct efficacy trials. A human infection challenge may significantly advance or accelerate vaccine development, but may conversely hamper product development if clinically meaningful endpoints are not used or the threshold for efficacy is too high. (Darton et al. 2016) A critical role for human challenge studies is to better understand the nature and type of immune responses required for vaccine induced protection from disease, and to identify correlates of protection, such as the application of a Shigella CHIM to identify of serum anti-LPS IgG as a correlate of protection against Shigellosis. (Jin et al. 2020; WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION 2016) (Cohen et al. 2019)

There are a number of instances where human challenge models have accelerated vaccine candidates on a pathway to licensure, in particular for enteric pathogens. For example, the live attenuated cholera vaccine CVD 103-HgR was licensed as a travel vaccine on the basis of safety, immunogenicity and efficacy data arising from CHIM studies. (Chen et al. 2016)

Another example is using a Vi-TT conjugate against typhoid fever (Typbar-TCV®, Bharat Biotech). This first demonstrated efficacy of 57% against culture confirmed typhoid fever in a human challenge model conducted in the UK. Data from the human challenge model contributed in part to recommendations from WHO SAGE to support programmatic use of Vi-TT in high burden settings in children aged 6 months and over. The same vaccine progressed to phase 3 clinical trials in Nepal, where it demonstrated efficacy of 82% against culture confirmed typhoid fever in children aged 6 months to 16 years. Notably, the efficacy in a clinical setting was comparable to a higher efficacy estimate of 89% was achieved in the challenge model using a secondary disease endpoint.(Jin et al. 2017)

Despite these notable examples, human challenge studies are – in general – considered by regulators and policy makers as studies that can be suggestive of efficacy, but that typically require further evidence from field trials before directly contributing to licensure. In part, this stems from concerns that such studies fail to accurately reflect natural disease, with reference to the population under study, the inoculum used, and the endpoints measured. Nevertheless, the malaria and typhoid cases-studies indicate that efficacy signals from human challenge studies conducted in adults in high-income settings can – in some instances – translate to comparable efficacy readout in children in low-and-middle income countries.

2.7. *Salmonella* human challenge studies

Human challenge studies using *Salmonella* enterica have existed for several decades. The majority of these have used the typhoidal *Salmonella* serovars Typhi and Paratyphi A. (Gibani et al. 2019; Waddington et al. 2014) Recently, we have established a first in human challenge study using the non-typhoidal *Salmonella* serovar *Salmonella* Typhimurium.

2.7.1. Non-typhoidal *Salmonella* challenge studies

Unlike human host-restricted S. Typhi, NTS serovars typically have a broad host range and several animal models have been developed to assess NTS candidate vaccines. (Higginson et al. 2016) *Salmonella* spp. are frequently used as model organisms to study bacterial pathogenesis and host-microbe interactions. Many animal models have been described, including colonisation models; lethality models; gastroenteritis models and invasive *Salmonella* models. These include immunocompetent mouse models (such as those using NRAMP deficient strains e.g BALB/c or C57BL/6); immunodeficient mouse models; humanised mice; guinea-pig; chicken; rat and rabbit models; streptomycin-treated mouse models; calf ileal loop and rhesus macaque models. Unfortunately, many of these models do not fully

recapitulate the manifestations of disease in a clinically relevant context. (Ault et al. 2013)

Wild-type NTS challenge for gastroenteritis has been performed in a small number of volunteers. In the earliest published study from 1936, Hormaeche et al challenged a total of five volunteers with *Salmonella* Typhimurium administered in water, at a dose level of 2 x 109 (n=3) and 4 x 109 (n=2). Four out of five developed gastrointestinal disease and the fifth participant developed fever. Other studies have described challenge with *Salmonella* Anatum, *Salmonella* Pullorum and *Salmonella* Meleagridis administered in eggnog. (Hormaeche et al. 1939) Previous reports have described challenge of healthy volunteers with attenuated S. Typhimurium as candidate oral vaccines. Attenuated strains include **Δ**phoP/**Δ**phoQ and **Δ**aroC/**Δ**ssaV (WT05) variants. Challenge with attenuated S. Typhimurium was reportedly safe, immunogenic and – notably – not diarrhoeagenic. However, in some instances oral challenge with WT05 was associated with prolonged stool shedding of vaccine lasting up to 21 days. (Hindle et al. 2002)

2.7.2. Challenge with attenuated strains

Post responses to *Salmonella* Typhimurium have been studied in vaccine trials where *Salmonella* Typhimurium was administered as a live attenuated formulation.

The WT05 *Salmonella* live attenuated vaccine has been tested in a phase I study in 2002. It was derived from the wild-type S. Typhimurium strain TML, isolated from a gastroenteritis patient, with *Δ*aroC *Δ*ssaV deletions disrupting purine and aromatic acid synthesis as well as SpI-2 effector functions. although generally well-tolerated, persistent bacterial shedding was observed for up to three weeks at high doses, raising concerns about vaccine strain transmission. (Hindle et al. 2002)

Angelakopoulos and Hohmann (2000) evaluated a S. Typhimurium vaccine candidate as a vectored vaccine to deliver heterologous antigens from Helicobacter pylori. The vaccine vector was derived from S. Typhimurium reference strain ATCC 14028, with *Δ*phoP/phoQ and *Δ*purB deletions, leading to impair resulting in a strain that is highly attenuated and purine auxotrophic. In a Phase I clinical trial, six adult volunteers received a single oral dose of 5 - 8 x 107 CFU administed with sodium bicarbonate. The strain was reportedly well tolerated with 2/6 volunteers developing transient fever. All participants experienced transient faecal shedding – 3/6 cleared within four days, and 3/6 had persistent shedding until day 7 -10, requiring antibiotic treatment to enhance clearance. There was evidence of strong systemic and mucosal immunity. Five out of six volunteers had a ≥4 fold-rise in serum anti-S. Typhimurium-LPS IgG. five out of six volunteers had detectable IgA-producing antibodies secreting cells against S. Typhimurium LPS, which peaked at 7-10 days post-vaccination, indicative of local mucosal immune responses. This candidate has not been taken forward as a stand-alone S. Typhimurium vaccine but demonstrates the feasibility of live attenuated NTS strains as oral vectors for heterologous antigen delivery in humans.

2.7.3. The CHANTS study

In 2023, Imperial College London began a first-in-human challenge model using two strains of S. Typhimurium (4/74 and D23580); the study was called Challenge Non-Typhoidal *Salmonella* study (CHANTS). Healthy volunteers aged 18-50 were enrolled in a dose escalation study, ranging from 1-5 x 103 to 1-5 x 105 CFU. Participants were randomised to challenge with the ST19 lineage strain (4/74) or the ST313 lineage strain (D23580). (Smith et al. 2024)

A total of 50 participants were enrolled and received oral challenge with one of the two strains. A dose-response relationship was observed for both strains, with attack rates increasing at higher doses. The primary endpoint was defined as either a bloodstream infection or a sustained fever (oral temperature ≥38.0°C for ≥12 hours).

At the lowest dose (1–5 x 10^3 CFU), no participants met the primary endpoint. At the intermediate dose (1–5 x 10^4 CFU), D23580 had a 50% attack rate (2/4 participants), while 4/74 had a 0% attack rate (0/3 participants). At the highest dose administered (1–5 x 10^5 CFU), the model-estimated attack rates were 57.9% for D23580 and 47.4% for 4/74, with no statistically significant difference between the strains.

The symptom profile following challenge was consistent with that expected after acute *Salmonella* gastroenteritis. The most frequently reported symptoms were headache, malaise, abdominal pain, and diarrhoea, with rates increasing at higher challenge doses (10^4–10^5 CFU). There were no serious adverse events (SAEs). Five participants met the protocol-defined criteria for severe salmonellosis. These included three cases of transient and self-limiting gastrointestinal bleeding (two following challenge with strain 4/74 and one with D23580) and two grade 4 laboratory abnormalities (one participant with a peak ALT of 374 U/L, considered unlikely to be treatment related, and one with a CRP of 218.9 mg/L, considered definitely related). All cases resolved without long-term sequelae.

Microbiological relapse was observed in five participants, characterised by the re-emergence of *Salmonella* on stool culture after apparent clearance. All episodes were asymptomatic, and none were associated with clinical illness or further adverse events.

The study was stopped at the 1–5 x 10^5 CFU dose level, and the maximum dose of 1–5 x 10^6 CFU was not administered, as the observed attack rate was deemed appropriate to take forward.

2.8. RATIONALE FOR CURRENT STUDY

This rationale for this human challenge and re-challenge study aims to assess whether prior exposure to *Salmonella* confers a protective effect against a subsequent exposure. The primary objective is to compare the rate of infection following initial (naïve) challenge with the rate of infection on secondary challenge (re-challenge). The secondary and exploratory objectives are to assess similarities and difference between the clinical, microbiological and immunological response to re-challenge. The

specific focus is to identify immune correlates and mechanisms of protection that will support the development of effective iNTS vaccines.

In this study, participants will first undergo primary exposure to the S. Typhimurium D23580 strain, using the infectious dose (1-5 x 10^5 CFU) established in the CHANTS study. This dose has been selected to achieve a reliable infection rate, providing an optimal platform for studying immune responses without compromising participant safety.

Following the initial challenge, volunteers will undergo a homologous re-challenge with the same D23580 strain of S. Typhimurium 3 to 6 months after their primary exposure. This re-challenge timeframe is intended to allow adequate development of immunological memory while also enabling the assessment of immune responses before significant waning occurs. By using a homologous (same strain) re-challenge, the study will specifically evaluate the effectiveness of immune memory developed from the initial exposure in providing protection against the same pathogen.

2.8.1. Re-challenge considerations

This study aims to determine the protective effect of prior exposure to S. Typhimurium on the rate of infection following secondary exposure. To maintain a degree of consistency, the challenge dose and the challenge strain will be consistent between primary and secondary challenges.

Another important variable is the interval between primary and secondary exposure. In this study, the interval between primary and secondary challenge will be between 3 to 6 months. The decision to choose this interval between primary and secondary challenge has been dictated by several considerations. Participant retention should also be taken into consideration. Variables to be considered include:

- Participant acceptability
- Retention rate
- Serum antibody titres
- Maturation of the adaptive immune response following primary challenge
- Recovery of the microbiota following primary exposure and/or antibiotic treatment
- Availability of the challenge space and challenge staff also need to be taken into consideration.

The primary consideration should be acceptability to the participants, coupled with theoretical considerations about maturation of the immune response following primary exposure.

2.8.2. Rationale for challenge strain selection

S. typhimurium strains belonging to the lineage ST313 are thought have adapted to an extra-intestinal lifestyle and are responsible for the majority of iNTS cases across sub-Saharan Africa. ST313 consists of three discrete phylogenetic groups, denoted lineage 1, 2 and 3. Strains belonging to lineage 2 are the most frequently isolated from invasive infections in sub-Saharan Africa.

We have manufactured challenge stocks from the ST313 lineage 2 reference strain D23580, which is considered as an archetypal isolate representative of strains causing invasive disease in sub-Saharan Africa. D23580 was originally isolated from a blood culture taken from a 24-month-old child at Queen Elizabeth Central Hospital, Blantyre, Malawi. This strain has been extensively characterised at a genomic, transcriptomic, proteomic and phenotypic level. It has reduced catalase activity, cannot grow on melibiose, L-tartaric acid or dihydroxyacetone as sole carbon sources, and presents a negative red, dry and rough (RDAR) phenotype. D23580 possesses five prophages (BTP1, Gifsy-2, ST64B, Gifsy-1, and BTP5), of which Gifsy-2, Gifsy-1, and ST64B are inactivated, and BTP1 is highly spontaneously induced. The strain carries four plasmids, including pSLT-BT (a virulence plasmid pSLT with a Tn21-like element carrying antibiotic resistance genes); pBT1; pBT2 encoding CysS and pBT3.

Several lines of evidence indicate that this strain is adapted to an extra-intestinal lifestyle, including disruption of genes associated with intestinal persistence (PipD, RatB, MacAB) and increased dissemination (SseI), alongside transcriptomic changes associated with escape from immune surveillance and complement resistance. D23580 possess a distinct prophage and plasmid repertoire as compared with ST19, which is – in turn - distinct from, ST313 lineage 1 and lineage 3 isolates. D23580 is sensitive to ciprofloxacin, azithromycin and third generation cephalosporins, ensuring that a range of treatment options are available. The virulence plasmid pSLT encodes resistance to chloramphenicol, ampicillin, and sulphonamides – none of which would be used for treatment. Notably, D23580 is distinct from ST313 strains isolated from the UK and appears to associated with a higher risk of bloodstream infection.

S. Typhimurium (D23580) was manufactured under GMP conditions at the Walter Reed Army Institute of Research, Maryland, USA (WRAIR) in partnership with PATH. Full antibiotic sensitivity of the strain has been demonstrated and further characterization work including genome-sequencing has been completed. The D23580 strain has been used to safely challenge 25 participants at Imperial College London in 2024/5.

3. STUDY OBJECTIVES

Table 1 - Study objectives and endpoints

	Objective(s)	Endpoint(s)
Primary	To determine the attack rate of systemic *Salmonellosis* after challenge and re-challenge with *Salmonella* Typhimurium.	The proportion of participants who develop either: Fever ≥38°C on ≥2 occasions ≥12 hours apart **AND/OR** *Salmonella* Typhimurium bacteraemia
Secondary	To describe the *Salmonella* colonisation rate following oral challenge and re-challenge with *Salmonella* Typhimurium	The proportion from whom *Salmonella* Typhimurium is isolated from stool on ≥2 occasions ≥48 hours from challenge OR re-challenge.
	To describe the rate of gastroenteritis following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants at different doses at each strain developing any of: Severe diarrhoea **and/or** Moderate diarrhoea and/or Mild Diarrhoea
	To determine the fever rate following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants who develop any fever ≥38°C
	To describe the *Salmonella* bacteraemia rate following oral challenge or re-challenge with *Salmonella* Typhimurium	The proportion of participants developing *Salmonella* Typhimurium bacteraemia at any timepoint following challenge or re-challenge.
	To describe the rate of systemic Salmonellosis according to **an alternative composite diagnostic criteria** following oral challenge or re-challenge with *Salmonella* Typhimurium.	The proportion of participants meeting the criteria for a composite diagnosis of *Salmonellosis* defined as any of 1) *Salmonella* Typhimurium is isolated from stool on ≥2 occasions ≥48 hours from challenge and/or 2) *Salmonella* gastroenteritis and/or 3) fever ≥38°C on ≥2 occasions ≥12 hours apart and/or 4) *Salmonella* Typhimurium bacteraemia detected by blood culture and/or novel molecular methods
	To describe the safety of challenge and re-challenge with *Salmonella* Typhimurium	The proportion of participants at different doses of each strain reporting Adverse events, Adverse events of special interest, SAEs, SUSARs. Concomitant medication usage as outlined in the study protocol

	To compare **clinical** features following oral challenge or re-challenge with *Salmonella* Typhimurium	A comparison of the clinical features of *Salmonella* infection after challenge and re-challenge with D23580 strain, with specific reference to The proportion of participants in each group developing any diarrhoea. The severity of diarrhoea in each group, as measured by: Frequency of bowel motions/24hrs Total gastrointestinal symptom severity score calculated by summing numerical values assigned to the severity of all solicited symptoms between Day 0 to Day 14 Total systemic symptom severity score calculated by summing numerical values assigned to the severity of all solicited and unsolicited gastrointestinal and systemic symptoms between Day 0 to Day 14 (graded 0-4). Area under the curve for gastrointestinal and systemic symptom severity score between Day 0 to Day 14 Time to onset of diarrhoea in each group, measured in hours since challenge to the first grade 6/7 stool. The proportion of participants in each group developing any fever ≥38°C Fever clearance time of febrile participants in each group measured by time in hours to first temperature <38°C lasting ≥12hrs The proportion of participants in each group reporting the following solicited symptoms at any time: • headache, • malaise • diarrhoea • abdominal pain • nausea • vomiting • myalgia • arthralgia • cough • tenesmus Duration of solicited symptoms measured in days from first symptom onset to complete resolution of symptoms Severity of solicited and unsolicited symptoms measured by: The proportion of participants with maximum symptom severity score graded 0-4.

		The proportion of participants meeting the criteria for severe *Salmonellosis* Symptom severity scores for individual solicited and unsolicited symptoms calculated by summing numerical values assigned to the severity of symptoms between Day 0 to Day 14 (graded 0-4).
	To compare **microbiological** features of **gastrointestinal infection** following oral challenge or re-challenge with *Salmonella* Typhimurium	Gastrointestinal *Salmonella* infection after challenge or re-challenge, with specific reference to: Time to onset in days in each group from challenge to first stool sample positive for *Salmonella* Typhimurium by culture and/or PCR Duration of stool shedding in each group measured in days from first stool sample positive for *Salmonella* The duration of stool shedding in each group measured in days from first stool sample positive by culture and/or for *Salmonella* Typhimurium to first persistently negative stool sample (defined as three consecutive samples negative) The magnitude of stool shedding measured in CFU/ml in quantitative stool culture analysis
	To compare **microbiological** features of **bloodstream infection** following oral challenge and re-challenge with *S.* Typhimurium	*Salmonella* bloodstream infection after challenge or re-challenge, with specific reference to The proportion of participants in each group developing any bacteraemia The severity of bacteraemia in each group as measured by: The duration of bacteraemia measured by time in hours from the first positive blood culture to first persistently negative blood culture Blood culture clearance time measured by time in hours from initiation of antibiotics treatment to first persistently negative blood culture Magnitude of bacteraemia measured in CFU/ml in quantitative blood culture samples collected immediately prior to the initiation of treatment The time to onset of bacteraemia in each group, measured by hours since challenge
	To compare biochemical and haematological laboratory parameters following oral challenge or re-challenge with *S.* Typhimurium	Absolute values of laboratory parameters from time of challenge to Day 28 and/or Day 90, with specific reference to Total haemoglobin (d/L) Haemoglobin change from baseline (Hb g/l D0 – nadir Hb g/l) Total white cell count ($x10^9$/l)

		Neutrophil count (x10^9/l) Lymphocyte count (x10^9/l) Eosinophil count (x10^9/l) Monocyte/lymphocyte ratio Urea and electrolytes (Na+,K+, Urea, Creatinine – mmol/l) C-reactive protein (mg/l) Liver function tests (Bilirubin [umol/l], aspartate transaminase (AST IU/l), alkaline phosphatase (ALP IU/l), alanine transaminase (ALT IU/l), Albumin (g/l)
	To describe and compare the serum antibody response following oral challenge and re-challenge with *S.* Typhimurium	Absolute values of laboratory assays on serum samples measured at baseline and post challenge time points, including but not limited to *S.* Typhimurium O-specific polysaccharide serum IgG and IgA concentration measured by ELISA *S.* Typhimurium specific serum IgG and IgA concentration against other S. Typhimurium antigens measured by ELISA Serum bactericidal antibody titres against *Salmonella* Typhimurium Other binding and functional antibody activity measurements including (but not limited to) antigen arrays, systems serology platforms etc.
Exploratory	To investigate how the human microbiota interacts with oral challenge of *S.* Typhimurium	Pathogen specific and metagenomic analysis of stool and/or saliva to measure constituent microbiological flora (and their metabolome, transcriptome and anti-microbial resistance profile) at baseline and post-challenge time points
	To investigate the impact of diet on the outcome of *Salmonella* challenge.	Rate of challenge outcomes stratified by dietary composition, as assessed by a food diary.
	To describe and compare cell-mediated immune response following oral challenge with *S.* Typhimurium	To describe and compare cell-mediated immune response following oral challenge with S. Typhimurium Absolute values and inter-group comparison of laboratory assays performed on peripheral blood mononuclear cells measured at baseline and post challenge time points including (but not limited to) Description of lymphocyte populations at baseline and following challenge as measured by flow cytometry and/or CyTOF Frequency and magnitude of S. Typhimurium specific cell-mediated immune responses as measured by

		ELISPOT and flow cytometry and/or CyTOF
	To describe and compare the mucosal antibody response following oral challenge and re-challenge with *S.* Typhimurium	Absolute values of laboratory assays on saliva (and/or stool copro-antibodies) samples measured at baseline and post challenge time points using laboratory assays including (but not limited to) S. Typhimurium specific IgG and IgA concentration measured by ELISA Bactericidal antibody titres Other functional antibody activity measurements including systems serology platforms
	To investigate novel diagnostic methods for detecting *S.* Typhimurium infection	Exploratory analysis of blood and faecal samples, including novel molecular techniques
	To describe and compare additional host immune responses following oral challenge and re-challenge of *S.* Typhimurium D23580 strains.	Analysis of B-cell repertoire, T-cell repertoire, plasma cytokine profile and kinetics, additional exploratory immunology
	To evaluate host-pathogen interactions following *Salmonella* infection including the presence and mechanisms of *Salmonella* persister infected macrophages	Exploratory analysis of infected immune cells from blood including, but not limited to, single-cell RNA-seq.
	To describe participant experience following oral challenge or re-challenge with S. Typhimurium	Descriptive statistics following administration of a questionnaire post-challenge
	To described epigenetic markers associated with susceptibility to *Salmonella* infection.	Identification and quantification of epigenetic modifications (e.g., DNA methylation, histone modifications) in whole blood and specific leukocyte sub-populations that correlate with susceptibility to *Salmonella* infection.

4. STUDY DESIGN

This study is a phase 1 challenge-rechallenge controlled human infection model of S. Typhimurium infection. After screening and a pre-challenge visit, participants will undergo their first challenge with S. Typhimurium D23580 at a dose of 1-5 x 105 CFU, termed the primary challenge. We will enrol healthy volunteers aged 18 to 50. After a period of 3-6 months, participants will undergo a second challenge with S. Typhimurium D23580 at a dose of 1-5 x 105 CFU. The total study duration will be 6 – 9 months.

4.1. STUDY OUTCOME MEASURES

4.2. Primary outcome measures

The primary outcome measures are:

- Fever ≥38°C on ≥2 occasions ≥12 hours apart
- *Salmonella* Typhimurium bacteraemia at any time point

4.3. Secondary clinical endpoints

The secondary disease endpoints are:

- *Salmonella* Colonisation - Isolation of *Salmonella* Typhimurium from stool on ≥2 occasions ≥48 hours from challenge and/or
- *Salmonella* gastroenteritis
- Any diarrhoea
- Mild, moderate or severe diarrhoea
- Fever ≥38°C on ≥2 occasions ≥12 hours apart
- Any fever ≥38°C
- *Salmonella* bacteraemia alone
- *Salmonella* Typhimurium bacteraemia with or without fever

4.4. Challenge agent dose

Based on data from the CHANTS study, the dose used for the challenge will be 1-5 x 10^5 CFU. In volunteers without prior exposure to *Salmonella*, this dose is estimated to cause an attack rate of 57.9% (95%CI 37.3-73.8%) using a primary endpoint of fever ≥38°C and/or bacteraemia, when administered with sodium bicarbonate.

4.5. Challenge strain

This study will use the ST313 Lineage D23580 strain of S. Typhimurium. We will use GMP grade frozen batches of challenge stocks used in the CHANTS study. Stability testing has confirmed stable challenge doses over 2 years since manufacture.

5. PARTICIPANT ENTRY

5.1. PRE-REGISTRATION EVALUATIONS

All participants will attend a screening visit prior to enrolment in the study. Screening procedures are outlined below. Prior to enrolment participants will undergo the following investigations:

- Full blood count
- Urea and electrolytes
- Liver function tests
- C-reactive protein
- Serum IgA
- Coeliac serology

- HLA-B27 screening
- Coagulation screen
- Haemoglobinopathy screen
- HIV 1&2 antibody
- Hepatitis B surface antigen
- Hepatitis C IgG
- HbA1c
- Ultrasound of the biliary tract
- Ultrasound of the abdominal aorta.
- Stool culture and PCR for *Salmonella* spp.[1]
- Malaria screen (for participants who have travelled to malaria endemic areas within the preceding 6 months from the date of screening).
- Urine pregnancy test
- Urine dipstick test
- ECG
- Resting heart rate, respiratory rate, blood pressure, oxygen saturations and oral temperature.

5.2. INCLUSION CRITERIA

Participants must satisfy all the following criteria to be considered eligible for the study:

- Agree to give informed consent for participation in the study.
- Aged between 18 and 50 years inclusive at time of challenge.
- In good health as determined by medical history, physical examination, and clinical judgment of the study team.
- Agree (in the study team's opinion) to comply with all study requirements, including capacity to adhere to good personal hygiene and infection control precautions.
- Agree to allow his or her General Practitioner to be contacted regarding participation in the study.
- Agree to allow study staff to access their medical history and vaccination records via a shared electronic health record and/or to contact their GP to access medical records.
- Agree to allow UKHSA and the local health protection team to be informed of their participation in the study.
- Agree to give his or her close contacts written information informing them of the participant's involvement in the study.
- Agree to a period of inpatient quarantine in accordance with study protocols.
- Agree to make every effort to be contactable 24/7 by study staff during the four weeks post challenge and re-challenge. Participants will make every effort to ensure that they are contactable by mobile phone and/or email and/or instant messaging service for the duration of the study period.

[1] Testing will be performed at Day-7 visit and will comprise a multiplex PCR to assess for Salmonella spp., Shigella spp., Campylobacter spp., E. Coli O157, Giardia spp and Cryptosporidum spp.

- Agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period after quarantine (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant after discharge from inpatient quarantine.
- Agree to avoid antipyretic/anti-inflammatory treatment from the time of challenge (Day 0) until advised by a study doctor or until 14 days after challenge and re-challenge.
- Agree to refrain from donating blood for the duration of the study.
- Agree to provide their National Insurance/Passport number for the purposes of Trial over-volunteering prevention system (TOPS) registration and for payment of reimbursement expenses.
- Proficient in English at a level sufficient to understand, retain, weigh up and communicate the study details as outlined in the participant information sheet, in the opinion of the study investigator.

5.3. EXCLUSION CRITERIA

The participant will not be enrolled if any of the following apply:

- History of microbiologically confirmed *Salmonella* infection prior to the primary challenge.
- History of significant[2] organ-specific and/or systemic disease that could interfere with trial conduct or completion. Including, for example, but not restricted to:
    - Cardiovascular disease, including specifically
        - Atherosclerotic disease
        - Stable or unstable angina
        - Previous myocardial infarction
        - Valvular heart disease
    - Vascular disease, including specifically
        - Documented aneurysmal arterial disease
        - Peripheral arterial disease
        - Endovascular prosthesis
    - Respiratory disease[3]
    - Haematological disease including sickle cell disease.
    - Endocrine disorders, including specifically Diabetes mellitus
    - Renal or bladder disease, including history of renal calculi
    - Biliary tract disease, including specifically
        - biliary colic,
        - asymptomatic gallstones, or

[2] Individuals with specific medical co-morbidities (e.g. childhood asthma) will be considered for enrolment after review with the principal investigator and clinical oversight group, following discussion with their GP and/or consultant as appropriate. The rationale for enrolment in these cases will be recorded in the CRF.

[3] Participants with well controlled asthma may be considered for enrolment at the discretion of the study investigators and following consultation with their general practitioner

- previous cholecystectomy
- Gastro-intestinal disease including specifically,
  - a current requirement for antacids, H2-receptor antagonists, proton pump inhibitors or laxatives
  - Inflammatory bowel disease
  - Confirmed diagnosis of irritable bowel syndrome
- Neurological disease
- Metabolic disease
- Autoimmune disease
- Psychiatric illness requiring hospitalisation
- Known or suspected drug and/or alcohol misuse disorder
- Chronic/Active Infectious disease including active tuberculosis
- Severe infection requiring hospitalisation for intravenous antibiotics within the last 10 years. Exceptions to this would include a short course of intravenous antibiotics for appendicitis, biliary sepsis, diverticulitis, and cellulitis.
- History of recent malaria infection within the past 12 months from screening.[4]
- History of joint replacement
- History of any orthopaedic/osseous implanted prosthesis
- Presence of other internal implanted device e.g. permanent pacemaker
- Have any known or suspected impairment of immune function (as defined in the green book), alteration of immune function, or prior immune exposure that may alter immune function to *Salmonella* infection resulting from, for example:
  - Congenital or acquired immunodeficiency, including IgA deficiency
  - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
  - Evidence of severe primary immunodeficiency, for example, severe combined immunodeficiency, Wiskott-Aldrich syndrome, and other combined immunodeficiency syndromes.
  - Currently being treated for malignant disease with immunosuppressive chemotherapy or radiotherapy, or who have received such treatment within at least the last six months.
  - Individuals who have received a solid organ transplant and are currently on immunosuppressive treatment
  - Individuals who have received a bone marrow transplant, until within 12 months of finishing all immunosuppressive treatment,
  - Individuals receiving systemic high-dose steroids until at least three months after treatment has stopped.[5]

[4] Volunteers with distant history of malaria infection may be considered for enrolment if they have been treated and can demonstrate a negative malaria test at screening.
[5] Defined as those receiving at least 40mg of prednisolone per day for more than one week or equivalent

    - Individuals receiving other types of immunosuppressive drugs[6] (alone or in combination with lower doses of steroids) until at least six months after terminating such treatment.
- Receipt of immunoglobulin or any blood product transfusion within 3 months of study start.
- History of cancer (except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ).
- Moderate or severe depression or anxiety at screening or challenge that is deemed clinically significant by the study doctors.[7]
- Weight less than 50kg.[8]
- Anyone taking long-term medication (e.g. analgesia, anti-inflammatories or antibiotics) that may affect symptom reporting or interpretation of the study results.
- Contraindication to cephalosporin, fluroquinolone, or macrolide antibiotics.
- Female participants who are
    - pregnant,
    - lactating or
    - who are unwilling to ensure that they or their partner use effective contraception 30 days prior to challenge and re-challenge
- Full-time, part-time, or voluntary occupations or students involving:
    - Clinical healthcare work[9]
    - Social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or other clinically vulnerable children, adolescents
    - Clinical or social work with direct contact with highly susceptible patients or persons in whom *Salmonella* infection would have particularly serious consequences.
    - Commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating)
- Close household contact with:
    - Young children (defined as those attending pre-school groups, nursery or those aged less than 2 years)
    - Any individual who is immunocompromised.
    - Pregnant women
    - Household contacts aged over 70 years
- Scheduled elective surgery or other procedures requiring general anaesthesia during the study period.

[6] e.g. azathioprine, cyclosporin, methotrexate, cyclophosphamide, leflunomide and other cytokine inhibitors.
[7] If elevated scores are due to temporary significant life events, the questionnaire may be repeated after resolution of the event with a view to inclusion if normal
[8] Or a Body Mass Index (BMI) that, in the opinion of the study doctors, may adversely impair the interpretation of the study results or affect the safe performance of any study procedures.
[9] This includes medical, dental, nursing or midwifery students (or other allied-healthcare professionals) with direct patient contact. Such students who are not undertaking patient-facing rotations until demonstrated not to be infected with *Salmonella* species may be considered for enrolment.

- Participants who have participated in another research study involving an investigational product that might affect risk of *Salmonella* infection or compromise the integrity of the study within the 30 days prior to enrolment (e.g. significant volumes of blood already taken in previous study).[10]
- Detection of any abnormal results from screening investigations, unless deemed not clinically significant.
- Any other social, psychological or health issues which, in the opinion of the study staff, may
    - Put the participant or their contacts at risk because of participation in the study,
    - Adversely affect the interpretation of the primary endpoint data,
    - Impair the participant's ability to participate in the study.
    - Having previously received any experimental *Salmonella* vaccine as part of a clinical trial
- Have participated in previous *Salmonella* challenge studies (with ingestion of challenge agent).
- Have a prolonged corrected QTc interval (>450 milliseconds) on ECG screening
- Screening blood test positive for HLA-B27.
- Any employee of the sponsor or research site personnel directly affiliated with this study or their immediate family members.
- Inability to comply with any of the study requirements (at the discretion of the study staff).

5.4. Temporary exclusion criteria at challenge

Participants will be temporarily excluded from challenge if presenting at the challenge visit with the following:

- Significant acute or acute-on-chronic infection within the previous 7 days or have experienced fever (>37.5oC) or subjective febrile symptoms within the previous 3 days.
- History of any antibiotic therapy during the previous 5 days.
- Any systemic corticosteroid (or equivalent) treatment in the previous 14 days, or for more than seven consecutive days within the past 3 months.
- Therapy with antacids, proton pump inhibitors or H2-receptor antagonists within 24 hours prior to challenge.
- Detection of gastrointestinal pathogens in stool culture/PCR collected at the pre-challenge assessment (Ch1_D-7, Ch2_D-7) including Shigella spp., Campylobacter spp., E. Coli O157, Giardia spp and Cryptosporidum spp, until two subsequent samples are negative. Detection of *Salmonella* spp. in stool prior to challenge (Day – 7) will result in exclusion from the study. Detection of *Salmonella* spp. in stool prior to re-challenge Ch2_D – 7 will result in deferment of challenge until spontaneously cleared.

[10] As assessed by both participant questioning and registration with The Over Volunteering Prevention System (TOPS) database.

5.5. Temporary exclusion due to positive stool samples

The result of the stool sample collected at Day-7 will be reviewed prior to the challenge visit. Detection of gastrointestinal (other than *Salmonella*) in stool culture/PCR collected at the pre-challenge assessment (Day-7) will result in temporary exclusion from the study. This included detection of the following organisms:

- Shigella spp.,
- Campylobacter spp.,
- E. Coli O157,
- Giardia spp
- Cryptosporidum spp,

Participants will be managed according to the local standard operating procedures (SOPs) for management of incidental medical findings. They may subsequently be eligible for challenge at the discretion of the chief investigator if they are able to provide two subsequent samples, 48 hours apart, that test negative. These samples would be collected at ad hoc study visits arranged directly with participants.

Detection of *Salmonella* spp. in stool prior to challenge will result in exclusion from the study. Sampling procedures prior to re-challenge are outlined elsewhere in this protocol.

5.6. Pregnancy and contraception

The possible adverse effects of *S*. Typhimurium infection or the effect of some antibiotics on the outcome of pregnancy are unknown. Therefore, pregnant and breastfeeding participants will be excluded from the study. If relevant, female participants will be required to use an effective form of contraception from 30 days prior to challenge until deemed to be clear of infection.

Participants of childbearing potential will be required to use an effective form of contraception. A woman is considered of childbearing potential (i.e. fertile) from the point following menarche until becoming post-menopausal, unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as having no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient, and effective contraception would need to be used.

Contraception should have been initiated at least for one month prior to receiving the challenge agent and for the duration of the study. Acceptable forms of effective contraception for participants of childbearing potential include:

- Established use of oral, injected or implanted hormonal methods of contraception.
- Intrauterine device (IUD).
- Intrauterine hormone-releasing system (IUS).
- Barrier methods of contraception (condom or occlusive cap with spermicide).
- Bilateral tubal occlusion.
- Vasectomised male partner if the vasectomised partner is the sole partner for the participant.
- Sexual abstinence when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to challenge agent, and withdrawal methods are NOT acceptable methods of contraception.
- Exclusive sex with a female partner(s).

Should a volunteer become pregnant during the study this will be recorded and the Sponsor delegate and the DSMB will be notified. No further non-essential study procedures will be performed (i.e. inoculation), however, procedures such as appropriate antibiotic treatment may be required if pregnancy is detected following challenge.

Male participants with female partners are not required to use barrier methods for the purposes of contraception.

In the event of a female participant becoming pregnant during the study this outcome will be recorded and the Sponsor and the DSMC will be notified if appropriate. No further non-essential trial procedures will be performed (i.e. appropriate antibiotic treatment, screening for stool carriage and/or referral may be required but not further blood sampling etc.).

5.7. WITHDRAWAL CRITERIA

Each participant can exercise their right to withdraw from the study at any time. If, however, the participant decides to withdraw after they are challenged, they may require additional follow up for public health reasons. In some instances, antibiotic treatment may be required, and participants may be required to attend additional hospital/non-study visits to ensure compliance.
In addition, the investigator may terminate a participant's involvement in the study at any time if the investigator considers it necessary for any reason including, though not exclusive to, the following:

- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening),
- Significant protocol deviation,
- Significant non-compliance with study requirements or risk to public health,
- Any adverse event which requires discontinuation of the study procedures or results in an inability to continue to comply with study procedures,
- Lost to follow up.

- Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. The reason for withdrawal, if given, will be recorded in the CRF.

In the unlikely event that the participant loses capacity to consent, identifiable data and samples will be withdrawn from the study.

5.8. Trial over-volunteering prevention system

The Over-volunteering Prevention System (TOPS) is a database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs or blood donations. Participants will be registered with this system at screening using their national insurance number or passport number if they do not have a national insurance number. The system will be updated in the event of the participant not entering the trial, being withdrawn, or excluded. Alternatively, TOPS will be updated on the participant's last visit.

6. ADVERSE EVENTS

6.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

6.2. REPORTING PROCEDURES

Adverse events graded two or more will be recorded (Grade 1 events have no impact on participant safety or quality of life). Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

6.3. Non serious AEs

All such events, whether expected or not, would be recorded- any reporting should be consistent with the purpose of the trial end points.

6.4. Serious AEs

In the event of an SAE, the CI must be notified immediately or within 24 hours of the SAE being noticed. The initial report can be made verbally but must be promptly followed with a detailed, written report using an SAE form. The SAE form must record the event with an assessment of seriousness, (along with causality, expectedness and severity) on a trial SAE form. Follow-up information must be provided when available. Where supporting documents are sent with this form, these must be anonymised/pseudonymised. Where the information available is incomplete at that time, as much information as can be ascertained should be sent to ensure timely reporting, with additional information provided as soon as it is known. Additional information received for an event (follow-up or corrections to the original event data) needs to be detailed on a new Serious Adverse Event Reporting Form.

The PI/CI must send all SAE reports to the Research Governance and Integrity Team, Imperial College AHSC immediately or within 24 hours after becoming aware of the event at the below address: RGIT@imperial.ac.uk

All SAEs should be reported to the approving REC where, in the opinion of the Chief Investigator, the event was 'related' (ie resulted from the administration of any of the research procedures) and 'unexpected' (ie an event that is not listed in the protocol as an expected occurrence). Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies.

Local investigators should report any SAEs as required by their Local Research Ethics Committee (REC), Sponsor and/or Research & Development Office.

6.5. Contact details for reporting SAEs

RGIT@imperial.ac.uk
CI email Dr Malick Gibani (m.gibani@imperial.ac.uk)

Please send SAE forms to: RGIT@imperial.ac.uk (sponsor) and fulham.rec@hra.nhs.uk (REC).
Tel: 020 7594 9480 (sponsor) and 0207 104 8084 (REC) (Mon to Fri 09.00 – 17.00)

Hospitalizations for elective treatment of a pre-existing condition do not need reporting as SAEs.

6.6. Notes on recording adverse events

Pre-existing medical conditions (present prior to enrolment into the study) are considered "concurrent medical conditions" and will not be recorded as AEs. However, if the participant experiences a worsening or complication of the condition, the worsening or complication will be recorded as an AE. Study staff will ensure that the AE term recorded captures the change in the condition (e.g., "worsening of").

AEs may also be recorded as a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) will not be recorded as additional AEs.

6.7. Expected adverse events

Any adverse event related to symptomatic *Salmonella* Typhimurium infection will be deemed an 'Expected Adverse Event'. These symptoms will be considered 'Expected Adverse Events' if they occur from Day 1 and Day 28 post-challenge and re-challenge.

If these symptoms listed occur outside Day 1 and Day 28 post-challenge and re-challenge they will still be recorded as adverse events, but not as Expected Adverse Events.

Events which will be attributed to development of symptomatic *Salmonella* infection are:

- Diarrhoea
- Abdominal pain
- Loss of appetite
- Nausea
- Vomiting
- Tenesmus
- Headache
- Fever >37.5°C but <40.0°C (fever ≥40.0°C will be considered a Grade 4 AE)
- Malaise
- Myalgia
- Arthralgia
- Cough

6.8. Causality

Causality assessment will follow the guidelines from existing SOPs, except for causality attribution and classification that will be based on the following criteria.

- No relationship - No temporal relationship to S. Typhimurium ingestion, and alternative aetiology (clinical, environmental or other intervention), and does not follow pattern of recognised response to NTS infection.

- Unlikely - There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after S. Typhimurium ingestion). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).

- Possible - Reasonable temporal relationship to S. Typhimurium ingestion, or event not readily explained by alternative aetiology (clinical, environmental, or other interventions), or similar pattern of response to that seen to NTS infection.

- Probable - Reasonable temporal relationship to S. Typhimurium ingestion, and event not readily produced by alternative aetiology (clinical, environment, or other interventions), or known pattern of response with NTS infection.

- Definite - Reasonable temporal relationship to S. Typhimurium ingestion, and event not readily produced by alternative aetiology (clinical, environment, or other interventions), and known pattern of response to NTS infection.

- Not assessable - There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship

6.9. Severe *Salmonella* Infection

Severe *Salmonella* infection will be defined as illness that includes any of the criteria

- Severe diarrhoea is defined ≥6 loose/liquid stools in 24 hours or ≥2 stools with gross blood in 24 hours
- Severe Salmonellosis is diagnosed if ANY of the following apply
- Oral temperature > 40.0oC
- Systolic blood pressure < 78 mmHg
- Significant lethargy or confusion (GCS <12)
- Severe diarrhoea lasting >72hrs
- Gastrointestinal bleeding lasting >24hrs
- Gastrointestinal perforation
- Extra-intestinal seeding of *Salmonella* Typhimurium

6.10. Adverse Events of Special Interest (AESI)

The following events will be considered AESIs and reported to the DSMC by email notification within four weeks of occurrence.

- Severe *Salmonella* infection
- Clinical or microbiological relapse of *Salmonella* gastroenteritis following completion of therapy
- Clinical or microbiological relapse of *Salmonella* bacteraemia following completion of therapy
-
- Reactive arthritis
- Pregnancy
- Transmission of S. Typhimurium to a contact of a participant
- AEs requiring a physician visit or Emergency Department visit which, in the opinion of study staff, are related to the challenge with S. Typhimurium.

Participants with severe *Salmonella* Typhimurium infection will be closely monitored during the initial study phase, until a course of antibiotic is completed, minimising the risk of complications.

The possible adverse effects of S. Typhimurium infection or the effect of some antibiotics on the outcome of pregnancy are unknown. Therefore, pregnant women will be excluded by history and laboratory tests, and female participants will be specifically instructed to prevent conception during the challenge period of the study until completion of antibiotic therapy and clearance of *Salmonella* infection is confirmed. Should pregnancy occur, information about outcome of the pregnancy will be sought.

6.11. Data safety monitoring committee

A Data and Safety Monitoring Committee (DSMC) will be appointed to provide real-time oversight of safety and trial conduct.

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. The DSMC will have access to data and, if required, will monitor these data and make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. The DSMC will particularly review the attack rate and advise on any adjustments to the cohort size to ensure confidence in the study results. A summary of all AESIs and SAEs to date will be provided to the DSMC at each review or upon request. The DSMC will also be notified if the study team have any concerns regarding the safety of a participant or the general public (e.g. if a participant is not contactable after S. Typhimurium challenge and potentially infectious to others).
The outcome of each DSMC review will be communicated directly to the study investigators and documentation of all reviews will be kept in the Trial Master file (TMF). The Chair of the DSMC will also be contacted for advice when the Chief Investigator feels independent advice or review is required.

6.12. Stopping and pausing rules

Further challenge of participants will be temporarily paused if a participant develops a SAE or at the advice of the DSMC. All AESIs and cases of bacteraemia will be reviewed by the DSMC. The DSMC will provide recommendations to the trial steering committee as to whether if it is safe and appropriate to challenge further participants and/or if protocol adjustments are required.

6.13. Review of bacteraemia

All cases of S. Typhimurium bacteraemia will be discussed with the DSMC. The DSMC will make recommendations to the trial steering committee if it is safe and appropriate to challenge further participants and/or if protocol adjustments are required.

6.14. Trial Management Committee

The trial investigators will form the trial management committee and will provide on-going management of the trial.

6.15. Procedure to be followed in the event of an abnormal finding

Abnormal clinical findings from medical history, examination, or blood tests, will be assessed as to their clinical significance using the severity grading criteria for Adverse Events (see Appendix). If a test result is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed, and appropriate medical care will be arranged with the permission of the participant. Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the study team.

7. ASSESSMENT AND FOLLOW-UP

The study will involve two challenge periods, referred to as challenge one and challenge two.

Before taking part, volunteers will attend a screening visit at the outpatient clinic to check eligibility.

Challenge one (Ch1) will begin after screening tests confirm eligibility. Participants will return to the clinic seven days before the challenge for a check-up and then be admitted to the hospital on the day of the challenge. They will remain in hospital quarantine for seven days so that the study team can monitor them closely. On day 7 they will be discharged home. Some participants can be discharged earlier than Day 7 if they meet pre-specified criteria.

After discharge, participants will receive a daily telephone call for six days. They will then return for an in-person clinic visit seven days after discharge. During this period of telephone follow-up, participants may be asked to come into the clinic sooner if they develop any symptoms. After this, they will attend further outpatient visits on day 14 and day 28 after the challenge.

Challenge two (Ch2) will take place 3–6 months later, following the same pattern: an outpatient visit seven days before the challenge, a seven-day hospital stay, discharge on day 7, daily telephone calls for six days, and an in-person clinic visit seven days after discharge. Further outpatient visits will take place on day 14, day 28, and day 90 after Ch2.

7.1. Timepoint 1: Screening visit

Participants will attend an in-person screening visit to (a) provide informed consent and (b) complete baseline assessments to confirm eligibility (inclusion/exclusion criteria).

Assessments and information collected:

- Demographics: age, sex, ethnicity, country of birth.

- Medical history: relevant medical and surgical history (participant report, supplemented where needed by GP records and/or shared NHS care records, e.g. the Shared London Care Record). Where clarification is required, medical records may be obtained and/or clinicians contacted.
- Blood donation: prior donations and plans for future donation during the study period.
- Immunisation history: with particular focus on prior oral typhoid vaccination.
- Contraception: current use (female participants only).
- Concomitant exposures/medications: prescribed medicines, over-the-counter medicines, vitamins/supplements, herbal supplements, and recreational drug use.
- Physical examination (minimum): cardiovascular, respiratory, abdominal, gross neurological examination, and BMI.
- Investigations:
- Bloods: FBC, U&E, LFTs, CRP, serum IgA, coeliac serology, HLA-B27, coagulation screen, haemoglobinopathy screen, HIV-1/2 antibody, hepatitis B surface antigen, hepatitis C IgG, HbA1c.
- Malaria screen: for participants who have travelled to malaria-endemic areas within the preceding 6 months.
- Urine: dipstick (with laboratory analysis if indicated) and pregnancy test (female participants only).
- 12-lead ECG.
- Abdominal ultrasound: to screen for gallbladder disease and abdominal aortic aneurysm.
- Questionnaires: GAD-7 and PHQ-9.
- Documents collected: 24-hour contact details for the participant and a nominated emergency contact.

Additional consent and participant information:

- Consent will be obtained to register participants on the Over-Volunteering Prevention System (TOPS).
- Participants will be asked to inform close contacts of participation and will be given written information to share.
- Participants will be offered optional consent for the Imperial College Healthcare Tissue Bank.

If a participant is found to be ineligible, the reason will be explained. Clinically relevant abnormal findings will be communicated to the participant and, where appropriate, their GP will be informed.

7.2. Timepoint 2 - Pre-challenge visit 1 - Ch1_D-7

Volunteers will be invited to attend a study visit one to two weeks (Day -14 to Day -4) prior to challenge (designated as the “Day -7 visit”), after which they will be formally enrolled in the study.

The primary purpose of this visit is to prepare participants for the challenge period and to test stool for *Salmonella* spp and other gastrointestinal pathogens. We will repeat a medical history and assessment to ensure that there have been no changes to participant health since the screening visit. Study procedures are outlined in and sample collection is outlined in the sample collection table.

7.3. Timepoint 3 - Primary challenge visit & Admission to Inpatient Quarantine - Ch1_D0

Participants will be administered the S. Typhimurium challenge agent for primary challenge on Day 0. The procedures surrounding challenge are outlined in below. They will be admitted on the morning of challenge and remain in quarantine for the next 7 days. The inpatient quarantine beds will be in the Imperial Clinical Research Facility (ICRF) at the Hammersmith Hospital Campus or Ward 15N/S at Charing Cross Hospital Campus (Imperial College Healthcare NHS trust).

7.4. Timepoints 4 to 10 - Inpatient quarantine #1 (Days 1 - 7 post primary challenge) - Ch1_D1/D2/D3/D4/D5/D6/D7

Participants will undergo daily in-person assessments whilst in the quarantine unit following the challenge.

Participants will be discharged from quarantine on Day 7. Participants who have not received antibiotics will be discharged with antibiotics to be taken if and how instructed by a study doctor.

Participants who meet the primary endpoint prior to day 7 will be eligible for early discharge if they meet discharge criteria outlined below. An in-person visit will be arranged at day 7.

7.5. Timepoints 11 to 17 - Post quarantine observation #1 (Days 8 - 14 post primary challenge) - Ch1_D8/D9/D10/D11/D12/D13/D14

On Days 8, 9, 10, 11, 12, and 13 post primary challenge, participants will have remote assessments conducted via telephone or a secure video call using an appropriate platform (e.g., Microsoft Teams).

Participants will be reviewed in person on Day 14 (see section 9.11 for study visit windows).

If participants develop symptoms, including but not limited to fever or diarrhoea, or at the discretion of the study investigators, an additional in-person visit may be scheduled between Days 8 and 13.

The procedures for observations during this period are outlined below.

7.6. Timepoint 18 - Convalescent visit #1 (Day 28 post primary challenge) – Ch1_D28

At Day 28 post primary challenge, participants will attend in person for clinical assessment as well as blood and stool collection.

7.7. Timepoint 19 - Pre-challenge visit #2 Ch2_D-7

Secondary challenge (re-challenge) will be undertaken 3-6 months after primary challenge. When the re-challenge date has been set, participants will be seen approximately one week prior to re-challenge, repeating the “D-7” assessment.

7.8. Timepoint 20 – Secondary Challenge visit & Admission to Inpatient Quarantine -Ch2_D0

Secondary challenge (re-challenge) will be undertaken 3-6 months after primary challenge. The procedures surrounding re-challenge will match those surrounding the primary challenge.

7.9. Timepoints 21 to 27 – Inpatient quarantine #2 (Days 1 - 7 post-secondary challenge) - Ch2_D1/D2/D3/D4/D5/D6/D7

Participants will be admitted to quarantine for a second period during which they will undergo re-challenge. The monitoring considerations for the secondary quarantine admission will match those of the primary inpatient quarantine period.

7.10. Timepoints 28 to 34 - Post quarantine observation #2 (Days 8 - 14 post-secondary challenge) – Ch2_D8/D9/D10/D11/D12/D13/D14

Participants will be discharged from quarantine between at 7 days after re-challenge and/or upon fulfilling discharge criteria, whichever is sooner. They will then undertake post-quarantine observation assessments to match those of the observation assessments undertaken after primary challenge.

7.11. Timepoint 35 – Convalescent visit #2 (Day 28 post secondary challenge) – Ch2_D28

Participants will be reviewed in person 28 days after the secondary challenge (re-challenge).

7.12. Timepoint 36 - Convalescent visit #3 (Day 90 post-secondary challenge) – Ch2_D90

Participants will be reviewed in person at day 90 following secondary challenge (re-challenge).

7.13. Study visit window periods

Every effort will be made to perform the visits on time as indicated in the study protocol. Based on previous experience with challenge studies and similar trial

protocols, we are aware that in some instances this may be difficult. A window of time to complete the visits will be as follows:

- Timepoint 0: Within 180 days of timepoint 2 (C1_D-7)
- Timepoint 2: -7 days/+3 days
- Timepoint 17: +/- 4 days
- Timepoint 18: +/- 6 days
- Timepoint 19: -7 days/+3 days
- Timepoint 34: +/- 4 days
- Timepoint 35: +/- 6 days
- Timepoint 36: +/- 14 days

7.14. Unscheduled visits

Up to 10 unscheduled visits may be made at the discretion of the study team as deemed necessary for safety or the participant and/or to confirm clearance of the infection. Additional safety/research bloods may be collected at unscheduled visits for safety monitoring and data quality. Participants will be paid for extra unscheduled visits on an ad hoc basis.

7.15. End of study

The study will end at last participant has completed the final scheduled study visit (LPLV), once all protocol-required assessments are complete, key safety follow-up (including any required microbiological clearance assessments, where applicable) has been performed, and all serious adverse events and ongoing clinically significant adverse events have been appropriately followed to resolution or clinical stability. Study close-out will be confirmed following database lock and completion of required regulatory reporting.

8. STUDY INTERVENTIONS

8.1. S. Typhimurium challenge

We have selected to undertake bacterial challenge and re-challenge with *Salmonella* Typhimurium strain D23580, which belongs to the ST313 lineage.

The challenge strain has been manufactured to a GMP standard, equivalent to that used for IMP production. Manufacture was undertaken at an accredited facility (Pilot Bioproduction Facility [PBF], WRAIR). The challenge strain has a detailed record of isolation and storage, and have been deposited in the UK National Collection of Type Cultures (NCTC).

The D23580 (NCTC14677) strain was originally isolated from a blood culture taken from a 24-month-old child at Queen Elizabeth Central Hospital, Blantyre, Malawi. It was supplied to the University of Liverpool from the Malawi-Liverpool-Wellcome unit and was deposited in the NCTC under the terms of the material transfer agreement. Vials of both strains were supplied by the University of Liverpool to the PBF at

WRAIR, who undertook manufacture to GMP standard of both strains. Batch production records have been produced for both strains. Microbial limits testing was performed to confirm absence of other specified pathogenic bacteria, namely Escherichia coli, Pseudomonas spp., Bacillus cereus, Staphylococcus aureus, *Salmonella* spp. (other than S. Typhimurium), yeasts and moulds and <100 non-pathogenic bacteria and <20 fungi/ ml of the strain material. The culture media and selective media used are outlined in the batch production record. Microbial limits testing was performed by WRAIR. Full antibiotic sensitivity and microbial purity of both strains in the master cell banks was demonstrated and further characterisation work including genome sequencing has been completed at the University of Liverpool. Samples have been taken at defined time points to evaluate the stability of the frozen GMP manufactured product. Stability testing has been performed by WRAIR, in accordance to established SOPs.

8.2. Storage

The S. Typhimurium D23580 strains is stored as a frozen suspension in soya tryptone medium containing 10% sucrose. Suspensions have been labelled with the contents (S. Typhimurium 4/74 or S. Typhimurium D23580), date of manufacture, CFU information, storage conditions, batch and vial number.

Vials of the required concentration of S. Typhimurium will be thawed and diluted immediately prior to use. The actual challenge dose administered will be back calculated after each administration. Details of sample storage and accountability are outlined in the Laboratory Analysis plan and in local SOPs.

8.3. Accountability for the challenge strain

The investigator will be responsible for adequate and accurate accounting of S. Typhimurium vials prepared for administration to participants. The investigator or designee will administer the study S. Typhimurium vials only to individuals included in this study following the procedures set out in this study protocol and the associated local SOPs and Study Plans. The date, dosage and time of administration will be recorded.

The study team will track all vials of S. Typhimurium that have been used, administered to participants, and wasted within an accountability log.

8.4. Preparation of challenge agent

The solution for ingestion containing wild type S. Typhimurium D23580 will be prepared in a Class II biological safety cabinet within containment level 2 laboratory. Challenge solution preparation is conducted by laboratory staff and checked by two laboratory staff members. Two clinical study team members check the challenge solution immediately prior to ingestion by the participant. The water, 0.9% saline and bicarbonate used for the preparation will be commercially available food products. The strain will be prepared, checked, and given to participants as outlined in relevant SOPs and Laboratory and Clinical Study Plans. Following solution ingestion, the

single-use containers will be returned to the laboratory for inspection, autoclaving, and disposal.

8.5. Administration of S. Typhimurium D23580 (ST313)

Challenge agent used in this study will be administered by the oral route with sodium bicarbonate at a dose of 1-5x105 CFU. Participants will fast for 90 minutes before and after challenge.

The procedure for administration is:
- Remove the S. Typhimurium suspension from the BIOJAR.
- Check label to ensure contents of solution match participant allocation.
- Ask the participants to drink the 120 ml of bicarbonate solution.
- Ask the participants to ingest the 30ml S. Typhimurium 0.9% saline solution.
- Return containers to the laboratory for disposal in accordance with local SOPs.

8.6. Compliance with Trial Treatment

The challenge agents will be administered by trained doctors and nurses. Administration will be documented according to Good Clinical Practice (GCP) guidelines and relevant local SOPs. Issues related to compliance are therefore the responsibility of the study staff that have received appropriate training.

8.7. Concomitant Medication

Any medication taken by the participant at the time of enrolment into the study or during the first 90 days of the study period will be recorded on the eCRF. Study medications as outlined in will be provided by study staff according to clinical indication.

8.8. Post-trial Treatment

Study medication will not be continued beyond the study period. Unused medications prescribed during the study will be returned to the study team for disposal 28 days after NTS challenge.

9. STATISTICS AND DATA ANALYSIS

9.1. Sample size

Data and all appropriate documentation will be stored for a minimum of 10 years after study completion (including the follow-up period).

The primary endpoint is binary (infection versus no infection as defined by the primary endpoint). This will be analysed within participants using McNemar's test at a two-sided significance level of 0.05. Based on data from the CHANTS study, the attack rate at the 1–5 × 10^5 CFU dose was approximately 57%. For the present

study, we assume that re-challenge will reduce the attack rate by 50% (i.e. from 57% to 28.5%).

Power for a paired design depends on the proportion of discordant outcomes between the first and second challenge. We assume that few participants who are negative at the first challenge will become positive on re-challenge, and that most of the difference will arise from participants who are positive initially and negative at re-challenge.

Under these assumptions, with 30 paired participants the study will achieve approximately 61–83% power to detect the expected reduction, depending on the exact proportion of discordant pairs. With 50 paired participants the corresponding power is approximately 82–97%.

As a sensitivity check, if the two challenges were analysed as independent groups rather than paired data, power to detect a reduction from 57% to 28.5% would be about 64% with 30 per group and about 85% with 50 per group. This illustrates the greater efficiency of the paired analysis, which will form the primary analysis strategy.

On this basis, a total sample size of 30–50 paired challenges is considered sufficient to detect a 50% reduction in attack rate at re-challenge with adequate statistical power.

For planning purposes, we assume an attrition rate of approximately 10–15% between primary and secondary challenge.

Table 2 - estimated statistical power for paired re-challenge design under varying assumptions of discordant pairs. Power is calculated using McNemar's test at a two-sided significance level of 0.05. The baseline attack rate is assumed to be 57%, with a 50% relative reduction at re-challenge (to 28.5%). Power depends on the proportion of discordant outcomes, particularly participants who were negative at primary challenge but positive at re-challenge (p01). Most of the observed effect is expected to arise from participants who were positive initially and negative at re-challenge (p10). The table shows power estimates for total sample sizes of 30 and 50 paired participants across a plausible range of p01 values.

Total N (paired)	Assumed p01	Power (two-sided, α=0.05)
30	0	83%
30	0.02	78%
30	0.05	71%
30	0.1	61%
50	0	97%
50	0.02	94%
50	0.05	90%
50	0.1	82%

9.2. Analysis Populations and Statistical Approach

The primary analysis will focus on participants who completed both the primary and re-challenge phases of the study. Analyses will be conducted using both the modified intention-to-treat (mITT) and per-protocol (PP) populations, as defined below. Secondary subgroup analyses will examine the relationship between infection status at the first and second challenges.

For the primary objective, the mITT population is defined as participants who:

- Have received both the primary and re-challenge doses of *Salmonella* Typhimurium D23580 at the specified dose;
- Were not culture-positive for *Salmonella* spp. prior to their first challenge (baseline negative); and
- Have completed all post-challenge follow-up for both challenge periods sufficient to assess the primary endpoint (systemic Salmonellosis).

This population therefore represents volunteers who have undergone full study participation and for whom outcomes of both challenge and re-challenge can be directly compared.

The Per-Protocol (PP) population will include participants within the mITT set who completed all protocol-specified visits and assessments without any major deviation that could affect endpoint assessment (for example, early antibiotic administration, missed sampling, or failure to meet dosing criteria). Participants will be analysed according to the actual challenge received.

The Safety Set will include all participants who underwent at least one challenge (primary or re-challenge).

Safety data will be analysed according to the challenge received, and all data collected prior to withdrawal will be included in the analysis.

The primary analytical subset of interest comprises those participants who developed systemic Salmonellosis following their first (primary) challenge. Within this group, the analysis will examine the rate and severity of infection after re-challenge to quantify the extent of protection conferred by prior exposure.

In addition, three predefined secondary subgroups will be examined:

- Participants infected at both the primary and re-challenge phases.
- Participants infected at only one of the two challenge phases.
- Participants infected at either challenge (combined infection population).

These exploratory subgroups will be used to evaluate immunological, microbiological, and clinical correlates associated with protection or susceptibility between challenges.

All population definitions will be confirmed and detailed in the Statistical Analysis Plan (SAP), which will be finalised and approved by the Chief Investigator before database lock.

9.3. Analysis of Demographics and Baseline Characteristics

Descriptive statistics (mean ± SD, median [IQR], counts and percentages) will summarise demographic and baseline clinical characteristics for the FAS population. No formal hypothesis testing will be conducted to compare baseline characteristics between challenge periods.

9.4. Primary Outcome Analysis

The primary objective is to determine the attack rate of systemic Salmonellosis after primary and re-challenge with S. Typhimurium D23580.

For each challenge, the attack rate will be defined as the proportion of participants meeting criteria for systemic Salmonellosis (fever ≥ 38 °C on ≥ 2 occasions ≥ 12 hours apart and/or confirmed S. Typhimurium bacteraemia).

Comparisons between the two challenge periods will use a paired design, focused on the primary analytical subset (participants infected at the first challenge).

Null hypothesis (H_0): Attack rate (re-challenge) ≥ Attack rate (primary challenge)

Alternative hypothesis (H_1): Attack rate (re-challenge) < Attack rate (primary challenge)

The primary comparison will employ McNemar's test for paired binary data with 95 % confidence intervals for discordant pairs. Relative risk reduction and 95 % confidence intervals will be presented.
Exploratory logistic regression with participant-level random effects will evaluate covariates (e.g. sex, baseline antibody titre, magnitude of initial illness).

A supplementary time-to-event (Kaplan–Meier) analysis will explore time from challenge to infection onset, censoring participants who withdraw, receive early antibiotic therapy, or remain uninfected at the end of follow-up.

9.5. Secondary Outcome Analyses

Secondary endpoints (including gastroenteritis rate, stool shedding, fever duration, shedding kinetics, and immunological correlates) will be analysed as follows:

Binary outcomes (e.g. infection or colonisation) → Chi-square or Fisher's exact tests.

Continuous outcomes (e.g. duration, antibody titre, cytokine concentration) → mean ± SD or median [IQR]; comparisons via t-test or Mann–Whitney U test.

Correlations between immune parameters and protection → logistic or linear regression using $\log_{10}$-transformed titres.

Subgroup analyses will explore differences between the predefined infection status categories (infected both / one / either challenge).

A two-sided α = 0.05 will be used for significance testing unless otherwise stated. All model specifications will be detailed in the SAP.

9.6. Analysis of Safety Outcomes

Safety analyses will be descriptive and conducted on the Safety Set.

Solicited AEs will be summarised daily for the first 7 days post-challenge and post-re-challenge, with maximum severity summarised over the 14-day post-challenge period.

Unsolicited AEs, SAEs and AESIs will be listed individually with onset, duration, relationship to challenge, outcome, and seriousness assessment.

Expected AEs (e.g. mild to moderate gastroenteritis) will be summarised separately.

All safety data will be summarised by challenge phase, severity, and relatedness to the challenge agent.

9.7. Analysis of secondary endpoints

The analysis for secondary endpoints will follow the primary endpoint analysis. For the time-to-event analyses of secondary endpoints (e.g. positive blood culture, oral temperature ≥ 38.0°C etc.) all participants will be included. Participants not meeting the criteria for an individual secondary endpoint will be censored in the analysis at, the time of withdrawal, the time of diagnosis or Day 14, whichever is earlier.

9.8. Subgroup analysis

We will conduct prespecified subgroup analyses to explore patterns of susceptibility and protection across challenge episodes. Subgroups will be defined according to diagnostic outcome at primary and secondary challenge.

1. Diagnosed at both challenges (P11): participants who met the diagnostic criteria at both primary and secondary challenge.
2. Diagnosed only at primary challenge (P10): participants who met diagnostic criteria at the first challenge but not at the second (suggesting protection on re-challenge).

3. Diagnosed only at secondary challenge(P01): participants who did not meet diagnostic criteria at the first challenge but did at the second (representing de novo events).
4. Not diagnosed at either challenge(P00): participants who remained negative at both challenges.

Comparisons of attack rates will be made between primary and secondary challenge within these subgroups, with exact binomial confidence intervals reported. Discordant pairs (categories 2 and 3) will be explicitly examined, as these contribute most directly to the paired analysis under McNemar's test. The primary focus will be on the proportion of participants protected from re-infection (category 2), while also quantifying the frequency of de novo events (category 3).

Exploratory analyses may additionally compare baseline demographic and immunological characteristics between subgroups, in order to generate hypotheses about correlates of protection and susceptibility.

9.9. Endpoint definitions

- Diarrhoea – The passage of three or more loose or liquid stools per day.
- Loose/Liquid stool – Stool score of 6 or 7 on the Bristol stool scale 34
- Type 6 stool – Fluffy pieces with ragged edges, a mushy stool
- Type 7 stool – Watery, no solid pieces, entirely liquid
- Diarrhoea severity
- Mild - 3 loose/liquid stools in a 24-hour period
- Moderate - 4–5 diarrheal stools in 24 hours
- Severe - ≥6 loose/liquid stools in 24 hours or ≥2 stools with gross blood in 24 hours
- Time to onset of symptoms – Time (Hours & Days) to first recorded solicited symptoms in the diary card OR first temperature ≥38oC
- Duration of illness – Time (Hours & Days) from first recorded individual solicited symptoms to complete resolution of individual recorded symptoms in the diary card.
- Fever clearance time – Time (Hours & Days) from first dose of treatment until temperature ≤37.5°C for a 48 hour period.

Symptom severity

- The proportion of participants with maximum symptom severity score graded as mild, moderate or severe following challenge. See Appendix.
- The proportion of participants meeting the criteria for severe Salmonellosis
- The proportion of participants recording one or more severe solicited or unsolicited symptoms following challenge.
- Total symptom scores calculated by summing numerical values assigned to the severity of all solicited symptoms between Day 0 to Day 14 (graded 0-4).

- Individual symptom severity scores calculated by summing numerical values assigned to the severity of individual solicited symptoms between Day 0 to Day 14 (graded 0-4).

- Time to diagnosis – Time (Hours/Days) from challenge until fulfilment of diagnostic criteria (taken as date/time Gram negative rods are detected in blood culture AND/OR recorded temperature ≥38oC for 12hours)

- Time to onset of bacteraemia – Time (Hours/Days) from challenge until the date/time first positive blood culture collected.

- Duration of bacteraemia – Time (Hours/Days) from collection of first positive blood culture until date/time of the first negative blood culture and blood cultures are persistently negative.

- Gastrointestinal bleeding - overt bleeding originating from any part of the gastrointestinal tract. This includes visible blood in vomit (hematemesis), blood passed through the rectum (haematochezia), or black, tarry stools (melena). This definition does not include non-visible (occult) bleeding detected solely by faecal occult blood testing.

- Time to onset of stool shedding - Time from challenge (Hours/Days) to the first positive stool culture.

- Time to onset of diarrhoea - Time from challenge (Hours/Days) to the first grade 6/7 stool.

- Duration of stool shedding – Cumulative number of days where positive stool samples test positive for *Salmonella* Typhimurium by culture and/or PCR

- Pattern of stool shedding – Descriptive

- Haematological and biochemical parameters
    - Total Haemoglobin (g/L)
    - Haemoglobin change from baseline (Hb g/l D0 – Hb g/l D14)
    - Total White Cell Count (x109/l)
    - Platelet counts (x109/l)
    - Neutrophil count (x109/l)
    - Lymphocyte count (x109/l)
    - Monocyte count (x109/l)
    - Eosinophil count (x109/l)
    - Monocyte/Lymphocyte ratio
    - Urea & Electrolytes (Na, K+, Urea, Creatinine –mmol/l)
    - C-reactive protein (mg/l)
    - Liver function tests (Bilirubin [umol/l], aspartate transaminase (AST IU/l), alkaline phosphatase (ALP IU/l), alanine transaminase (ALT IU/l), Albumin (g/L)

9.10. Level of statistical significance

The main study outcome is a proportion, which will be quoted with 95% confidence intervals. 95% confidence intervals will be used throughout.

9.11. Criteria for termination of the study

The trial steering committee with the Data and Safety Monitoring Committee will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the clinical study team will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the Sponsor, Imperial College Healthcare NHS Trust and the relevant Ethics Committee will be notified within 15 days of this occurring.

9.12. Procedure for accounting for missing, unused and spurious data

All available data will be used in the analyses. Participants will be analysed according to the type of challenge received for summaries by group.

9.13. Procedures for Reporting deviations from the Original Statistical Plan

Any additional analysis or deviations from the analysis plan will be documented and updated according to the statistical standard operating procedure.

10. STUDY PROCEDURES

10.1. Recruitment

Several strategies may be employed to recruit the required cohort of participants, including:

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor's surgeries, tertiary education institutions and other public places with the permission of the owner/ proprietor.
- Direct mail-out: This will involve direct mailing of the study information sheet to adults whose names and addresses have been obtained via the Electoral register. Those people who have indicated they do not wish to receive postal mailshots will have their names removed prior to the study team being given the names and addresses. The company providing this service is registered under UK GDPR.
- E-mail communication: We will contact representatives of local tertiary education establishments, local employers, and advocacy groups and ask them to circulate posters and information, and to circulate a link to study information on the study website by email.
- NIHR Imperial Clinical Research Facility healthy volunteer database – Direct email and link to members of the public who have registered their interest in

potentially volunteering for clinical trials conducted by the ICRF. This secure database is maintained by the NIHR ICRF and members of the public registered here have given consent to have their details recorded and be contacted expressly for this purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to volunteering for any trial they are contacted about.

- Media advertising: Local media, newspaper, website, and social media advertisements placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- Website advertising: Description of the study and copy of information booklet on the study website.
- Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

10.2. Expression of interest

Potential volunteers will be asked to read the PIS (available to download from the website) and then complete a pre-screening questionnaire on the study website and submit their contact details and consent for us to request their SCR. If any answers a participant provides in the questionnaire mean they are ineligible to take part, they will receive an automated message and no personal data will be captured.

We will send the information booklet by email or post to read at their leisure upon request.

Volunteers who are interested in study participation will be able to contact the study team by telephone, text message, instant message service (e.g. WhatsApp) or email, or by visiting the study website online registration.

10.3. Initial eligibility assessment of potential study participant

We will obtain consent to contact their GP (or other clinician involved in their long-term care) to access a shared electronic NHS care record to confirm their medical and immunisation history and participation in the study. If their medical history excludes them from taking part, they will be contacted to explain why and their medical history and other personal details (such as contact details) held by us deleted.

If participants are willing to proceed, they are invited for a screening and consent visit, where a member of the clinical research team will assess their eligibility. To further assess understanding, a consent quiz will be provided at the screening visit. Participants must complete the quiz as part of the consent process, ensuring they fully comprehend the study requirements and procedures before proceeding.

10.4. Informed Consent

The participant will personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Consent will be sought as described in relevant local SOPs and the clinical study plan.

Written and verbal versions of the participant information booklet and informed consent form will be presented to the participant, detailing no less than:

- the exact nature of and the rationale for performing the study
- implications and constraints of the protocol
- The risks and benefits involved in taking part.
- To contact their GP (or other clinician involved in their long-term care) to inform them of their participation in the trial.
- For a study staff member to hold the name and 24-hour contact number of a close friend, relative or housemate who lives nearby and will be kept informed of the study participant's whereabouts after the study. This person is to be contacted if study staff are unable to contact the participant for clinical follow up. The 24-hour contact will receive written information, and complete and sign a reply slip that the participant will give the study doctor/nurse before challenge.
- For UKHSA and the local health protection team to be informed of study participation, challenge outcome, commencement and completion of antibiotic course, and clearance results.
- It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. The participant will be allowed adequate time to consider the information, and the opportunity to question the researcher, their GP, or other independent parties to decide whether they will participate in the study.
- The study team will conduct in-person consenting using the GCP-compliant OpenClinica v4.0 e-Consent module, with paper consent forms available as an alternative when needed.

Written informed consent will be obtained by means of a dated signature of the participant and a signature of the study staff member who presented informed consent. A copy of the signed informed consent will be given to the participant and if on paper, the original signed form will be retained at the study site. A doctor or nurse, who has been trained in the consent process, will conduct the informed consent discussion.
Participants will be asked to complete a consent quiz before completing the informed consent form to ensure they have properly understood the study.

10.5. Management of Incidental medical findings

All laboratory results will be reviewed and collated by the study team who will record these in the electronic source database. If a test result is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the participant will be informed, and appropriate medical care arranged with the permission of the participant in liaison with their GP.

Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Chief and Co-Investigators.

10.6. Eligible participants

After all screening procedures are completed, the study investigators will review all results. An eligibility checklist will be documented in the CRF by two separate study investigators.

If eligibility checklist is satisfactory, a volunteer will be deemed eligible to participate. Volunteers will at that point be invited to attend a pre-challenge visit, when they will be enrolled in the study.

10.7. Eligible but declined to participate

Some individuals may be deemed eligible to participate after screening, but subsequently decline to participate. For the purposes of CONSORT reporting, these participants will be designated as 'Declined to Participate'. This will be recorded in the CRF for the purposes of maintaining a screening log.

10.8. Ineligible participants

After screening, some volunteers may be deemed ineligible to participate if they meet any of the study exclusion criteria or if screening investigations are abnormal. Decisions to exclude potential participants from enrolling in the trial or to withdraw a participant from the trial will be at the discretion of the Chief and Co-Investigators. Participants will be informed of the reason for screening failure and if any further action is required. They will be reimbursed for the screening visit and investigations as outlined below. For the purposes of CONSORT reporting, these participants will be designated as screening failures. The reason for screening failure will be documented in the CRF for the purposes of maintaining a screening log.

10.9. Enrolment

A participant is considered enrolled at the time of the Pre-challenge visit (Timepoint 2). A maximum period of 180 days between initial screening and enrolment into the study will be accepted. If the period between screening and enrolment exceeds 180 days, rescreening will consist of repeat informed consent, interim medical history, and blood tests as a minimum. Additional screening investigations, including repeat ultrasound gallbladder scan, may be performed at the discretion of the study doctor following consultation with the senior study investigator.

10.10. Stool collection

Participants will be asked to provide a stool sample prior to challenge. The purpose of this is to ensure that participants are not colonised with *Salmonella* spp. or other enteric pathogens prior to challenge.

To facilitate sample collections, participants will be issued with a stool collection kit at the screening visit or by post. This stool collection kit includes an opaque transport bag, cool packs, stool pots, transport tubes, toilet seat stool collection paper and stool collection instructions.

Participants will be asked to return the stool sample at the Day –7 visit, and if missed, no later than 72 hours prior to challenge.
The stool sample will be processed for Stool *Salmonella* PCR for *Salmonella* spp. and other enteric pathogens.

The result of the stool sample will be recorded in the CRF.

10.11. Diary card

At the Day –7 visits, participants will be provided with access to an electronic diary. They will be instructed in how to complete the diary and when to report to the study team. The diary will be accessible via web browser or mobile application and designed to comply with data protection requirements under UK GDPR and Imperial College London sponsor standards.

10.12. Diary card Recording Schedule

Solicited and unsolicited symptoms will be recorded from Day –7 (seven days prior to challenge) through to Day 14 post-challenge.

Participants will be asked to record entries once daily and additionally whenever they experience new or worsening symptoms.

Oral temperature will be recorded at least twice daily and at any additional time points if participants perceive a subjective fever.

The diary will be reviewed daily during the inpatient stay and during the telephone consultations from day 8 to day 13. Participants may be contacted outside of clinic visits by telephone or email to confirm that the diary card has been completed and to review solicited and unsolicited symptoms.

10.13. Solicited symptoms

Participants will be specifically prompted to record the following solicited symptoms

- Diarrhoea
- Abdominal pain
- Loss of appetite
- Nausea
- Vomiting
- Tenesmus
- Headache

- Malaise
- Myalgia
- Arthralgia
- Cough

10.14. Unsolicited Symptoms

In addition to the above, participants will be able to record any unsolicited symptoms. Unsolicited symptoms will be recorded from challenge up to 6 months post-secondary challenge (re-challenge).

10.15. Food diary

Dietary information will be collected to provide both a snapshot of participants' habitual dietary intake and repeated measures of short-term intake during the study. This will enable integration of dietary data with microbiota analyses, recognising that diet is a key driver of gut microbial composition and function.

Data will be obtained using validated tools capable of capturing habitual dietary patterns over weeks to months, complemented by repeated 24-hour dietary assessments that provide resolution on day-to-day variation. We aim to assess both long-term dietary habits and proximal intake to allow analysis of dietary diversity, food group consumption, and overall dietary patterns1. These will inform to microbiota outcomes,

- Day Ch1_D–7 (pre-challenge baseline): Participants complete a validated Food Frequency Questionnaire (FFQ) (for example, the EPIC-Norfolk FFQ) or equivalent instrument to characterise habitual diet over the preceding weeks/months.
- Days CH1_D0 through CH1_D14 and CH2_D0 through CH2_D14 (challenge period + defined recovery window): Participants complete daily 24-hour dietary recalls (or food records) via an electronic e-diary system. Each day's recall pertains to the prior 24 hours (midnight to midnight or specified window).
- Optional extended assessment: At later timepoints (e.g. 1 month, 3 months), repeat a FFQ or short food frequency instrument to detect shifts in habitual diet.
- Data processing: Recalls and FFQ data will be converted into food-group and nutrient outputs, used to derive dietary diversity indices or diet quality scores for incorporation as covariates in microbiome models.

10.16. Pre-challenge assessment

Check observations and perform physical examination if clinically indicated; complete continued consent form; check eligibility criteria (specifically for temporary

exclusion criteria to challenge) and check for occurrence of any new adverse or medically significant events since previous visit.

- Check details of the 24-hour contact (who will be kept informed by the participant of their whereabouts for the subsequent 28 days)
- Record oral temperature, pulse, and blood pressure.
- Perform urinary pregnancy test for all females.
- Collect clinical specimens
- Confirm the participant has fasted for a minimum of 90 minutes prior to ingestion of the challenge agent.

10.17. Assessment after challenge

- Admit the participant to the quarantine unit.
- Fast for a further 90 minutes.
- Participants who vomit for any reason within 90 minutes of the challenge will be withdrawn from the trial and treated with antibiotics.
- Instruct participant to notify staff any serious adverse events/reactions that occur prior to next review.
- Instruct participant not to use antipyretics.
- Instruct participant to notify study team if they require the use of any medications.
- Instruct participant to notify study team if they have a temperature ≥38.0°C.
- Provide participant with a link to an electronic diary for recording systemic effects and oral temperatures.
- Check details of a mobile telephone number that the participant will be carrying with them for the 14 days post-challenge. Counsel the participant on the importance of maintaining contact with the study team and keeping the mobile always switched on and with them. Explain to the participant that if they are uncontactable, and their 24-hour contact is unsure of their whereabouts, the study team will make every effort to locate them.
- Issue participant with information on enteric precautions.
- Educate participant on correct hand washing technique, including demonstration and observation.
- Advise participant to inform the study team if any breaches of enteric precautions occur such that another individual meets excreta from the participant.
- Issue participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.
- Instruct participant on obtaining stool specimens (as outlined in the relevant SOPs) and provide the participant with sampling equipment.
- Notification of challenge will be provided to the local UKHSA health protection team, and to the participant's GP.

10.18. Inpatient quarantine

Participants will be admitted to a quarantine facility immediately after challenge. The inpatient quarantine beds will be in the ICRF at the Hammersmith Hospital Campus or Ward 15N/S at Charing Cross Hospital Campus (Imperial College Healthcare NHS trust).

During the inpatient quarantine period, the participants will be reviewed at least daily by a member of the study team. Designated assessments will take place during this time, including the following procedures:

- Check continued consent,
- Review symptoms profile, diary entries, and laboratory blood results (if available),
- Check for occurrence of any new adverse or medically significant events since previous visit,
- Record oral temperature, pulse, and blood pressure
- Perform GAD-7 Anxiety Test questionnaire and PHQ-9 Depression Test questionnaire (only on day of discharge)
- Perform sample collection as per Table 9,Table 10
- Prescribe and issue concomitant medication for symptom control if required
- Re-iterate participant requirements such as completion of the diary, refraining from use of antipyretics, notification of any medication administration, and notification of any fever ≥38.0°C (where appropriate).
- Re-iterate hand hygiene precautions

The quarantine facility will be staffed 24/7 by trained nurses who will work alongside the research team. A clinician will be on-call 24/7 and will be alerted by the staff nurses in case of any abnormal observations. Out of hours visits will be arranged as necessary.

10.19. *Salmonella* diagnosis

At any point post challenge a participant may meet the criteria for *Salmonella* diagnosis (SD) and/or the criteria for commencing treatment initiation (Table 4). These criteria will be assessed at each assessment.

10.20. Discharge

All participants will be admitted for inpatient quarantine immediately after challenge. The planned duration is 7 days. Early discharge on Days 4–6 is permitted only if the protocol-specified discharge criteria are met and public health requirements are satisfied as outlined in Table 3. If these criteria are not met on Day 7, then participants will be asked to remain in quarantine until the discharge criteria are met.

Upon discharge from the hospital, participants will be issued a rescue pack of antibiotics. Daily reconciliation will be performed remotely to ensure participants have not taken antibiotics outside the study protocol.

Table 3 - Criteria for discharge. 1 – Resolution of diarrhoea will be taken as 48hrs measured from the time of first type 1-5 stool, with no type-6-7 stools in the interim.

1) *Salmonella* Diagnosis (SD) Treatment Pathway
Medically fit for discharge in the opinion of the study physician AND
Antibiotic treatment has been initiated AND
Complete resolution of diarrhoea (Bristol stool type 6-7) for 48 hours[1] AND
Resolution of *Salmonella* Typhimurium bacteraemia (if applicable)
2) Not diagnosed pathway
Medically fit for discharge in the opinion of the study physician AND
No fever ≥38 °C AND
No bacteraemia AND
Complete resolution of diarrhoea (Bristol stool type 6-7) for 48 hours AND
Issued with antibiotic rescue pack to start on clinician advice after same-day clinical contact.

10.21. Post quarantine outpatient observation

From timepoints 11 to 16, the default mode of assessment will be conducted remotely via phone or a secure video call using an appropriate platform, such as Microsoft Teams. However, an in-person visit may be scheduled at any time at the discretion of the study investigators. At timepoint 17, all participants will be reviewed in person.

If participants develop symptoms, including but not limited to fever or diarrhoea, or if deemed necessary by the study investigators, an additional in-person visit may be scheduled between Days 8 and 13.

The next scheduled in-person visit will occur on Day 14 post challenge (Timepoint 17).

Designated assessments will take place during this time, including the following procedures:

- Check continued consent,
- Check for occurrence of any new adverse or medically significant events since previous visit,
- Review symptoms profile, diary entries, and laboratory blood results (if available),
- Record oral temperature, pulse, and blood pressure
- Perform sample collection as per Table 9
- Instigate monitoring of faecal shedding as outlined in Table 6
- Daily reconciliation will be performed remotely to ensure participants have not taken antibiotics outside the study protocol.
- Prescribe and issue concomitant medication for symptom control if required.

- Re-iterate participant requirements such as completion of the diary, refraining from use of antipyretics, notification of any medication administration, and notification of any fever ≥38.0°C (where appropriate).
- Re-iterate hand hygiene precautions
- Check details of a mobile telephone number that the participant will be carrying with them.
- Counsel the participant on the importance of keeping the mobile always switched on and with them. Explain to the participant that if they are uncontactable, and their 24-hour contact is unsure of their whereabouts, the study team will make every effort to locate them. This may include notifying the police.
- Provide details of next clinic appointment.

10.22. Convalescent visit #1 (Day 28 Post primary challenge)

At Day 28 post primary challenge, participants will attend in person for clinical assessment as well as blood and stool collection. Designated procedure include:

- Check continued consent,
- Check for occurrence of any new adverse or medically significant events since previous visit,
- Review symptoms profile, diary entries, and laboratory blood results (if available),
- Record oral temperature, pulse, and blood pressure
- Perform sample collection
- Instigate monitoring of faecal shedding as outlined in Table 6
- Medicine reconciliation to ensure participants have not taken antibiotics outside the study protocol.
- Prescribe and issue concomitant medication for symptom control if required.
- Re-iterate participant requirements such as completion of the diary, refraining from use of antipyretics, notification of any medication administration, and notification of any fever ≥38.0°C (where appropriate).
- Re-iterate hand hygiene precautions
- Check details of a mobile telephone number that the participant will be carrying with them.
- Counsel the participant on the importance of keeping the mobile always switched on and with them. Explain to the participant that if they are uncontactable, and their 24-hour contact is unsure of their whereabouts, the study team will make every effort to locate them. This may include notifying the police.
- Provide details of next clinic appointment.

10.23. Pre-challenge #2

Secondary challenge (re-challenge) will be undertaken 3-6 months after primary challenge. When the re-challenge date has been set, participants will be seen approximately one week prior to re-challenge, repeating the “D-7” assessment. The same inclusion and exclusion criteria (including temporary) as the primary challenge

will apply and the clinical team will check there have been no changes that make the participant ineligible.

Stool samples will be collected at Ch1_D14, Ch1_D28, and during any subsequent monitoring visits. The aim is to ensure that participants have cleared *Salmonella* before undergoing re-challenge, which is scheduled 3–6 months after the primary challenge (with a target interval of 3 months).

Detection of *Salmonella* spp. in stool prior to re-challenge will result in a temporary delay to re-challenge. If *Salmonella* is still detected three months after the primary challenge, stool cultures will be performed fortnightly or monthly until two consecutive negative results are obtained. Re-challenge may only proceed once clearance is confirmed by two consecutive negative samples before the re-challenge visit.

Participants who remain culture-positive at day 180 following the primary challenge will not be eligible for re-challenge. All stool culture results will be reviewed before each challenge visit.

Detection of gastrointestinal (other than *Salmonella*) in stool culture/PCR collected at the pre-challenge assessment (Day-7) will result in temporary exclusion from the study. This included detection of the following organisms:

- Shigella spp.,
- Campylobacter spp.,
- E. Coli O157,
- Giardia spp
- Cryptosporidum spp,

Participants will be managed according to the local SOP for management of incidental medical findings. They may subsequently be eligible for challenge at the discretion of the chief investigator if they are able to provide two subsequent samples, 48 hours apart, that test negative. These samples would be collected at ad hoc study visits arranged directly with participants.

10.24. Secondary Challenge - Ch2_D0

Secondary challenge (re-challenge) will be undertaken 3-6 months after primary challenge. The procedures surrounding re-challenge will match those surrounding the primary challenge.

10.25. Inpatient quarantine #2

Participants will be admitted to quarantine for a second period during which they will undergo re-challenge. The monitoring considerations for the secondary quarantine admission will match those of the primary inpatient quarantine period.

10.26. Post quarantine outpatient observation #2

This will occur between Days 8 - 14 post-secondary. Participants will be discharged from quarantine between at 7 days after re-challenge and/or upon fulfilling discharge criteria, whichever is sooner. They will then undertake post-quarantine observation assessments to match those of the observation assessments undertaken after primary challenge.

10.27. Convalescent visit #2

This will occur at Day 28 Post secondary challenge. Participants will be reviewed in person.

10.28. Convalescent visit #3

Participants will be reviewed in person at day 90 following secondary challenge (re-challenge). In the unlikely event that a participant remains culture- or PCR-positive for *Salmonella* at their final scheduled visit following the second challenge, additional unscheduled follow-ups (see below) may be arranged. These unscheduled visits will be scheduled at intervals deemed clinically appropriate and will continue until at least two consecutive stool samples are negative for *Salmonella*. Participation in unscheduled follow-up will be optional, and reasonable compensation will be provided for any additional visits.

10.29. Participant Experience Questionnaire

Following completion of their last diary entry after re-challenge participants will be directed to an optional questionnaire regarding their experience of the study. The results of this questionnaire will be anonymised. The questionnaire will be closely based on one used in the previous challenge studies, which aimed to examine participant motivations, attitudes and factors influencing participation in human challenge research.92

10.30. Out of schedule visits

Unscheduled visits will be arranged, if required, to ensure participant safety and infection clearance as further history, examination and investigation may be needed. These visits will be at the discretion of the clinical study team. If participants are unwell and unable to attend the study site for a visit, they may be visited at home by one of the clinical study team members.

11. ANTIBIOTIC TREATMENT

Antibiotic therapy will be offered as soon as possible when one or more of the criteria in Table 4 are satisfied.

Table 4 - Antibiotic treatment criteria

Assessment Timepoint	Clinical & Microbiological Criteria	Required Action

Tier 1 – Immediate Antibiotic Treatment		
Anytime	Tier 1 criteria • Fever ≥38°C for ≥12hrs • *Salmonella* Typhimurium bacteraemia • Severe gastroenteritis (≥6 loose/liquid stools in 24 hours or >800 g of loose/liquid stools in 24 hours or ≥2 stools with gross blood in 24 hours) • Confirmed or suspected gastrointestinal perforation • Confirmed or suspected extra-intestinal seeding of *Salmonella*. • Participant Request • Any participant in whom antibiotic use is felt to be clinically necessary (as decided by a medically qualified study doctor)	Immediate Antibiotic Treatment
Tier 2 – Clinical and Microbiological Assessment		
Ch1_D28	Condition A: Stool cultures negative on Day 14 post challenge.	No Antibiotic Treatment
Ch1_D28	Condition B: Stool cultures positive on/beyond Day 14	Offer antibiotic treatment if ongoing systemic or gastrointestinal symptoms or participant request. Otherwise proceed to enhanced surveillance.
Ch2_90	Condition A: Stool cultures negative on Ch2_ 14 and Ch2_28 post re-challenge challenge.	No Antibiotic Treatment
Ch2_90	Condition B: Stool cultures positive on/beyond Ch2_28	Offer antibiotic treatment at investigator discretion and/or patient request.

Antibiotics treatment of *Salmonella* bacteraemia will be initiated on the identification of Gram-negative rods on microscopy from a positive blood culture taken from a challenged participant. Treatment will not await speciation.

Severe diarrhoea and is defined as ≥6 loose/liquid stools and/or ≥2 stools with gross blood in 24 hours.

Salmonella gastroenteritis is typically self-limiting in healthy immunocompetent adults and treatment may not be indicated in all patients. Antibiotic treatment can be associated with side effects and in mild cases maybe associated with a risk of prolonged shedding. Participants with *Salmonella* shedding not meeting the criteria for immediate treatment in Table 4 or those with mild diarrhoea, may not benefit from treatment. These participants will be offered the option of 1) no-treatment or 2) antibiotic treatment treated at Day 14 post challenge after being counselled about the risks and benefits of either approach. Treatment decisions will be recorded in the CRF.

11.1. Treatment for severe gastroenteritis

Participants with severe *Salmonella* gastroenteritis will receive treatment upon meeting the criteria for severe gastroenteritis as above. Treatment will comprise azithromycin 500mg once daily for 5 days. Second line treatment will comprise ciprofloxacin 500mg twice daily for 5 days.

11.2. Treatment for *Salmonella* bloodstream infection

Participants with *Salmonella* bloodstream infection will receive treatment if/when blood cultures test positive and Gram negative rods are identified on microscopy. Treatment will comprise ciprofloxacin 500mg twice daily for 10-14 days. Second line treatment will comprise ceftriaxone IV OD for up to 14 days.

11.3. Systemic illness

Participants may be treated for severe *Salmonella* or systemic illness at the discretion of the attending physician following S. Typhimurium challenge as outlined in Table 4. This will comprise azithromycin 500mg once daily for 5 days. Second line treatment will comprise ciprofloxacin 500mg twice daily for 5 days.

11.4. Treatment of clinical or microbiological relapse

Additional course(s) of antibiotic treatment may be offered to participants in instances where *Salmonella* Typhimurium is isolated from faecal specimens after a course of primary treatment (with or without diarrhoea and/or systemic symptoms compatible with *Salmonella* disease). In such instances, the antibiotic offered will belong to a different antibiotic class to that offered for primary treatment. For example, if ciprofloxacin is offered for primary treatment then azithromycin will be offered for the second course of treatment (and vice versa). In instances of microbiological or clinical relapse, enhanced hand washing precautions will be emphasised. We will confirm clearance by collected three faecal specimens taken at least 48hrs apart, the first of which will be collected at least 7 days after completing the re-treatment course. If, *Salmonella* is persistently isolated from faecal specimens after re-treatment, then a third course of treatment will not be routinely offered within the remit of the study, as asymptomatic shedding with spontaneous clearance is well recognised. Further faecal cultures will be collected as outlined in Table 6.

11.5. Concomitant medication for treatment of *Salmonella* infection

Concomitant medication can be provided for symptomatic control of *Salmonella* infection, before and after diagnosis. Concomitant medication after *Salmonella* diagnosis includes antibiotics and antipyretics if required.

Table 5 - Concomitant medication for treatment and symptom control.

Drug	Indication	Dose	Route	Frequency
Ciprofloxacin	*Salmonella* bloodstream infection (1st line) and *Salmonella*	500mg	Oral	BD

	gastroenteritis (2nd line)			
Azithromycin	*Salmonella* gastroenteritis (1st line)	500mg	Oral	OD
Ceftriaxone	*Salmonella* bloodstream infection (2nd line)	2g	Intravenous	OD
Paracetamol	Fever and discomfort	500mg - 1 gram	Oral	PRN, max QDS
Codeine	Pain including headache	30-60mg	Oral	PRN (max. 240mg/24 hours)
Senna	Constipation	2-4 tablets	Oral	PRN
Cyclizine	Nausea and/or vomiting	50mg	Oral	PRN (max. 150mg/24 hours)
Chlorpheniramine	Allergy	4mg	Oral	PRN TDS-QDS (max. 24mg/24 hours)
Oral rehydration solution	Prevention of Dehydration	200-400ml after each loose stool – approx 2000ml daily	Oral	PRN
IV fluids – Sodium chloride 0.9% +/- 20-40mmol KCl	Dehydration – unable to tolerate oral fluids	250-1000ml	IV	PRN

12. FAECAL SHEDDING MONITORING

Faecal shedding will be monitored systematically throughout both the primary and secondary challenge phases. At the pre-challenge assessment (TP2), a stool culture will be obtained, and the primary challenge will be deferred if positive. During the inpatient period following the primary challenge (TP3–TP10), daily stool cultures will be collected. No routine cultures will be performed during the subsequent outpatient period (TP11–TP16), unless participants develop symptoms or if clinically indicated. A mandatory stool culture will be obtained at TP17; if negative, no further cultures will be collected until the TP18 eligibility assessment. If positive, enhanced surveillance with weekly cultures will be initiated until Day 28. At TP18, another mandatory stool culture will be obtained; if negative, no further sampling is required until TP19. If positive, enhanced surveillance with monthly cultures will continue until two consecutive negative up to TP19.

For the secondary challenge, a stool culture will be obtained approximately one week prior to re-challenge (TP19 – allowing for study visit windows), and participants will only be eligible for re-challenge if microbiological clearance has been documented prior to TP19 (defined as two consecutive culture-negative stools at least 24 hours apart, without antibiotics for at least 7 days). During the inpatient period following the second challenge (TP20–TP27), daily stool cultures will again be

collected. No routine outpatient cultures will be performed during TP28–TP33, unless clinically indicated. Mandatory stool cultures will be collected at TP34 and TP35, with clearance sampling initiated after TP35, consisting of weekly cultures until two consecutive negative results at least 24 hours apart are obtained. A final review visit at TP36 will confirm clearance; if stool cultures remain positive, ongoing management will be determined by the Principal Investigator and medical monitor.

Table 6 - Faecal shedding monitoring schedule

Challenge Phase	Timepoint(s)	Setting	Stool Culture Schedule	Conditions / Actions
Primary Challenge	TP2	OP	Mandatory culture	Pre-challenge assessment. Defer primary challenge if positive.
	TP3 to TP10	IP	Daily (1 per day)	Routine monitoring during quarantine
	TP11 to 16	OP	None routinely	Culture only if symptomatic or clinically indicated
	TP17	OP	Mandatory culture	**If negative**: no further cultures until TP18 eligibility check.
		OP		**If positive**: initiate enhanced surveillance (weekly until Day 28).
	TP18	OP	Mandatory culture	**If negative**: no further cultures until TP19 eligibility check.
		OP		**If positive**: initiate enhanced surveillance (monthly until two consecutive negative up to TP19).
Secondary Challenge	TP19	OP	Mandatory culture	1 week prior to second challenge. Re-challenge only if microbiological clearance documented (2 negatives ≥24 h apart without antibiotics for ≥7 days).
	TP20-27	IP	Daily (1 per day)	Routine monitoring during quarantine
	TP28-33	OP	None routinely	Culture only if symptomatic or clinically indicated
	TP34	OP	Mandatory culture	Standard surveillance
	TP35	OP	Mandatory culture	Begin clearance sampling thereafter: monthly cultures until 2 negatives ≥24 h apart
	TP36	OP	Review visit	Confirm clearance. If still positive, reviewed by PI/monitor for ongoing management.

13. INFECTION CONTROL CONSIDERATIONS

13.1. Enteric precautions

During the inpatient quarantine participants will be managed using the local infection control protocol appropriate for enteric diseases.

13.2. Hand hygiene

Study investigators will perform hand hygiene using soap-and-water before and after contact with the patient, the patient's immediate environment, and any contaminated

articles. Gloves should be removed between procedures, hands washed and dried thoroughly. Typically, alcohol hand rub should not be used in patients with loose stool and diarrhoea.

Participants will be instructed on hand hygiene during the inpatient quarantine stay. Hand washing should be performed regularly, particularly after defecation. Participants will be issued with disinfectant wipes to clean toilet handles, toilet seats and other touch points after toilet use.

13.3. Personal protective equipment in Side rooms

Study investigators will wear a disposable apron and gloves during study procedures. Gloves will be changed between procedures. Hand hygiene will be performed after contact with the patient, contaminated articles, or the patient's immediate environment. Participants will be instructed on hand washing procedures. Face masks or other facial protection are not strictly needed unless splashing of body fluids is anticipated. Local guidance may recommend routine face mask use for routine clinical care (including outpatient clinic visits) as per local infection prevention and control team/UKHSA guidance.

13.4. Side rooms

Participants will be managed in single-occupancy side rooms with dedicated toilet facilities.

13.5. Linen

Linen will be treated as contaminated, according to local policies. Linen changes should take place daily.

13.6. Crockery and cutlery

Crockery and cutlery will be handles according to local SOPs and washed using a routine domestic hot wash.

13.7. Clinical waste

Clinical waste will be disposed of in an infectious waste bag inside the isolation area. If the outside of the bag becomes contaminated, this should be placed inside a second infectious waste clinical waste bag at the door of the isolation room.

13.8. Room cleaning

Room cleaning will take place daily in accordance with local procedures. Door handles, taps, toilet flushes and bed rails should be cleaned regularly throughout the quarantine period in accordance with local policies.

Terminal cleaning of the room will take place at the end of the quarantine period. All clinical waste must be removed prior to terminal cleaning. The specifics of terminal

cleaning are outlined in local policies, but typically this will involve washing of all horizontal surfaces, equipment, taps, door handles and toilet flushes. The floor will be mopped thoroughly with a 1000ppm chlorine solution (or equivalent disinfectant according to local SOPs).

13.9. Bedpans

Disposable cardboard bedpans will be used to collect stool. The bedpan will be covered after use. After collection of the stool samples, the bedpan will be disposed of as per local policies for infectious waste. The toilet seat will be cleaned with disinfectant wipes after each use and cleaned daily as per local policies

13.10. Visitors

Access for visitors will be limited, to minimise the risk of transmission. A limited number of visitors (one at a time) would be allowed if participants are not considered actively infectious- i.e. in the absence of ongoing type 6/7 stools for 48 hours. Any visitors would need to follow hospital policy for enteric precautions, including (but not limited to), wearing a disposable apron and gloves, plus handwashing with soap and water.

13.11. Food

There will be no specific restrictions to the food that can be eaten during the inpatient quarantine period. Participants will be provided with breakfast, lunch, and dinner during their inpatient stay. Participants will be allowed to bring snacks and order external meals. Participants will be asked to record details of their diet in their diary.

13.12. Stool culture for assessment of shedding

Clearance cultures following NTS infection are not typically indicated according to UKHSA guidance. This is in contrast to UK public health guidance following infection with the typhoidal *Salmonella* serovars (S. Typhi and Paratyphi) Prolonged shedding is known to occur and is thought to contribute to only a small proportion of onward transmission.

If >1 clearance samples test positive for *Salmonella* after the second challenge, further clearance stool samples will be collected until at least 2 consecutive samples test negative.

13.13. Reporting to local health protection team

The London Health Protection Team (UK Health Security Agency) will be informed of the name, address, and date of birth of all participants who have been challenged with S. Typhimurium

14. LABORATORY

Details regarding the process of blinding laboratory samples and the laboratory methods to be used are given in relevant SOPs and study plans. Samples processed by departments at the Northwest London Pathology laboratory will be processed using the participant site hospital medical record number (MRN) and NHS no.

14.1. Blood culture

After inoculation of aerobic broth with 10 mL of the participant's blood (BACTEC PLUS Aerobic/F culture vial; BD, Oxford, UK), culture will be performed using the BACTEC 9240 continuous monitoring system in the microbiology laboratory of the North West London Pathology Laboratory (Charing Cross Hospital, Imperial Healthcare NHS Trust; NWLP) according to current SOPs and UK Standards for Microbiology Investigations ID 24: identification of *Salmonella* species.93

Identification of organisms cultured will be by biochemical (API, Analytical Profile Index; bioMérieux, Basingstoke, UK) and serological methods, latterly by agglutination with *S*. Typhimurium anti-sera. Confirmed isolates will be tested for antibiotic susceptibility using standard methods. All *Salmonella* isolates will be sent to the gastrointestinal reference lab for whole genome sequencing.

14.2. Stool culture & PCR

Stool samples supplied by participants should be delivered to NWLP within 24 hours of being taken. If possible, the samples should be kept cool until delivered to NWLP and then stored at 2°C to 8°C. The time of sampling will be noted on the sample form.

Salmonella will be detected in stool by both PCR and culture.

All faecal samples will be routinely run on Serosep (real time PCR) for the detection of *Salmonella* sp., Shigella sp., Enteroinvasive E coli, Campylobacter jejuni/ lari/ coli, Cryptosporidium parvum/ hominis, Giardia lamblia and Verotoxin- producing E.coli (VTEC), according to local SOPs (MIC-LP-169-X Serosep Bacterial Enteric PCR).

Routine stool cultures and screening for enteric pathogens will be performed in parallel by the microbiology laboratory, according to relevant local SOPs (MIC-LP-009-X Enteric C&S SOP) and UK Standards for Microbiology Investigations ID 24: identification of *Salmonella* species.93. Stool will be inoculated directly onto XLD agar for semi-quantitative culture and into Selenite F enrichment broth for qualitative culture. After overnight incubation (at 37.0°C), each sample will be sub-cultured onto *Salmonella*-selective chromogenic agar. Suspicious colonies will be identified as per the blood culture method described above.

Stool cultures will be taken at Day 0 (challenge) through to Day 7, if they produce a bowel motion. A further sample will be collected at Day 14 day post-challenge

period. After the second challenge, participants will be required to supply further stool samples until proven not to be shedding S. Typhimurium in three consecutive samples.

Isolates of S. Typhimurium (from blood and stool culture) will be retained for whole genome sequencing or further investigation by the reference laboratory if challenge strain confirmation is required by UKHSA.

Additionally, quantitative stool cultures or PCR may be performed at Imperial, to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include whole genome sequencing (WGS) and/or metagenomic sequencing.

Analysis of the relative composition of bacterial populations within the bowel may be performed on collected stool samples. The faecal microbiome at baseline and its effects on challenge outcome, bacterial dynamics and response to antibiotic treatment may be assayed using techniques including pyrosequencing. After collection, stool will be stabilised in RNA-later and stored at -80°C, prior to further assessment.

14.3. Immunology assays

We will undertake a core and exploratory immunological assays to characterise host responses to S. Typhimurium challenge.

Core analyses:

- Serology: Serum IgG responses to S. Typhimurium O-antigen and other relevant antigens, measured using ELISAs.
- Functional antibody assays: Serum bactericidal activity (SBA) and opsonophagocytic assays.

Exploratory analyses

- Cytokines: Plasma cytokine profiles (e.g. IL-1β, IL-6, IL-8, IL-10, IL-17A, TNF-α, IFN-γ) using multiplex bead arrays.
- B-cell assays: Antibody-secreting cell and memory B-cell responses by ELISPOT.
- T-cell responses: Characterisation of cellular immunity using PBMCs with flow cytometry for activation, proliferation and cytokine production.
- Mucosal responses: Measurement of salivary IgA and stool IgA/IgG against S. Typhimurium antigens.
- Microbiome: Stool microbiota profiling linked with clinical outcome
- Systems serology for antibody Fc features and glycosylation.
- Proteome arrays for novel antigen discovery.
- Mass cytometry (CyTOF) for deep immune phenotyping.
- Transcriptomics (bulk RNA-seq, single-cell RNA-seq, TCR/BCR repertoire sequencing, whole blood gene expression).
- Host genetic analyses (candidate SNPs, epigenetic modifications).
- Metabolomics and proteomics from blood and urine.

- Comparative analyses with samples from other CHIMs (e.g. influenza, SARS-CoV-2, S. Typhi).

15. REGULATORY ISSUES

15.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the current version of the Declaration of Helsinki.

15.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

15.3. Ethics approval

The Study Coordination Centre has obtained approval from the London-Riverside Research Ethics Committee (26/LO/0316) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

15.4. Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

15.5. Confidentiality

The study staff will ensure that participants' anonymity is maintained. Study participants will be identified by initials and a participant ID number (which does not contain any identifiable data like initials or DOB) on the CRF. Any electronic databases and documents with participant identifying details will be stored securely and will only be accessible by study staff and authorised personnel. The study will

comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Data will be pseudonymised by using their Study ID on all records and samples collected.

Pseudonymised data will be transferred to the University of Oxford for analysis of endpoints.

15.6. Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

15.7. Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

15.8. Funding

Funding for the study has been provided by the Wellcome trust, funder reference 310277/Z/24/Z.

15.9. AUDITS

The study may be subject to audit by Imperial College London/ Imperial College Healthcare NHS Trust under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

16. RISKS AND BENEFITS

16.1. Clinical Profile in the Dose finding study

The risks and anticipated side effects of challenge are informed by the primary dose-escalation CHANTS study. A dose–response relationship was observed for both strains, with higher doses associated with higher attack rates.The primary endpoint was bloodstream infection or sustained fever (oral temperature ≥38.0°C for ≥12 hours), with attack rates per dose as follows:

- 1–5 × 10^3 CFU: no participants met the primary endpoint.
- 1–5 × 10^4 CFU: D23580 attack rate 50% (2/4); 4/74 attack rate 0% (0/3).

- 1–5 × 10^5 CFU (highest dose administered): model-estimated attack rates 57.9% for D23580 and 47.4% for 4/74, with no statistically significant difference between strains.

Symptoms were consistent with acute *Salmonella* gastroenteritis and increased with challenge dose. The most common symptoms were headache, malaise, abdominal pain, and diarrhoea. Severity was usually mild to moderate (Grade 1–2), with a small number of severe (Grade 3) events, most often headache, diarrhoea, abdominal pain, and malaise. Arthralgia, myalgia, and nausea were less frequent; tenesmus, cough, and vomiting were uncommon. Most symptoms were self-limiting and manageable, without long-term sequelae.

No serious adverse events occurred. Five participants met protocol-defined criteria for severe salmonellosis: three episodes of transient, self-limiting gastrointestinal bleeding (two after 4/74; one after D23580) and two Grade 4 laboratory abnormalities (ALT peak 374 U/L, considered unlikely to be treatment related; CRP 218.9 mg/L, considered definitely related). All resolved without long-term sequelae.

Stool colonisation (≥2 positive stool cultures ≥48 hours post-challenge) was common, occurring in 78% of participants, and varied by dose and strain.

- ST19 (4/74): 80.0% at 1–5 × 10^3 CFU; 80.0% at 1–5 × 10^4 CFU; 86.7% at 1–5 × 10^5 CFU.
- ST313 (D23580): 40% at 1–5 × 10^3 CFU; 60% at 1–5 × 10^4 CFU; 86.7% at 1–5 × 10^5 CFU.

Microbiological relapse occurred in five participants (reappearance on stool culture after apparent clearance). All relapses were asymptomatic, without further adverse events, and were managed per protocol. At the time of writing, 4/5 have subsequently cleared infection without sequelae; one remains under follow-up. Prolonged or intermittent convalescent faecal shedding is recognised after non-typhoidal *Salmonella* gastroenteritis, and routine convalescent stool culture is not recommended in UK public health guidance.

Overall gastroenteritis incidence was 30% (15/50), increasing with dose and differing by strain.

- ST19 (4/74): 0% at 10^3 CFU; 20% at 10^4 CFU; 40% (6/15) at 10^5 CFU.
- ST313 (D23580): 0% at 10^3 CFU; 20% at 10^4 CFU; 46.7% (7/15) at 10^5 CFU.

16.2. Potential benefits to study participants

Participants will not receive direct clinical benefit. The study may improve understanding of *Salmonella* infection and contribute to future prevention strategies. Participants may benefit from information on their general health status identified during screening and study assessments.

16.3. Potential risks to study participants

Risks include study burden, phlebotomy, symptomatic infection, and a small risk of complications. Risk mitigation measures are outlined below.

During the challenge phase (prior to antibiotic treatment), participants will be admitted to an inpatient quarantine facility with 24/7 observation and at least daily clinical review. Participants will be informed of potential symptoms and monitored closely. Symptoms will be actively assessed at each review, including fever, headache, malaise, anorexia, abdominal pain, nausea/vomiting, myalgia, arthralgia, cough, rash, diarrhoea, and constipation. Participants will record oral temperature twice daily if they feel feverish using a provided thermometer. If a participant becomes unexpectedly unwell, additional clinical review will be arranged. If required for safety following discharge, a study nurse or doctor may review the participant at home.

16.4. Study fatigue

Participants may experience fatigue due to intensive procedures, particularly during challenge. Participants are compensated, and procedures will be organised to minimise inconvenience.
Risk mitigation: visits and interventions will be kept to the minimum required and scheduled around participant commitments where practical.

Phlebotomy
Blood volumes are not expected to compromise healthy adults. Venepuncture or cannulation risks include tenderness, bruising, light-headedness, and rarely syncope or arterial puncture.

Risk mitigation: excluding unscheduled visits, up to 976 ml will be collected across the study (Table 9, Table 10). Recent blood donation history will be checked at enrolment to ensure total blood volumes remain safe. Venous cannulation may be used to reduce repeated venepuncture, although blood cultures will be taken from a fresh puncture to reduce contamination risk.

16.5. Symptomatic gastroenteritis

Most participants are expected to develop symptomatic *Salmonella* gastroenteritis after challenge; in community settings, a substantial proportion of infections are asymptomatic. A key aim is to compare symptomatic infection at re-challenge versus naïve exposure.

Incubation is typically 12–48 hours (range 4–120 hours). In CHANTS, onset was usually 1–3 days, with two delayed onsets at or beyond Day 7. Symptoms may include watery diarrhoea (occasionally bloody), abdominal pain, nausea, vomiting, headache, and fever. Stool frequency and severity may vary with infectious dose. Symptom duration is usually 4–7 days. Published estimates report diarrhoea duration around 3–13 days and fever around 1–2 days, with total illness duration ranging from 3–19 days.

Risk mitigation: inpatient quarantine enables early detection and management of severe diarrhoea or dehydration. Participants will have 24/7 observation and at least

daily clinical review, with assessment for dehydration. Daily stool output monitoring and daily stool cultures will be performed. Severe diarrhoea will trigger antibiotic treatment (Table 4). Oral rehydration will be provided, with intravenous fluids for severe hypovolaemia, transitioning to oral rehydration when clinically appropriate.

16.6. Invasive *Salmonella* infection

A proportion of participants may develop bloodstream infection. Potential complications include sepsis with haemodynamic compromise, pneumonia, anaemia, deep-seated infection, and gastrointestinal perforation or haemorrhage. (Hung et al. 2009; Bennett et al. 2019; Hohmann, n.d.-b; n.d.-c; Kotton and Hohmann, n.d.; Hohmann, n.d.-a)

Endovascular infection is a rare complication of invasive *Salmonella* infection. It is associated with atherosclerotic disease or endovascular prosthesis. It typically affects the infra-renal abdominal aorta but can affect any site of damaged endothelium. Endovascular infection is estimated to occur in up to 10% of over 50s with blood stream infection. (Cohen et al. 1978; Benenson et al. 2001; Bennett et al. 2019; Hohmann, n.d.-b; n.d.-c; Kotton and Hohmann, n.d.; Hohmann, n.d.-a)

Salmonella osteomyelitis is classically associated with sickle cell disease in children but can also occur in the context of other haemoglobinopathies and other immunosuppressed states (Bennett et al. 2019; Hohmann, n.d.-b; n.d.-c; Kotton and Hohmann, n.d.; Hohmann, n.d.-a; Kaplan et al. 2019)

Risk mitigation: invasive disease and complications occur predominantly in clinically vulnerable individuals and/or without timely antibiotic therapy. Higher-risk participants will be excluded. During quarantine, participants will have 24/7 observation and at least daily clinical review. Daily blood cultures will be collected to detect bacteraemia; confirmed bacteraemia will prompt antibiotic treatment. All cases of bloodstream infection require prompt antibiotic therapy. Treatment typically consists of a fluroquinolone or third generation cephalosporin, depending on antibiotic susceptibilities. Treatment duration is typically 10-14 days. Extra-intestinal focal infection or endovascular infections may require longer durations of treatment and additional surgical intervention.

16.7. Reactive arthritis

Reactive arthritis typically presents as mono- or oligoarthritis (often lower limb), with possible axial symptoms, enthesitis, and/or dactylitis. Most resolve or substantially improve by 6–12 months, though a minority may have symptoms beyond 12 months. (Ajene et al. 2013) (Hannu 2011; Aho et al. 1973; Leirisalo-Repo et al. 2003)

Risk mitigation: overall risk is low. HLA-B27 screening will be performed prior to enrolment. Suspected cases will be referred to Rheumatology at Imperial College Healthcare NHS Trust.

16.8. Post-infectious irritable bowel syndrome

There is a theoretical risk of post-infectious irritable bowel syndrome following bacterial gastroenteritis, estimated at 3–10% after bacterial diarrhoea. (Shane et al. 2017; Dupont 2011; Haagsma et al. 2010)

Risk mitigation: participants at increased risk of IBS will be excluded.

16.9. Relapse of *Salmonella* infection

Most immunocompetent adults recover fully. A small proportion may experience microbiological relapse or prolonged shedding, which may be exacerbated by antibiotic exposure. Relapse is more likely in immunocompromised states (for example advanced HIV, chronic granulomatous disease, specific cytokine pathway defects, and haematological malignancy).

Long-term carriage beyond 1 year is rare. Median shedding is estimated at around 5 weeks, with persistent excretion beyond 1 year in <1%. Some cohorts report a small proportion with shedding for ≥30 days, occasionally lasting months.

Chronic carriage is defined as the persistence of *Salmonella* in the stool for >12months from infection. The risk of chronic NTS carriage is lower than that for the typhoidal *Salmonella* serovars. The rate of carriage at 1 year is estimated to be <1%.(Buchwald and Blaser 1984) Chronic carriage is more common with host immunosuppression; in young children <5 and/or or in the context of biliary tract abnormalities. (Musher and Rubenstein 1973; Bennett et al. 2019; Hohmann, n.d.-b; n.d.-c; Kotton and Hohmann, n.d.; Hohmann, n.d.-a)

Risk mitigation: risk is primarily mitigated through exclusion of participants at increased risk. Stool cultures will commence two weeks after completion of antibiotics, with clinical review if symptoms or results suggest relapse. To confirm clearance and minimise chronic carriage risk, stool culture will be obtained at completion of the initial antibiotic course.

In the unlikely event that a participant remains culture- or PCR-positive for *Salmonella* at their final scheduled visit following the second challenge, additional unscheduled follow-up may be arranged. These unscheduled visits will be scheduled at intervals deemed clinically appropriate and will continue until at least two consecutive stool samples are negative for *Salmonella*. Antimicrobial treatment will not routinely be given in this context; participants will instead receive advice on enhanced hygiene measures and risk reduction. Participation in unscheduled follow-up will be optional, and reasonable compensation will be provided for any additional visits.

16.10. Antibiotics

Antibiotic therapy may include azithromycin, a fluoroquinolone (e.g. ciprofloxacin), or a third-generation cephalosporin. Risks include intolerance (gastrointestinal upset, nausea, vomiting), allergy (rash, angio-oedema, breathlessness, rarely anaphylaxis), QTc prolongation (azithromycin and fluoroquinolones), and biochemical abnormalities (including liver function derangement). Antibiotic exposure may, in

theory, increase carriage of drug-resistant organisms. (Onwuezobe et al. 2012) (Leinert et al. 2021; Marzel et al. 2016)

Ciprofloxacin may be used in this study under specific circumstances (e.g. confirmed *Salmonella* bacteraemia), where the investigator judges that it offers the most appropriate treatment option. Use of ciprofloxacin will follow current MHRA and national safety guidance on fluoroquinolones, including:

- awareness of the risk of serious, potentially long-lasting and disabling adverse reactions affecting the musculoskeletal and nervous systems (e.g. tendonitis, tendon rupture, neuropathy) and the risk of central nervous system effects
- avoidance in participants with a history of serious adverse reactions to fluoroquinolones
- caution and, where appropriate, avoidance in participants with known risk factors for tendon disorders (e.g. history of tendon rupture, concurrent corticosteroid use), a history of epilepsy or other predisposing factors for seizures, known QTc prolongation or uncorrected electrolyte disturbances, or other relevant risk factors as per MHRA guidance
- immediate discontinuation of ciprofloxacin and prompt clinical review if participants develop signs or symptoms suggestive of serious adverse reactions (e.g. tendon pain or swelling, muscle weakness, peripheral neuropathy symptoms such as pain, burning, tingling, numbness, or new or worsening psychiatric or neurological symptoms).

Risk mitigation: antibiotics will be avoided where risks outweigh benefits. Treatment duration will be minimised. Participants with known hypersensitivity to study antibiotics (ciprofloxacin, azithromycin or other macrolides, and cephalosporins) will be excluded. Suspected intolerance or allergy will be assessed promptly by a study doctor and an alternative antibiotic used if required. Participants remain in inpatient quarantine during challenge to enable rapid recognition and management of adverse reactions. Baseline ECG (QTc) will be performed; prolonged QTc will exclude participation. Liver function and electrolytes will be monitored regularly.

Ciprofloxacin will not be used routinely for diarrhoea or asymptomatic stool carriage. When used, participants will be counselled regarding these risks and provided with written information, consistent with MHRA recommendations on fluoroquinolone prescribing and risk minimisation.

16.11. Potential risk to close contacts

Person-to-person transmission can occur, particularly with symptomatic diarrhoea; transmission from asymptomatic individuals is less common. Infectiousness is greatest during diarrhoeal illness. Public health advice typically excludes individuals from high-risk activities until 48 hours after symptoms resolve; routine clearance cultures are not generally recommended due to common prolonged shedding. Household transmission may occur with poor hand hygiene after defecation and subsequent preparation of uncooked food. Bacterial proliferation can occur in food held at ambient temperature, increasing transmission risk.

Risk mitigation: secondary transmission after discharge is considered highly unlikely given the low infectivity of S. Typhimurium without bicarbonate buffer and high hygiene standards in the UK. Participants must agree to inform close contacts and will be given information to share. Strict hand hygiene will be required during the period of possible excretion, with soap, paper towels, and written hygiene guidance provided. Hygiene technique will be taught and observed at the challenge visit and reinforced at each follow-up.

To further reduce risk, food handlers will be excluded. Participants working closely with young children (pre-school groups or children <2 years), or with highly susceptible individuals (including older adults or those at high risk of severe outcomes), will be excluded unless they are willing to refrain from work until infection has been excluded.

17. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Imperial college London.

17.1. Quality assurance procedures

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and SOPs. Approved and relevant SOPs and Laboratory and Clinical Study Plans will be used at all clinical and laboratory sites.

17.2. Monitoring

Monitoring will be performed according to GCP by parties appointed by the Chief Investigator. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor and inspection by local and regulatory authorities.

17.3. Direct access to source data/documents

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

17.4. Modifications to the protocol

No modifications to this protocol will be made without consultation with and agreement from the Sponsor. Any modifications to the study that appear necessary during the study must be discussed with the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the

Chief Investigator and will be made a formal part of the protocol following ethical and regulatory approval.

The Investigator is responsible for ensuring that changes to an approved study, during the period for which NHS REC approval has already been given, are not initiated without NHS REC review and approval except to eliminate apparent immediate hazards to the participant.

17.5. Protocol deviation

Any deviations will be documented in a protocol deviation form and filed in the TMF.

17.6. Participant reimbursement

Participants who attend all routine study visits will receive up to £4,150 if they remain in the study for the entire period and attend all routinely scheduled study visits. All participants will be reimbursed for their time, travel and for inconvenience based on the following figures:

- Travel expenses: £16.30 per visit
- Inconvenience of blood tests: £10 per blood donation
- Inconvenience of screening ultrasound scan: £50
- Inconvenience of providing clearance stool samples: £10 per visit
- Time off work compensation – calculated using the London living wage rate of £14.80 an hour

Some participants may be asked to attend additional visits for the purposes of safety follow up. For example, if a participant is diagnosed with Salmonellosis at the end of the 14-day monitoring period, they will be asked to attend additional clinic visits and have additional blood tests taken. Additional reimbursement is provided for up to 10 extra visits, which will be reimbursed on a pro-rata basis at £48.50. If a participant attends all extra 10 visits, the maximum additional reimbursement will be £485. Time off work reimbursement is limited to the 10 days after challenge, to account for the 7 day quarantine period plus additional days off that may be required post quarantine. Payments will be made via bank transfer. Participants will be required to provide banking details including account name, sort code and account number. All personal banking details will be stored confidentially and retained by the study team while the participant is actively involved in the study. Consent will be obtained prior to requesting and storing personal bank account details.

Participant payments will be requested at the following visits: Screening, Ch1_D28, Ch2_D28, Ch2_D90 , and for each unscheduled visit when applicable.

Individual researchers do not receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research.

18. PUBLICATION POLICY

The Chief Investigator will co-ordinate dissemination of data from this study. The results of the study will be published via peer-reviewed scientific journals, conference presentations, and publications on website. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator and by the sponsor prior to submission. All communication or publications concerning the project, including at a conference or seminar, shall acknowledge the Parties and the Wellcome trust financial contribution.

19. CONFLICTS OF INTEREST

The investigators declare that they have no financial, professional, or personal conflicts of interest that could be perceived to influence the design, conduct, analysis, or reporting of this clinical trial. All study activities will be carried out with full adherence to ethical standards and in accordance with applicable regulatory requirements to ensure objectivity and integrity.

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21. APPENDIX 1 – SCHEDULE OF EVENTS

Table 7 - Schedule of events primary challenge

Day	*n/a*	*Screening*	*Ch1_D-7*	*Ch1_D0*	*Ch1_D1*	*Ch1_D2*	*Ch1_D3*	*Ch1_D4*	*Ch1_D5*	*Ch1_D6*	*Ch1_D7*	*Ch1_D8*	*Ch1_D9*	*Ch1_D10*	*Ch1_D11*	*Ch1_D12*	*Ch1_D13*	*Ch1_D14*	*Ch1_D28*
	TP0	*TP1*	*TP2*	*TP3*	*TP4*	*TP5*	*TP6*	*TP7*	*TP8*	*TP9*	*TP10*	*TP11*	*TP12*	*TP13*	*TP14*	*TP15*	*TP16*	*TP17*	*TP18*
Obtain SoC Record	x																		
Consent Quiz		x																	
Medical history		x	x																
Close contact information		x																	
Informed consent		x																	
Depression/anxiety questionnaires		x									x								
Food Frequency questionnaire		x																	
Vital signs[1]		x	x	x	x	x	x	x			x								
Physical examination[2]		x																	
Urine pregnancy test		x		x															
Urinalysis		x																	
12-Lead ECG		x																	
Ultrasound scan		x																	
Obtain 24-hour contact details			x																
TOPS registration		x																	
Concomitant medications check		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Screening blood samples		x																	
Admission to quarantine				x															
Continued consent ICF				x															

Challenge				x														
Antibiotic Treatment[3]					(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Discharge from quarantine[4]								(x)			(x)							
Telephone check-up									(x)	(x)		x	x	x	x	x	x	
Blood sample		x	x	x	x	x	x	x	(x)	(x)	x							x	x
Stool sample[5]		x	x	x	x	x	x	x	(x)	(x)	x							x	x
Saliva sample[6]				x							x							x	x
Adverse events recording				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
eDiary completion			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

1. **Vital signs may be taken and recorded at other times in the study if clinically indicated and at the discretion of the study team.**
2. **A Physical examination may be performed and recorded at other times in the study if clinically indicated and at the discretion of the study team**
3. **Antibiotic treatment may be started at any time post challenge as outlined in section 11**
4. **Participant will be discharged after 7 days. Early discharge days 4-7 is permitted if the criteria for discharge are met (section 10.20)**
5. **Stool samples will be collected when available - if there is no bowel movement, a sample cannot be procured.**
6. **Research samples are not integral to participant safety and may not be taken at every timepoint indicated.**

Table 8 - Schedule of events for re-challenge

Day	Ch2_ D-7	Ch2_ D0	Ch2_ D1	Ch2_ D2	Ch2_ D3	Ch2_ D4	Ch2_ D5	Ch2_ D6	Ch2_ D7	Ch2_ D8	Ch2_ D9	Ch2_ D10	Ch2 _D11	Ch2_ D12	Ch2_ D13	Ch2 _D14	Ch2_ D28	Ch2_ D90
	TP19	TP20	TP21	TP22	TP23	TP24	TP25	TP26	TP27	TP28	TP29	TP30	TP31	TP32	TP33	TP34	TP35	TP36
Vital signs[1]	x	x	x	x	x	x			x									
Urine pregnancy test		x																
Concomitant medications check	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Admission to quarantine		x																
Continued consent ICF		x																
Challenge		x																
Antibiotic Treatment[2]			(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Discharge from quarantine[3]						(x)			(x)									
Telephone check-up							(x)	(x)		x	x	x	x	x	x			
Blood sample	x	x	x	x	x	x	(x)	(x)	x							x	x	x
Stool sample[4]	x	x	x	x	x	x	(x)	(x)	x							x	x	x
Saliva sample[5]		x							x							x	x	x
Adverse events recording	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Participant feedback questionnaire																		x
eDiary completion	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		

1. **Vital signs may be taken and recorded at other times in the study if clinically indicated and at the discretion of the study team.**
2. **Antibiotic treatment may be started at any time post challenge as outlined in section 11**
3. **Participant will be discharged after 7 days. Early discharge days 4-7 is permitted if the criteria for discharge are met (section 10.20)**
4. **Stool samples will be collected when available - if there is no bowel movement, a sample cannot be procured.**
5. **Research samples are not integral to participant safety and may not be taken at every timepoint indicated.**

22. APPENDIX 2 – SAMPLE COLLECTION SCHEDULE

*Table 9 - Sample collection schedule primary challenge.† Stool samples will be collected when available - if there is no bowel movement, a sample cannot be procured. ‡Research samples are not required for clinical data integrity or participant safety so are not essential.*An extra one serum sample will be collected upon SD if it occurs.*

Day	n/a	Screening	Ch1_D-7	Ch1_D0	Ch1_D1	Ch1_D2	Ch1_D3	Ch1_D4	Ch1_D5	Ch1_D6	Ch1_D7	Ch1_D8	Ch1_D9	Ch1_D10	Ch1_D11	Ch1_D12	Ch1_D13	Ch1_D14	Ch1_28
	TP0	TP1	TP2	TP3	TP4	TP5	TP6	TP7	TP8	TP9	TP10	TP11	TP12	TP13	TP14	TP15	TP16	TP17	TP18
Hospital pathology lab (NWLP)																			
Blood culture (BACTEC)				10	10	10	10	10	(10)	(10)	10							10	
FBC		3	3	3		3		3			3							3	3
U&E/CRP/LFT		5	5	5		5		5			5							5	5
Screening serology tests – IgA/Coeliac/HIV/HBsAg/HCV IgG		15																	
Screening blood tests - malaria, HLA-b27, Haemoglobinopathy, coagulation, HBa1C		12																	
Faecal specimen†			x	x	x	x	x	x	x	x	x							x	x
Research Samples‡																			
Serum*			5	10	(10)	(10)	(10)	(10)	(10)	(10)	5							15	15
PBMCs			30	40				30			40							40	40
Paxgene			3	3	3	3	3	3			3							3	3
Faecal specimen			x	x	x	x	x	x	x	x	x							x	x
Saliva			x	x							x							x	x

*Table 10 - Sample collection schedule re-challenge. †Stool samples will be collected when available - if there is no bowel movement, a sample cannot be procured. ‡Research samples are not required for clinical data integrity or participant safety so are not essential. .*An extra one serum sample will be collected upon SD if it occurs.*

Day	Ch2_ D-7	Ch2_ D0	Ch2_ D1	Ch2_ D2	Ch2_ D3	Ch2_ D4	Ch2_ D5	Ch2_ D6	Ch2_ D7	Ch2_ D8	Ch2_ D9	Ch2_ D10	Ch2_ D11	Ch2_ D12	Ch2_ D13	Ch2_ D14	Ch2_2 8	Ch2_9 0
Timepoint	**TP19**	**TP20**	**TP21**	**TP22**	**TP23**	**TP24**	**TP25**	**TP26**	**TP27**	**TP28**	**TP29**	**TP30**	**TP31**	**TP32**	**TP33**	**TP34**	**TP35**	**TP36**
Hospital pathology lab (NWLP)																		
Blood culture (BACTEC)		10	10	10	10	10	(10)	(10)	10							10		
FBC	3	3		3		3			3							3	3	
U&E/CRP/LFT	5	5				5			5							5	5	
Faecal specimen†	x	x	x	x	x	x	(x)	(x)	x							x	x	
Research Samples‡																		
Serum*	5	10	(10)	(10)	(10)	(10)	(10)	(10)	5							15	15	15
PBMCs	30	40				30			40							40	40	40
Paxgene	3	3	3	3	3	3			3							3	3	3
Faecal specimen	x	x	x	x	x	x	(x)	(x)	x							x	X	X
Saliva	x	x							x							x	x	X

23. APPENDIX 3 - GRADING THE SEVERITY OF SOLICITED AND UNSOLICITED SYSTEMIC ADVERSE EVENTS

Table 11 - Grading the severity of solicited and unsolicited systemic Adverse Events

Symptom	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Vomiting	Not present	Present but no interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity OR requires outpatient IV hydration	ED visit or hospitalization for hypotensive shock
Nausea	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Diarrhoea	Not present	2-3 loose stools /24 hrs	4-5 loose stools /24 hrs	6 or more watery stools /24 hrs or requires outpatient IV hydration	ED visit or hospitalization for hypotensive shock
Abdominal pain	Not present	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ED visit or hospitalization
Tenesmus	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Headache	Not present	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ED visit or hospitalization
Fatigue/Malaise	Not present	No interference with activity	Some interference with activity not requiring	Significant; prevents daily activity	ED visit or hospitalization

			medical intervention		
Myalgia	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Arthralgia	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Cough	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Loss of appetite	Not present	No interference with activity	Some interference with activity not requiring medical intervention	Significant; prevents daily activity	ED visit or hospitalization
Unsolicited symptoms	N/A	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.	Moderate; minimal, local or noninvasive intervention indicated; limiting instrumental daily activities.	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care daily activities.	Life-threatening consequences; urgent intervention indicated.

24. APPENDIX 4 - GRADING THE SEVERITY OF VISIT OBSERVED ADVERSE EVENTS

Table 12 - Grading the severity of visit observed Adverse Events

Observation	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Oral temperature (℃)	35. 3-37.8	37.9 – 38.3	38.4 – 39.2	39.3-40.3	>40.3
Tachycardia (beats/min)	50-105	106-120	121-135	>135	ED visit or hospitalization for arrhythmia

Bradycardia (beats/min)	50-105	45-49	40-44	<40	ED visit or hospitalization for arrhythmia
Systolic hyper-tension (mmHg)	90-150	151-155	156-160	>160	ED visit or hospitalization for malignant hypertension
Diastolic hyper-tension (mmHg)	<95	95-99	100-104	>104	ED visit or hospitalization for malignant hypertension
Systolic hypo-tension (mmHg)	>88	83-88	78-82	<78	ED visit or hospitalization for hypotensive shock

25. APPENDIX 5 - GRADING THE SEVERITY OF LABORATORY ADVERSE EVENTS

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
Haemoglobin: decrease from baseline value (g/l)	< 10-19	20-29	30-49	>50
White cell count: elevated x10^9/l	10.7-20	21-28	29-39	>40
White cell count: depressed x10^9/l	3.1-5.1	2.1-3.0	2.0-1.1	≤1.0
Neutrophil count : x10^9/l	1.6-1.9	1.3-1.5	1.1-1.2	≤1.0
Platelets: x10^9/l	101-129	51-100	25-50	<25
Lymphocyte count: x10^9/l	0.7-1.0	0.4-0.69	0.0-0.39	N/A
Eosinophil count: x10^9/l	0.6-1.0	1.1-1.5	1.6-2.0	>2.0
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	147	148	149	>150
Potassium: hyperkalaemia (mmol/L)	5.4	5.5	5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.4	3.3	3.1–3.2	<3.1
Urea (mmol/L)	7.9–8.9	9.0–11	11-20	>21
Creatinine (µmol/L)	132-150	151-176	177-221	>221
ALT (U/L)	38-85	86-170	171-340	>340
Bilirubin (µmol/L)	23.1-31.5	31.6-42.0	42.1-63.0	>63
Alkaline phosphatase (IU/L)	143-260	261-360	361-1300	>1300
Albumin: hypoalbuminaemia (g/L)	28–34	25–27	<25	Not applicable
C-reactive protein (mg/L)	10-30	31-100	101-200	>200
Estimated glomerular filtration rate (eGFR) (mL/min/1.73m²)	45-60	30-44	15-29	<15