STUDY PROTOCOL

An oropharyngeal gonorrhoea controlled human infection model: a provisional protocol using a novel *Neisseria gonorrhoeae* challenge strain

[version 1; peer review: 1 approved, 2 approved with reservations]

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First published: 19 Nov 2025, **10**:636
https://doi.org/10.12688/wellcomeopenres.25103.1
Latest published: 19 Nov 2025, **10**:636

Open Peer Review

https://doi.org/10.12688/wellcomeopenres.25103.1

Abstract

Introduction

Gonorrhoea is a sexually transmissable infection caused by *Neisseria gonorrhoeae* that causes a significant global burden of disease. Urogenital infection can result in long-term impacts on reproductive, perinatal, and neonatal health. Little progress has been made in the public health control of gonorrhoea, and novel preventative strategies are urgently needed. Furthermore, future gonorrhoea management is threatened by increasing antimicrobial resistance (AMR). Oropharyngeal gonorrhoea is usually asymptomatic; likely plays an important role in development of AMR; and is a high-risk site for treatment failure. Here, we describe a protocol for an oropharyngeal gonorrhoea controlled human infection model (CHIM) that has been designed to maximize participant safety, with the aim of developing this as a platform to accelerate prevention and treatment strategies.

Methods and analysis

This dose-escalation CHIM study will enrol 20-35 healthy adult volunteers aged 18 to 50 years who were assigned male at birth and only have sex with people assigned male at birth. The primary objectives are to determine i) the safety and tolerability of an oropharyngeal gonorrhoea CHIM; and, ii) the minimum infectious dose of isolate AUSMDU00053933 required for 60–80% of participants to develop oropharyngeal *N. gonorrhoeae* infection. Secondary and exploratory endpoints include description of clinical, immunological, microbiological and pharmacometric responses. Participants will be monitored daily as outpatients during the five-day experimental infection phase. All participants will be treated with antibiotics, and followed up for three months. Statistical analysis and dose escalation/de-escalation decisions will follow a model-based continual reassessment method in a Bayesian statistical framework.

Ethics and dissemination

After scientific peer review of this provisional protocol, a detailed protocol will be submitted for human research ethics committee assessment. Protocol development was informed by feedback from community engagement. Study findings will be disseminated in peer-reviewed journals and at scientific meetings, with summaries provided to relevant stakeholders.

Plain Language summary

Approval Status ? ✓ ?

	1	2	3
version 1 19 Nov 2025	? view	✓ view	? view

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Any reports and responses or comments on the article can be found at the end of the article.

This report outlines a research study protocol using a controlled human infection model, where participants are inoculated in the throat with *Neisseria gonorrhoeae*, the bacteria that causes gonorrhoea. Throat infections usually have no symptoms but can still lead to unintentional spread to others. Genital gonorrhoea can cause painful symptoms. In rare cases, the infection can spread through the bloodstream to affect joints, tendons, and skin. It can also cause serious long-term health problems in women, including infertility. Although gonorrhoea can be treated with antibiotics, the only effective treatment is ceftriaxone and ceftriaxone-resistant gonorrhoea is increasingly reported. This increasing resistance makes it vital to develop new treatments. This initial study aims to confirm that the research approach is safe and to find the dose of bacteria that reliably causes throat infection within five days. All participants will be treated with antibiotics within five days of being exposed to the bacteria. As gonorrhoea can cause long-term reproductive impacts in people with a female reproductive system, only adults aged 18 to 50 who were assigned male at birth and have sex exclusively with others assigned male at birth will be eligible. Participants will attend the clinic as outpatients and must agree not to have any form of sexual contact, including oral sex and kissing, until they are cured. The study will explore clinical symptoms, immune responses, and how the bacteria behave in the throat. This will improve understanding of throat gonorrhoea. If this early research is successful, the model will be used to test new antibiotics, vaccines, and prevention strategies, with the ultimate goal of reducing the global burden of disease due to gonorrhoea.

Keywords
Controlled human infection model, gonorrhoea, sexually transmitted infection, study protocol, vaccine, antimicrobial resistance

This article is included in the Global Infectious Disease Ethics Collaborative (GLIDE) gateway.

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Competing interests: No competing interests were disclosed.

Grant information: This project was funded by Wellcome[203132, 221719, 320225] and Australian National Health and Medical Research Council [GNT2005380, GNT2009548, GNT2017383, GNT1172900, GNT2033299, GNT2025840, GNT2025960, GNT1174555, GNT2016396, GNT2038654, GNT2038630] and Medical Research Future Fund Clinician Researchers Applied Research in Health Grant [MRFAR000354]
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Williams E, Pollock GL, Price DJ *et al.* **An oropharyngeal gonorrhoea controlled human infection model: a provisional protocol using a novel *Neisseria gonorrhoeae* challenge strain [version 1; peer review: 1 approved, 2 approved with reservations]** Wellcome Open Research 2025, **10**:636 https://doi.org/10.12688/wellcomeopenres.25103.1

First published: 19 Nov 2025, **10**:636 https://doi.org/10.12688/wellcomeopenres.25103.1

Introduction

Neisseria gonorrhoeae is a significant global pathogen[1], estimated to infect approximately 88 million people per year[2]. The greatest burden of disease is carried by people in low- and middle-income countries where there has been no improvement in rates of infection in the past 30 years[2]. In high-income settings, there has been a dramatic increase in rates of infection over the past decade, with infection most prevalent in high-risk populations including men who have sex with men (MSM), transgender persons, sex workers and socially marginalised populations including indigenous populations[1]. *N. gonorrhoeae* can cause symptomatic urogenital gonorrhoea[3], sight-threatening gonococcal conjunctivitis[4], and uncommonly, disseminated gonococcal infection[5]. Left untreated, urogenital infection can result in adverse reproductive outcomes including pelvic inflammatory disease, infertility, and complications in pregnancy[6]. Infection therefore disproportionately impacts women and their children. Urogenital infection may also increase the risk of HIV acquisition[7]. *N. gonorrhoeae* also causes infection at extragenital sites including the oropharynx and anorectum; however, infection at these anatomical sites is usually asymptomatic[8].

Asymptomatic infection, particularly of the oropharynx, may play an important role in gonorrhoea transmission[9]. Antimicrobial resistance (AMR) in *N. gonorrhoeae* has been designated a critical threat to public health by the World Health Organization and by the United States Centers for Disease Control and Prevention[10,11]. *N.gonorrhoeae* exhibits a remarkable capacity to develop antimicrobial resistance (AMR), driven by its high genetic plasticity and ability to readily acquire resistance determinants through horizontal gene transfer[12]. Oropharyngeal infection is a high-risk site for acquisition of AMR, due to prolonged colonisation[13] and potential for horizontal gene transfer from commensal *Neisseria* species and other organisms[12].

Controlled human infection models (CHIMs) have contributed to the development of new vaccines and therapeutics for infectious diseases of concern such as typhoid, malaria, and influenza[14]. A male gonorrhoea urethritis CHIM, established in the United States in the 1980s[15], has predominantly been used to study pathogenesis. More recently, it has been used to evaluate vaccine efficacy[16]. An oropharyngeal gonorrhoea CHIM may be more acceptable to potential participants due to the lower likelihood of symptomatic infection and relatively non-invasive inoculation procedure compared to the urethritis model. An oropharyngeal gonorrhoea CHIM may play a key role in i) accelerating gonorrhoea vaccine development; ii) assessing the efficacy of novel antimicrobials at the important oropharyngeal site of infection; iii) identifying an immune correlate of protection to support future vaccine development and optimization; and, iv) characterising *N. gonorrhoeae* infection dynamics in the oropharynx[17].

Following multiple observational studies suggesting partial efficacy of an outer membrane vesicle (OMV) *Neisseria meningitidis* serogroup B vaccine (4CMenB) against gonorrhoea[18], and the increasingly urgent need for new preventative strategies, there is renewed interest in development of a gonococcal vaccine. However, results from the first randomised controlled trial of 4CMenB in MSM failed to demonstrate efficacy against gonorrhoea infection[19]. Despite these discouraging results, there remains hope for the development of an effective gonorrhoea vaccine. There are several gonorrhoea vaccines in early clinical and late preclinical development using OMV technology optimized specifically for *N. gonorrhoeae*, as well as vaccines incorporating novel vaccine antigens and adjuvants[20]. Novel antimicrobials have also recently been developed against *N. gonorrhoeae*, including zoliflodacin[21] and gepotidicin[22], with phase three studies demonstrating that these first-in-class antimicrobials are non-inferior to standard care for treatment of uncomplicated urogenital infection. However, these agents may have sub-maximal efficacy against oropharyngeal infection, the most challenging site for antimicrobial efficacy against gonorrhoea[23]. Unless new antimicrobials, vaccines, and other strategies have efficacy in the oropharynx, there is a risk that they will fail to control gonorrhoea at a population level[23,24].

Here, we describe the study design considerations, and a protocol for a first-in-human oropharyngeal gonorrhoea CHIM. Ethical considerations, including the scientific justification, safety and risk mitigation have been carefully considered and are described elsewhere[17]. The primary consideration in the development of this protocol has been to maximize participant and community safety. Development of this trial protocol has also followed best practice by incorporating community consultation into the study design phase[25,26]. This community engagement process has shaped protocol development by informing recruitment strategies, informed consent materials, and study procedures and will be described in a forthcoming publication. Other critical steps towards establishing a safe, and informative model have been: i) the genomics-based selection of a novel challenge strain from a large contemporary collection of clinical *N. gonorrhoeae* isolates[27]; and, ii) developing an animal-free liquid media strain manufacture that enables preparation of single-use frozen dose vials at various concentrations that can be thawed for use on the day of inoculation and in-depth release testing prior to participant inoculation, which will be published separately.

Study design considerations

The success of our first-in-human CHIM requires a clear rationale for study procedures and endpoints. Lessons may be drawn from the experience of several other CHIMs (Table 1). Critical to the design of an oropharyngeal gonorrhoea CHIM is building upon the decades of experience with the male urethritis model, which was established over 40 years ago[15]. However, given oropharyngeal gonorrhoea is predominantly an asymptomatic infection, many study design considerations are comparable with other human models of bacterial upper respiratory tract colonisation, such as *Neisseria lactamica*[28], *Bordetella pertussis*[29], and *Streptococcus pneumoniae*[30]. Much can also be learnt from the *Streptococcus pyogenes* model[31], the only other CHIM including an oropharyngeal challenge. Gastrointestinal bacterial CHIMs such as *Salmonella enterica* serovar

Table 1. Key design features of bacterial controlled human infection models relevant to the design of an oropharyngeal gonorrhoea model.

Organism	Participant eligibility	Inoculation (route, dose, attack rate achieved)	Setting	Duration between inoculation and treatment (or end of monitoring if treatment not provided)	Treatment (anti-microbial, dose, duration and timing)	Key features applicable to oropharyngeal gonorrhoea CHIM	Reference
Colonization studies							
Bordetella pertussis	Healthy adults aged 18–45 years without known or suspected recent pertussis infection	Nasal, ~10^5 cfu, 80% attack rate	Inpatient	14 days	Oral azithromycin 500mg daily for 3 days at day 14	Infectious upper respiratory pathogen, treatment at end of observation period	De Graaf *et al.*, Clin Infect Dis, 2020[32]
Streptococcus pneumoniae	Healthy adults aged 18–50 years	Nasal, ~10^5 cfu, 70% attack rate	Outpatient	14 days	Oral amoxicillin 500mg tds for 3 days at day 14	Infectious upper respiratory pathogen, treatment at end of observation period, outpatient design	Robinson *et al.*, Am J Respir Crit Car, 2022[33]
Neisseria lactamica	Healthy adults aged 18–45 years without active *N. lactamica* carriage or meningococcal vaccination in prior 5 years	Nasal, ~10^4 cfu, 60% attack rate	Outpatient	24 weeks	Nil	*Neisseria* species upper respiratory tract inoculation, 10^4 cfu dose with 60% attack rate. Subsequent CHIM involving genetically modified *N. lactamica* involving recruitment of contact participants as well as study participants	Evans, Clin Infect Dis, 2011[34] Gbesemete *et al.*, BMJ Open, 2019[35]
Clinical endpoint studies							
Neisseria gonorrhoeae	Healthy adults assigned male sex at birth, aged 18–35 years	Urethral, ~10^3 (MS11mkC), 50% attack rate; ~10^6 (FA1090), 80–90% attack rate	Inpatient-leave unit during day; Outpatient	5–10 days	Oral cefixime 400mg single dose at symptoms or day 5	*Neisseria gonorrhoeae* CHIM with 10^4–10^5 cfu inoculation at urethral site, treatment at end of observation period or development of symptoms	Hobbs & Duncan, Methods Mol Biology, 2019[15] Duncan *et al.*, clinicaltrials.gov[16]
Streptococcus pyogenes (Group A Strepto-coccus)	Healthy adults aged 18–40 years without risk factors for severe Group A Streptococcal disease	Pharyngeal, ~10^4 cfu, 85% attack rate	Inpatient	5 days	Intramuscular benzathine penicillin 900mg single dose and oral rifampicin 300mg bd for 4 days at development of symptomatic GAS pharyngitis or day 5	Infectious upper respiratory pathogen with potential for systemic illness, treatment at end of observation period or development of symptoms. Novel pharyngitis clinical assessment grading score	Osowicki *et al.*, Lancet Microbe, 2021[31]

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Organism	Participant eligibility	Inoculation (route, dose, attack rate achieved)	Setting	Duration between inoculation and treatment (or end of monitoring if treatment not provided)	Treatment (anti-microbial, dose, duration and timing)	Key features applicable to oropharyngeal gonorrhoea CHIM	Reference
Salmonella enterica serovar. Typhi	Healthy adults aged 18–60 years, without prior typhoid vaccine or resident in typhoid endemic regions for >6 months	Ingestion, ~10^4 cfu, 65% attack rate	Outpatient	14 days	Oral ciprofloxacin 500mg bd for 14 days at typhoid diagnosis or day 14	Infectious pathogen with potential for systemic illness, outpatient design with infection control procedures, treatment at end of observation period, development of symptoms (as well as bacteraemia)	Waddington *et al.*, Clin Infect Dis, 2014[36]
Salmonella enterica serovar. Paratyphi A	Healthy adults aged 18–60 years without prior typhoid vaccine or resident in enteric fever endemic regions for >6 months	Ingestion, ~10^3 cfu, 60% attack rate	Outpatient	14 days	Oral ciprofloxacin 500mg bd for 14 days at paratyphoid diagnosis or day 14	Infectious pathogen with potential for systemic illness, outpatient design with infection control procedures, treatment at end of observation period, development of symptoms (as well as bacteraemia)	Dobinson *et al.*, Clin Infect Dis, 2017[37]; McCullagh *et al.*, BMJ Open, 2015[38]
Non-typhoidal *Salmonella (Salmonella enterica* serovar Typhimurium)	Healthy adults aged 18–50 years without prior *Salmonella* infection or typhoid vaccine	Ingestion, ~10^1 – 10^6 , aim 60–75% attack rate	Inpatient (8 days) with subsequent outpatient phase (7 days)	14 days	Oral ciprofloxacin 500mg bd for 5–14 days at development of pre-specified symptoms or bacteraemia	Infectious pathogen with potential for systemic illness, dose escalation/de-escalation using continual reassessment model	Smith *et al.*, BMJ Open, 2024[39]

bd: bis die, twice daily; cfu: colony forming units; d: days; tds: ter die sumendum, three times daily.

Typhi[36], *Salmonella enterica* serovar Paratyphi A[37], and non-typhoidal *Salmonella*[39] are also informative in that each involve infection with a transmissible bacterial pathogen with a large and highly diverse genome, similar to *N. gonorrhoeae*.

Study protocol

Study synopsis

This study is a prospective dose-finding CHIM outpatient study which aims to establish the safety of an oropharyngeal gonorrhoea CHIM and identify the infectious dose required for healthy adult male participants to develop oropharyngeal *N. gonorrhoeae* infection following direct oropharyngeal application of *N. gonorrhoeae* strain AUSMDU00053933. A predicted total of approximately 20 to 35 eligible and consenting healthy male volunteers who exclusively have sex with people assigned male (assigned at birth) will be included in the study. Participants will be treated with ceftriaxone antimicrobial therapy for a maximum of 5 days after inoculation, undergo testing to confirm cure 7 and 14 days after treatment and will be followed up for 90 days after inoculation. Participant screening and recruitment is anticipated to commence in February 2026 and the study is anticipated to conclude in September 2026. This manuscript outlines the provisional protocol, version 1 (published October, 2025).

Community engagement

Community engagement activities, including a qualitative research study, have been conducted throughout the protocol design stage, resulting in the incorporation of insights from key stakeholders. This qualitative research comprised of one focus group and 27 semi-structured interviews conducted between July and November, 2024, with a broad range of stakeholders. Participants included two community-based organization representatives, eight subject matter experts including clinical infectious diseases, sexual health, public health physicians and industry professionals, and a sample of 22 participants who would be eligible for participation in the study (i.e., 18 to 50 year old MSM without chronic medical illness) recruited through university, sexual health and community organization study advertisements. These qualitative data will be published elsewhere[26]. A Clinical Trial Reference Committee including representatives from community-based organizations, a bioethicist, and infectious diseases experts will provide ongoing governance to maximize the ongoing acceptability of the trial. A process evaluation will be conducted in parallel with the CHIM to assess the experience of participants and to inform iterative improvement of the design and conduct of future trials (CURE-NG Participant Questionnaire).

Study objectives and outcomes

Study objectives and outcomes are summarised in Table 2. The primary objectives of this study are to establish i), the safety and tolerability of *N. gonorrhoeae* AUSMDU00053933 inoculation at the oropharynx; and ii), the dose of *N. gonorrhoeae* AUSMDU00053933 required to cause a reproducible microbiologically-confirmed *N. gonorrhoeae* oropharyngeal infection rate of 60 to 80% within five days of direct application by swab to the oropharynx. The secondary objectives of this study are to identify i) the proportion of participants at each dose level who develop symptomatic gonorrhoea pharyngitis; ii) the occurrence of severe, complicated or disseminated gonococcal infection among participants; and, iii) infection with *N. gonorrhoeae* at other mucosal sites during the study period. Exploratory objectives of this study include assessment of the microbiological characteristics and host immune responses of experimental human *N. gonorrhoeae* oropharyngeal infection, pharmacometric assessment of ceftriaxone treatment and acceptability of the study among participants.

Study recruitment

Several recruitment modalities will be employed to identify appropriate study participants. This will include advertising through local sexual health services and broader advertisement through higher education institutions, community-based organizations, social media and relevant websites. Advertising material used in this study will be co-designed with community-based organization representatives to maximize acceptability among potential participants. Participants will be offered reimbursement for their time for participation in the study.

Eligibility criteria

Consenting healthy adults assigned male at birth, aged 18 to 50 years without pre-existing risk factors for severe disease will be recruited as study participants. Strict eligibility criteria have been designed to mitigate the risk of severe disease (eg, exclusion of individuals with complement deficiency, eculizumab use, immunocompromise including HIV, diabetes, drug/alcohol misuse[40,41]) and transmission to individuals at risk of severe disease (eg, exclusion of household contacts of immunocompromised)[17] (Table 3). Detailed screening will include medical history, physical examination, baseline blood borne virus (BBV)/STI screening and blood tests. Individuals with a history of gonorrhoea infection in the preceding three months or who have previously received a *Neisseria meningitidis* serogroup B vaccine (eg. 4CMenB) will be excluded due to potential cross-protective immune responses against *N. gonorrhoeae*[42,43]. Individuals with a history of gonococcal-active antimicrobial usage in the prior three months will be excluded due to possible increased risk of antimicrobial resistance in subsequent gonorrhoea infections[44], due to carriage of antimicrobial resistance determinants in the oropharynx. Those with oropharyngeal *N. meningitidis* carriage detected in the week prior to inoculation will also be excluded due to the potential for transfer of virulence factors from *N. gonorrhoeae* to *N. meningitidis* in the oropharynx[45].

Only individuals assigned male at birth who exclusively have sex with people assigned male at birth will be recruited, due to the risk of acute and long-term reproductive health complications of urogenital gonorrhoea on the female reproductive system[6]. This population of adult MSM are a priority population to include in research exploring *N. gonorrhoeae* treatment and prevention strategies, as there is a high prevalence of *N. gonorrhoeae* infection in MSM both in Australia[46] and other high-income settings worldwide[1]. Although the oropharynx

Table 2. Primary and secondary objectives of the first oropharyngeal gonorrhoea controlled human infection model.

	Objectives	Outcomes
Primary objectives	1. To define the safety and tolerability of oropharyngeal inoculation of healthy volunteers with AUSMDU00053933 *N. gonorrhoeae*	Occurrence of solicited and unsolicited adverse events (as per the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) within the study period
	2. To establish the dose (defined in colony forming units per millilitre of the dose vial) of AUSMDU00053933 *N. gonorrhoeae* required to cause reproducible oropharyngeal infection rate of 60–80% within 5 days	Oropharyngeal *N. gonorrhoeae* infection is defined as: 1) Microbiologically-confirmed oropharyngeal *N. gonorrhoeae* (defined as detection of *N. gonorrhoeae* by NAAT using two different *N. gonorrhoeae* genomic targets from a combined posterior oropharynx, palatine tonsils and saliva swab) >48 hours after challenge strain inoculation, or 2) Microbiologically-confirmed symptomatic *N. gonorrhoeae* pharyngitis, defined as sore throat and examination score ≥2 or grade 3 pharyngitis (Figure 2) within 48 hours of inoculation of the challenge strain;
Secondary objectives	1. To identify the proportion of participants at each dose level who develop symptomatic gonorrhoea pharyngitis	Number of participants at each dose level who develop microbiologically-confirmed symptomatic *N. gonorrhoeae* pharyngitis
	2. To identify the occurrence of severe, complicated or disseminated gonococcal infection among participants	Identification of any participants that develop severe, complicated or disseminated gonococcal infection, defined as: 1) The detection of *N. gonorrhoeae* at sites other than the oropharynx and a clinical syndrome compatible with severe, complicated or disseminated gonococcal disease, or 2) A positive blood culture for *N. gonorrhoeae*.
	3. To assess for infection with *N. gonorrhoeae* at other mucosal sites during the study period	Detection of *N. gonorrhoeae* from other susceptible mucosalsites (rectal swab and first pass urine) from study participants during the study period via NAAT and/or culture
Exploratory objectives	To assess microbiological characteristics of experimental *N. gonorrhoeae* oropharyngeal infection	Microbiological detection (including qualitative culture and NAAT), phenotypic assessment (including presence of piliation), quantitation (including semi-quantitative culture, semi-quantitative (using the qualitative NAAT cycle threshold value) and quantitative NAAT (PCR)) and viability (by measuring the RNA-to-DNA ratio of a diagnostic target detected using NAAT) from throat swab during study period Genomic characterization of *N. gonorrhoeae* identified during study period Oropharyngeal microbiome characterization during the study period
	To assess the immune responses in healthy volunteers following experimental *N. gonorrhoeae* oropharyngeal infection	Changes in serological antibody responses during the study period Changes in mucosal antibody responses detected in saliva specimens during the study period Changes in systemic and oropharyngeal cytokine profile during the study period Changes in systemic cellular and mucosal response during the study period
	To assess pharmacometrics of intramuscular ceftriaxone for treatment of oropharyngeal gonorrhoea	Concentration of ceftriaxone in plasma and saliva after treatment and correlation of pharmacometric response with microbiological clearance
	To assess the motivations for participation and acceptability of participating in the oropharyngeal gonorrhoea CHIM	Identification of the motivations for participation; and acceptability of participating in the oropharyngeal gonorrhoea CHIM based on responses to a questionnaire completed before and after *N. gonorrhoeae* inoculation

NAAT: nucleic acid amplification test; PCR, polymerase chain reaction.

is the only site being inoculated with *N. gonorrhoeae* in this study, there is a risk of autoinoculation[47,48] or transmission to others via contact with the mouth or saliva[49]. As such, individuals with a female reproductive system and those with household or occupational contact with immunocompromised individuals or individuals < 18 years of age will be excluded[17]. Only prospective participants who are willing and able to adhere to study procedures to mitigate the risk of transmission of

Table 3. Eligibility criteria for participants in the oropharyngeal gonorrhoea controlled human infection model.

Inclusion criteria
Male (assigned at birth) aged ≥ 18 to ≤ 50 years old on the day of informed consent
Identifies as a person who has sex with people assigned male at birth and does not have sex with individuals assigned female at birth (past 12 months)
Proficient in English language
Able and willing to comply with all study requirements
Ability to read and understand the participant information, provide written informed consent to participate in the trial and demonstrate understanding of study requirements by passing a quiz
Provide written agreement to comply with infection control guidelines during the experimental gonococcal infection phase until the study team advise that *N. gonorrhoeae* eradication has been confirmed
Able and willing to abstain from the use of mouthwash from the day of screening until the end of the study
Exclusion criteria
History of any clinically important cardiac, endocrinologic, haematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, renal or other major disease, as determined by the Investigator
History of hospitalization for illness within the six months prior to enrolment into study or major surgery within the 12 months prior to enrolment into study
History of severe infectious disease including i) requirement for hospitalization for intravenous antibiotics; ii) prior *N. meningitidis* infection such as meningococcal meningitis or meningococcal bacteraemia; iii) ocular or disseminated gonococcal infection
History of cancer (except adequately treated squamous cell or basal cell carcinoma of the skin >5 years prior)
Any known or suspected immunodeficiencies or impairment/alteration of immune function including: • Congenital or acquired immunodeficiency including complement deficiency, antibody deficiency, chronic granulomatous disease, HIV infection or asplenism • Receipt of any immunosuppressive therapy such as anti-cancer chemotherapy or radiotherapy within the preceding 12 months • Any known or suspected autoimmune disorders (mild autoimmune disorders, such as eczema, are not exclusionary and will be determined by the Investigator)
Presence of implants or prosthesis (e.g. artificial joints, pacemakers)
History or presence of current alcohol abuse (defined as regular alcohol consumption of more than 40g per day), illicit drug use, or any prior intravenous usage of an illicit substance
Significant acute or chronic infection within 14 days prior to inoculation that the Investigator deems may compromise participant safety
Clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion, e.g. gastrectomy
History of tonsillectomy or adenoidectomy
Prior history of *N. meningitidis* serogroup B (e.g. 4CMenB) vaccination
A history of confirmed *N. gonorrhoeae* infection at any other site (urogenital, anorectal or oropharyngeal) in the three months prior to inoculation, including at screening or pre-enrolment testing
Detection of *N. meningitidis* on oropharyngeal swab at screening or pre-enrolment testing
Ex-smoker with >10 pack/year smoking history or a current active smoker defined as having smoked a cigarette or cigar in the four weeks prior to challenge
Any use of gonococcal-active antimicrobial therapy in the three months prior to inoculation
Any vaccination within the 28 days prior to challenge
Participation in a research study that involves blood sampling of more than 450ml/unit of blood, received or donated blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation within three months before study, plans for donation at any time during the study and up to three months after the last blood test

Any clinically significant abnormal finding on laboratory screening investigations, with specific exclusion criteria including: • Serum creatinine level >1.1x upper limit of normal (ULN) and deemed clinically significant by the study physician • Serum ALT level >1.25x ULN and deemed clinically significant by the study physician • White blood count (WBC) <2.5 or >15.0 x 109/L and deemed clinically significant by the study physician • Haemoglobin level <110 g/L or above ULN and deemed clinically significant by the study physician • 50% complement haemolytic activity (CH50) outside normal limits • Positive serologic results for human immunodeficiency (HIV) antibodies, hepatitis B surface antigen (HBsAg), and/or hepatitis C virus (HCV) antibodies (+ PCR positive if Hepatitic C virus antibodies detected), syphilis serology (with evidence of active untreated infection) • A positive urine drug test at screening or pre-enrolment (e.g. amphetamines, barbituates, benzodiazepines, cannabinoids, cocaine, and opiates) unless there is an explanation acceptable to the Investigator (e.g. the participant has stated in advance that they consumed a prescription or over the counter product which contained the detected drug) and the participant has a negative urine drug screen on repeat testing • A positive alcohol breath test In the event of abnormal test results, confirmatory repeat tests will be requested
Known hypersensitivity or other contraindications to the use of cephalosporins, carbapenems or macrolides (including treatment with other medications that are contraindicated with ceftriaxone, azithromycin and ertapenem and these antimicrobials and cannot be safely withheld)
Participation in another research study involving an investigational product or other intervention within 12 weeks prior to enrolment, at any time during the study and up to 12 weeks after completion of the study
Use of any systemic immunomodulatory treatment including eculizumab, corticosteroids (topical corticosteroids acceptable), anti-inflammatories (beside sporadic use of non-steroidal anti-inflammatory drugs), anticoagulants (aspirin acceptable), investigational products, interleukins, interferons or growth factors within the previous three months, or anticipated use of such drugs during the study period.
Known hypersensitivity to soya protein or any other component of the liquid culture media used for inoculation
Use of inhaled or intranasal corticosteroid from 14 days prior to inoculation until 28 days after inoculation, or confirmation of *N. gonorrhoeae* eradication, whichever is later
Use of non-prescription drugs and herbal supplements (such as St John's Wort) within 14 days or 5 half-lives (whichever is the longer) prior to inoculation. Use of vitamin supplements taken at standard doses is allowed.
Any other significant disease or disorder, which, in the opinion of the investigator, may either put the participants at risk because of participation in the study, or may influence the results of the study, or the participant's ability to participate in the study
Intolerance of throat swab procedure (exaggerated gag reflex)
Occupational, household or intimate contact with immunocompromised individuals (including HIV infection, asplenia, malignancy, recurrent, severe infections and chronic immunosuppressant medication within the past 6 months)
Occupational or household contact with individuals <18 years of age
Residence in unstable or emergency housing
Any employee of the sponsor or research site personnel directly affiliated with this study or their immediate family members defined as spouse, parent, sibling or child whether biologic or legally adopted

N. gonorrhoeae to others will be included. Specific infection control procedures will be included in participant information and informed consent materials (Table 4). Participants will also be required to demonstrate understanding of the study procedures and requirements before participating in the study. Due to increased risk of disseminated gonococcal infection described in some studies[40,50,51], people living with HIV (PLHIV) will be excluded from this initial study.

Study-specific exclusion criteria focus on mitigating the risk of participants developing complications and on reducing the risk of bias related to primary, secondary or exploratory outcomes. These include a history of disseminated gonococcal infecion; complement deficiency or other known or suspected congenital or acquired immunodeficiency; history of tonsillectomy; intolerance of throat swab procedure (e.g. exaggerated gag reflex) and known hypersensitivity or contraindication to b-lactam antimicrobials, soya protein (included in the bacterial culture medium) or other constituent in the bacterial culture medium (Table 5). Exclusion criteria include restriction of specific concomitant medications in the month prior to inoculation until confirmed cure, specific vaccines, systemic and intranasal corticosteroids, immunomodulators and anti-inflammatory therapy.

Infection control considerations

Participants will be managed using infection control protocols observing contact and droplet precautions in the trial

Table 4. Infection control guidelines for study participants in the oropharyngeal gonorrhoea controlled human infection model.

Day of inoculation at clinical trials centre
• Participants must wear a surgical mask at all times unless within their personal room until discharge from the clinical trials centre
• Participants must not enter the personal rooms of other participants
• Participants must remain in the clinical trials centre for the duration of the inoculation procedure and a 1 hour monitoring period thereafter
• Participants must wash their hands with soap for 30 seconds or use alcohol based hand rub before leaving their room
• Participants must not have contact with known immunosuppressed individuals
• Participants must not have any contact that could involve saliva or respiratory secretions to others during the clinical trials centre attendance
From inoculation to confirmed *N. gonorrhoeae* eradication
From inoculation to confirmed *N. gonorrhoeae* clearance:
• Participants must not engage in sexual activity including contact with the mouth (including deep kissing and saliva use in sexual activity), or contact with the penis, urine, semen or anorectal region with any other individual
• Participants must not have any contact with any other individual that has a high risk of transmission of saliva, including:
○ Sharing objects placed in the mouth such as cutlery or drinking vessels
○ Sharing of sex toys
• Participants must not use illicit drugs or alcohol
• Participants must avoid contact with known immunosuppressed individuals and individuals 18 years of age
• Participants must wash their hands with soap for 30 seconds or use alcohol based hand rub after contact with their own mouth or saliva

Table 5. Constituents in the bacterial culture media.

Agar	L-glutamine	Sodium hydroxide
Ammonium bicarbonate	L-ornithine	Sodium lactate
Corn starch	Nicontinamide adenine dinucleotide	Soya peptone
Di-potassium hydrogen phosphate	Oxaloacetate	Spermidine
Ferric nitrate	Peptone	Thiamine hydrochloride
Glucose	Potassium dihydrogen phosphate	Thiamine pyrophosphate
Glycerol	Sodium acetate	Uracil
Hydrogen chloride	Sodium bicarbonate	
Hypoxanthine	Sodium chloride	

centre on the day of inoculation. As *N. gonorrhoeae* is culturable from the saliva of individuals with oropharyngeal gonorrhoea[52,53] and transmission via fomites has been reported[54], contact and droplet precautions have been adopted to mitigate risk to trial personnel. Because the inoculation procedure may induce coughing due to a gag reflex, a more stringent infection control protocol including droplet precautions has been instituted for the day of inoculation for this study. Participants will be managed in single-rooms on the day of inoculation at the trial centre. All high-touch surfaces will be decontaminated after patient discharge from the clinical trials unit as per local policy.

Study participants will receive clear verbal and written instruction regarding infection control procedures in informed consent materials (Table 4), and be instructed to adhere to these instructions until *N. gonorrhoeae* eradication is confirmed.

Information sheets, detailing access to free gonorrhoea testing and treatment services, will be provided to the participants that can be given to any contacts in the case that infection control procedures have been breached during the relevant period (from inoculation until confirmed eradication). In the event that participants advise study staff of infection control breaches during the relevant period (inoculation until confirmed eradication), study staff will also facilitate contact tracing, including anonymous notification of *N. gonorrhoeae* exposure and referral to free testing and treatment services. As gonorrhoea is a notifiable disease in Australia, the jurisdictional public health department have also been notified of the study.

Study procedures and schedule

Study procedures and schedule of events are described in Figure 1 and Table 6, respectively. Study participants will be screened for eligibility between two months and five days (day -60 to day -5) prior to enrolment. Study participants will undergo pre-enrolment screening on day -5 to day -4 to confirm eligibility prior to enrolment in the study on day 0.

After confirmation of informed consent by the investigator or delegated study team member, eligibility and collection of baseline blood for exploratory immunological assessment, inoculation will take place on day 0 at the clinical trials centre. Building on established oropharyngeal inoculation procedures from the *Streptococcus pyogenes* CHIM, each participant will undergo a single inoculation, delivered by applying *N. gonorrhoeae* from a thawed single-dose vial to the oropharynx using a sterile swab[55]. Inoculation will take place in a dedicated room at the clinical trials facility. The designated single-dose vial will be thawed from storage at ≤-70°C, and inoculated within 0 to 10 minutes of thawing. Participants will be required to fast (inclusive of all food and drink) for 90 minutes before and after inoculation. The participant will be prepared by being seated in a semi-recumbent position. The vial will be prepared by performing gentle inversion ten times to mix the inoculum and the sterile swab will be dipped in the vial for ten seconds. The participant will be instructed to tilt their head backwards and open their mouth widely. Using a tongue depressor to hold the tongue in place, the operator will apply the inoculum by rolling the swab back and forth over the tonsillar arches and posterior oropharynx. After inoculation, the single-dose vial will be recapped, marked as used with the time, date of inoculation and study participant identification number, and returned to the laboratory for storage. Study monitoring procedures will track all doses received, dispensed, inoculated and returned to pharmacy and these data will be reconciled at the end of the study.

Participants will be observed in the clinical trials unit for one hour after inoculation, and attend the trial unit daily for up to

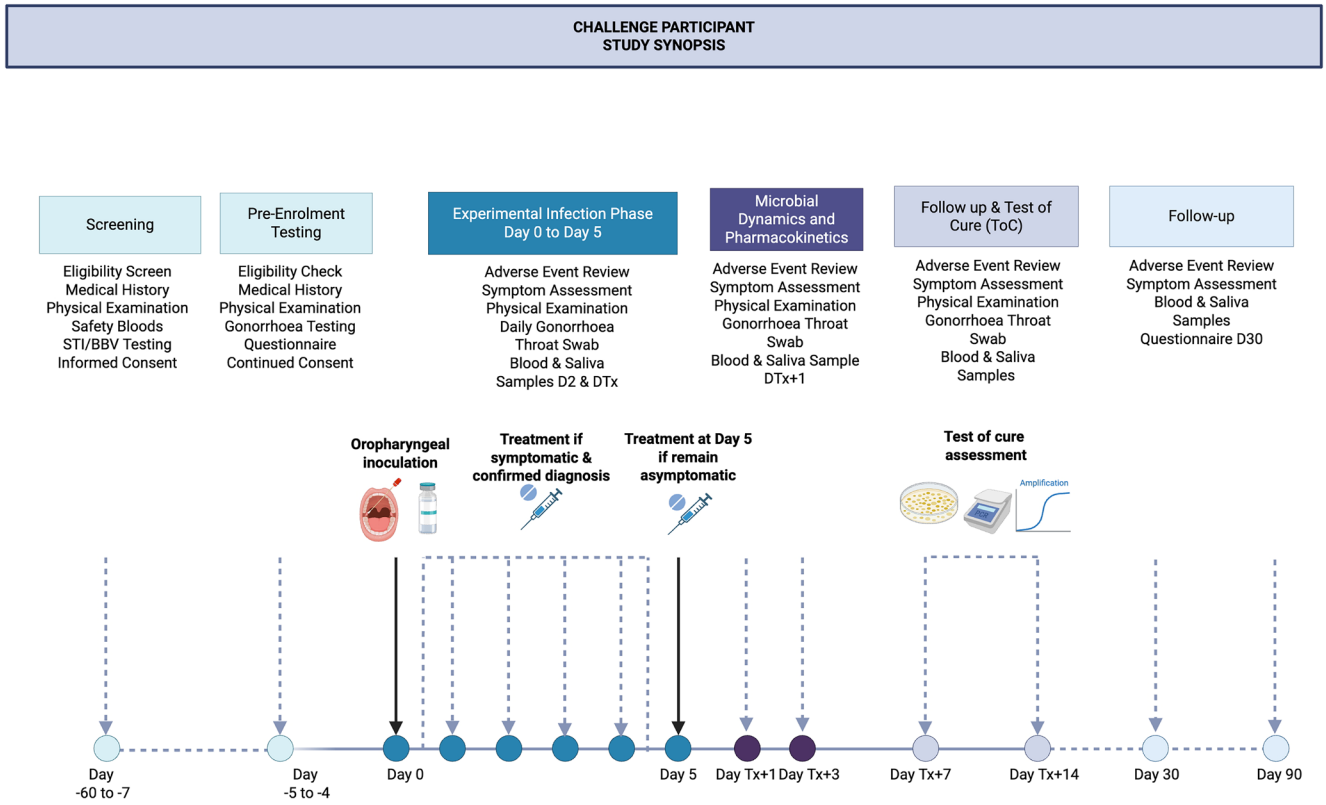

Figure 1. 1. Overview of participant timeline from screening to study completion. DTx, day of treatment; BBV, blood borne virus; STI, sexually transmitted infection; ToC, test of cure. Created in BioRender. Williams, E. (2025) https://BioRender.com/w9c1nrb.

Table 6. **Study procedures for participants for the oropharyngeal gonorrhoea controlled human infection model.**

Schedule of Activities															
Procedures	**Phone Screening**	**Screening**	**Pre-enrolment Testing**	**Experimental Infection Period**			**Follow-up Visits**						**Persistent Carriage Confirmation**	**Treatment for Persistent Carriage**	**Eradication Testing After Treatment for Persistent Carriage**
Study Day	**D-60 to D-7**	**D-60 to D-7**	**D-5 (+/-1)**	**Challenge: D0**	**Daily Monitoring (D1 to D4)[a]**	**Symptomatic Infection / Treatment (DTx)[b]**	**DTx +1**	**DTx+3**	**DTx +7: Test of cure (+/-1)**	**DTx+14[c]: Test of cure (+/-1)**	**D30 (+/-3)**	**D90 (+/-5)**	**+7D after initial positive test of cure (+/-1)**	**Within 24hrs of lab notification of confirmed persistent carriage**	**+7, +14, and +21[d] days after treatment for persistent carriage (+/-1)**
Day of Week			**Tue**	**Sun**	**Mon-Thu**	**Mon-Fri**					**Tue**	**Sat**			
Consent, history and examination															
Review eligibility	X	X	X	X											
Verbal informed consent	X														
Written informed consent		X													
Confirmation of consent			X	X											
Demographics	X	X													
Medical history	X	X	X	X											
Social history	X	X	X	X											
Concomitant medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptom check					X	X	X	X	X	X	X	X	X	X	X
Weight & height		X													
Vital signs		X	X	X	X	X	X	X	X	X	X		X	X	X
Comprehensive clinical examination		X													

Schedule of Activities															
Procedures	**Phone Screening**	**Screening**	**Pre-enrolment Testing**	**Experimental Infection Period**			**Follow-up Visits**						**Persistent Carriage Confirmation**	**Treatment for Persistent Carriage**	**Eradication Testing After Treatment for Persistent Carriage**
Study Day	**D-60 to D-7**	**D-60 to D-7**	**D-5 (+/-1)**	**Challenge: D0**	**Daily Monitoring (D1 to D4)[a]**	**Symptomatic Infection / Treatment (DTx)[b]**	**DTx +1**	**DTx+3**	**DTx +7: Test of cure (+/-1)**	**DTx+14[c]: Test of cure (+/-1)**	**D30 (+/-3)**	**D90 (+/-5)**	**+7D after initial positive test of cure (+/-1)**	**Within 24hrs of lab notification of confirmed persistent carriage**	**+7, +14, and +21[d] days after treatment for persistent carriage (+/-1)**
Day of Week			**Tue**	**Sun**	**Mon-Thu**	**Mon-Fri**					**Tue**	**Sat**			
Targeted examination			X	X	X	X	X	X	X	X	X		X	X	X
Symptom-directed physical examination					X	X	X	X	X	X	X				
Adverse events				X	X	X	X	X	X	X	X	X	X	X	X
Clinical tests															
Alcohol breath test		X	X	X											
Urine drug screen		X	X												
Hematology blood tests		X				X							X		
Biochemistry blood tests		X				X							X		
CH50 determination		X													
Serum (blood test) for storage				X											
Serology (blood test) for immunological assessment				X	X[l]	X			X	X	X	X			
PBMCs (blood test) for immunological assessment				X	X[l]				X		X	X			

Schedule of Activities															
Procedures	**Phone Screening**	**Screening**	**Pre-enrolment Testing**	**Experimental Infection Period**			**Follow-up Visits**						**Persistent Carriage Confirmation**	**Treatment for Persistent Carriage**	**Eradication Testing After Treatment for Persistent Carriage**
Study Day	**D-60 to D-7**	**D-60 to D-7**	**D-5 (+/-1)**	**Challenge: D0**	**Daily Monitoring (D1 to D4)[a]**	**Symptomatic Infection / Treatment (DTx)[b]**	**DTx +1**	**DTx+3**	**DTx +7: Test of cure (+/-1)**	**DTx+14[c]: Test of cure (+/-1)**	**D30 (+/-3)**	**D90 (+/-5)**	**+7D after initial positive test of cure (+/-1)**	**Within 24hrs of lab notification of confirmed persistent carriage**	**+7, +14, and +21[d] days after treatment for persistent carriage (+/-1)**
Day of Week			**Tue**	**Sun**	**Mon-Thu**	**Mon-Fri**					**Tue**	**Sat**			
Plasma for drug levels						X	X								
BBV Serology (HIV Ag/Ab, HBsAg, HCV Ab, syphilis Ab)		X													
ECG													X		
Combined oropharyngeal swab (CT/NG PCR; culture)		X	X	X	X	X	X	X	X	X			X	X	X
Combined oropharyngeal swab (viability assessment)						X	X	X	X	X			X		X
Nasopharyngeal swab for immunology				X					X		X	X			
Saliva (immunological assessment)				X	X[i]	X			X	X	X	X			
Saliva (microbiological assessment)				X	X[i]	X			X	X	X	X			
Saliva (drug levels)						X	X								
Rectal swab CT/NG PCR[e]		X	X	X		X[f]			X[f]	X[f]			X		X
Urine CT/NG PCR[e]		X[g]	X[g]	X[g]		X[f]			X[f]	X[f]			X		X

Schedule of Activities															
Procedures	**Phone Screening**	**Screening**	**Pre-enrolment Testing**	**Experimental Infection Period**			**Follow-up Visits**						**Persistent Carriage Confirmation**	**Treatment for Persistent Carriage**	**Eradication Testing After Treatment for Persistent Carriage**
Study Day	**D-60 to D-7**	**D-60 to D-7**	**D-5 (+/-1)**	**Challenge: D0**	**Daily Monitoring (D1 to D4)[a]**	**Symptomatic Infection / Treatment (DTx)[b]**	**DTx +1**	**DTx+3**	**DTx +7: Test of cure (+/-1)**	**DTx+14[c]: Test of cure (+/-1)**	**D30 (+/-3)**	**D90 (+/-5)**	**+7D after initial positive test of cure (+/-1)**	**Within 24hrs of lab notification of confirmed persistent carriage**	**+7, +14, and +21[d] days after treatment for persistent carriage (+/-1)**
Day of Week			**Tue**	**Sun**	**Mon-Thu**	**Mon-Fri**					**Tue**	**Sat**			
Blood culture						X[f]									
CHIM procedures															
Photo of oropharynx				X		X			X	X			X		X
Review infection control adherence					X	X	X	X	X	X			X	X	X
Participant experience quesetionnaire			X								X				
Inoculation				X											
Antibiotic treatment[h]						X								X	

[a] Daily monitoring between Inoculation and Symptomatic Infection/Treatment visits. If Symptomatic Infection occurs earlier than D5, this will replace and stop the Daily Monitoring Visits.

[b] DTx = Day of Treatment. This visit will not occur any later than D5.

[c] Day Tx+14 will not be undertaken if the participant has remained uninfected with oropharyngeal gonorrhoea

[d] +21 timepoint will not be required if test of cure returns negative at +14 timepoint

[e] Self-collected sample

[f] If *N. gonorrhoeae* infection is established

[g] First pass urine collection

[h] Treatment may also be provided at any time upon participant request

[i] Day 2 only

Ab, antibody; Ag/Ab, antigen/antibody; BBV, blood borne virus; CH50, total complement activity; CHIM, controlled human infection model; CT, *chlamydia trachomatis*; ECG, electrocardiogram, HIV, human immunodeficiency virus; HBsAg, Hepatitis B surface antigen; HCV, hepatitis C virus; NG, *Neisseria gonorrhoeae*; PCR, polymerase chain reaction

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five days after inoculation. As oropharyngeal gonorrhoea is predominantly an asymptomatic infection with limited clinical impact, an oropharyngeal gonorrhoea CHIM can be safely performed in the outpatient setting with risk mitigation procedures in place. As transmission is expected to be negligible with appropriate infection control procedures in place and there is a high prevalence of oropharyngeal gonorrhoea in the target recruitment population of MSM in Melbourne, inpatient admission and confinement is not considered necessary for this CHIM. The prevalence of oropharyngeal gonorrhoea in a cross-sectional study of 3,677 MSM in Melbourne in 2016-2017 was 6.2%[56]; and oropharyngeal gonorrhoea prevalence was up to 16% in a prospective cohort study of 100 MSM with increased risk of oropharyngeal gonorrhoea (including HIV PrEP usage or oropharyngeal gonorrhoea infection in the prior three months) in Melbourne in 2019[57]. Daily visits will include review of adverse events, participant symptoms and sexual activity/personal contact activities, physical examination, recording of vital signs and collection of a combined oropharyngeal swab (collected from the posterior oropharynx, palatine tonsils and saliva) (Figure 1). Participants will be treated with 1g ceftriaxone by intramuscular injection in the following circumstances: i) at day five after inoculation; ii) within 24 hours if they develop symptomatic pharyngitis, defined as sore throat and examination score ≥2 or grade ≥3 pharyngitis (Figure 2) with microbiologically-confirmed *N. gonorrhoeae* infection; or, iii) upon participant request.

On the day of treatment, blood for haematology, biochemistry and exploratory immunological assessment will be collected. Blood cultures, first pass urine and rectal swabs for *N. gonorrhoeae* NAAT will be collected from individuals in whom oropharyngeal gonorrhoea infection has been established. Participants will attend an outpatient review on days 1, 3, 7 and 14 after antimicrobial therapy for clinical assessment and microbiological sampling including test of cure (combined oropharyngeal swab for gonococcal culture and *N. gonorrhoeae* NAAT). Study participants will also attend an outpatient review at one and three months after inoculation for clinical assessment to assess for the development of post-infection complications and undergo further sampling for immunological assessment. Supportive therapy will be provided as required for symptom control, however most infections are expected to be asymptomatic. Throughout the trial, participants will have access to 24-hour telephone support and advice from clinical trials staff, including medical personnel, and access to urgent review and sepsis treatment through the partner adult tertiary hospital in the unlikely event that acute infectious complications occur.

Outcome measures

As this is a first-in-human experimental oropharyngeal gonorrhoea infection study, it has two primary outcomes, one being safety and tolerability and the other dose-finding of the *N. gonorrhoeae* challenge strain, AUSMDU00053933, at this anatomical site. The occurrence of serious adverse events and solicited and unsolicited adverse events within the study period will be monitored by the Trial Management Group (TMG) overseen by the medical monitor and Safety Monitoring Committee (SMC) throughout the study to establish the safety of the CHIM.

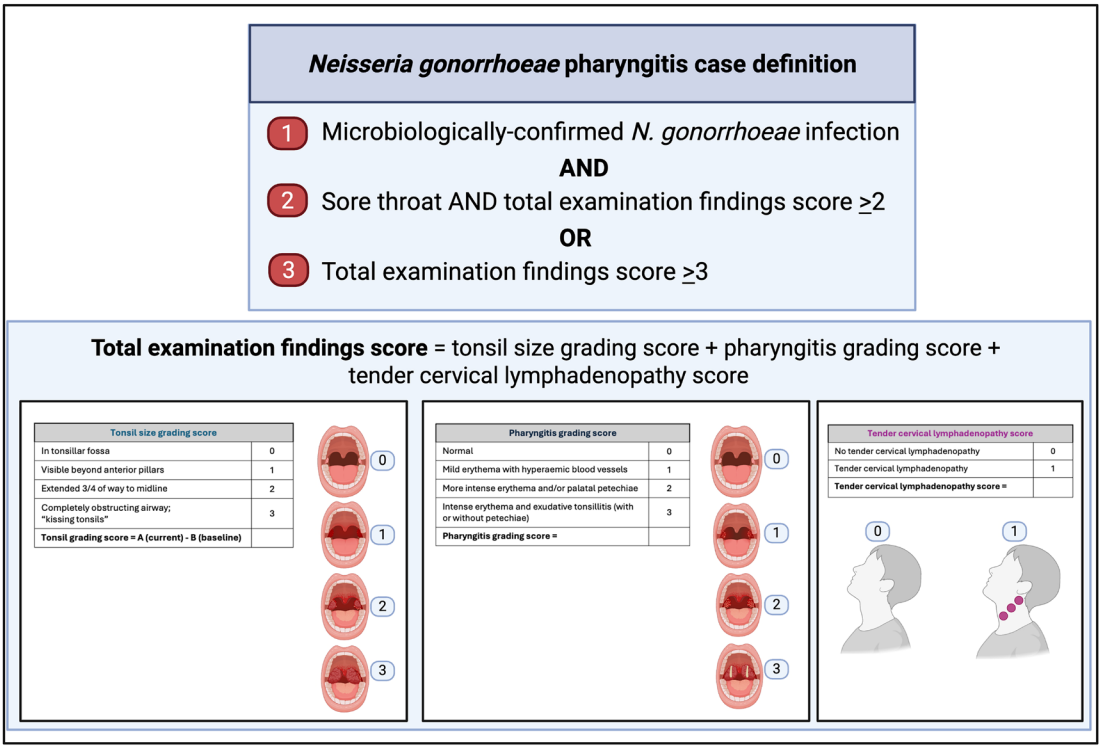


Figure 2. *Neisseria gonorrhoeae* pharyngitis case definition. Created in BioRender. Williams, E. (2025) https://BioRender.com/c61x06a.

Oropharyngeal gonorrhoea is predominantly an asymptomatic infection. As such, the case definition of oropharyngeal *N. gonorrhoeae* infection utilized to establish the dose of AUS-MDU00053933 required to cause a reproducible oropharyngeal infection rate of 60-80% within five days for this study can be established via microbiological criteria alone, or combined clinical and microbiological criteria. This case definition includes i) microbiologically-confirmed oropharyngeal *N. gonorrhoeae* >48 hours after challenge strain inoculation; or, ii) microbiologically-confirmed symptomatic *N. gonorrhoeae* pharyngitis, defined as sore throat and examination score ≥2; or grade ≥3 pharyngitis (Figure 2) within 48 hours of inoculation of the challenge strain.

Microbiologically-confirmed *N. gonorrhoeae* will be defined as detection of *N. gonorrhoeae* by NAAT with two different *N. gonorrhoeae* genomic targets from a combined oropharyngeal swab collected from the posterior oropharynx, palatine tonsils and saliva. *N. gonorrhoeae* NAAT will be performed using the commercial multiplex Xpert CT/NG (Cepheid, Sunnyvale, CA) polymerase chain reaction (PCR) which has two *N. gonorrhoeae* targets that identify the presence of non-contiguous highly conserved chromosomal DNA regions in *N. gonorrhoeae*. The case definition for clinical *N. gonorrhoeae* pharyngitis used in this study was developed for use in the *S. pyogenes* CHIM[55] and is adapted from the Centor and McIsaac scores for prediction of streptococcal pharyngitis[58] (Figure 2). Although this score has not been validated for use for oropharyngeal gonorrhoea, it has been successfully implemented in the *S. pyogenes* CHIM[31] and provides a measure of pharyngeal inflammation including tonsillar size change, pharyngitis grading and cervical lymphadenopathy, which can be used to document and grade physical examination findings in conjunction with symptom assessment and microbiological testing.

Participants will have daily combined oropharyngeal swab collected from the posterior oropharynx, palatine tonsils and saliva for *N. gonorrhoeae* NAAT and culture during the experimental gonorrhoea infection phase. If the participant develops symptoms and signs consistent with pharyngitis, additional samples will be collected to facilitate testing for other common causes of pharyngitis in the community, including a nasopharyngeal swab for respiratory viruses and throat swab for *S. pyogenes*. These tests will be performed if *N. gonorrhoeae* is not detected on NAAT. In the event of asymptomatic microbiologically-confirmed *N. gonorrhoeae* infection, participants will be monitored daily in the clinical trials centre and treated on day five after inoculation. In the event that no infection has occurred by day five, a final combined oropharyngeal swab will be collected, followed by appropriate curative antimicrobial therapy for *N. gonorrhoeae* infection. Although *N. gonorrhoeae* culture will be performed concurrently with all NAAT sampling on throat swabs, results of these tests will not inform the primary microbiological outcome of the study, as *N. gonorrhoeae* culture for detection of *N. gonorrhoeae* infection is significantly less sensitive than NAAT at the oropharynx[59]. In observational studies, NAAT has been shown to be approximately five-fold more sensitive than culture[52]. Secondary and exploratory outcomes include clinical, microbiological, immunological, pharmacometric and process evaluation outcomes (Table 2).

Antimicrobial therapy

All participants will receive antimicrobial therapy aimed at eradicating the *N. gonorrhoeae* challenge strain from the oropharynx (Table 7). Antimicrobial therapy will comprise of single-dose intramuscular ceftriaxone 1000mg in 3.5mL of 1% lignocaine. This primary treatment regimen aligns with United States Center for Disease Control and British Association for Sexual Health and HIV recommendations for treatment of oropharyngeal gonorrhoea[60,61]. Although it does not align with Australian STI treatment guidelines, where dual therapy with 500mg intramuscular ceftriaxone and 2g oral azithromycin is recommended[62], it has been selected to align with local health service guidelines and reduce the probability of gastrointestinal adverse events associated with azithromycin therapy[63]. It also avoids the administration of two classes of broad-spectrum antimicrobial therapy to trial participants. The *N. gonorrhoeae* challenge strain is susceptible to all clinically-relevant anti-gonococcal antimicrobials (i.e ceftriaxone, azithromycin, ciprofloxacin and tetracycline).

Test of cure and additional antimicrobial therapy

Test of cure to confirm *N. gonorrhoeae* eradication will be performed for all participants who develop *N. gonorrhoeae* infection during the study. Test of cure will be undertaken by performing a *N. gonorrhoeae* culture and NAAT on a combined oropharyngeal swab collected from the posterior oropharynx, palatine tonsis and saliva on day 7 post antimicrobial therapy and confirmed by performing a *N. gonorrhoeae* culture and NAAT on combined oropharyngeal swab on day 14 post antimicrobial therapy. It is expected that *N. gonorrhoeae* culture will be negative at day 7 post antimicrobial therapy and *N. gonorrhoeae* NAAT will be negative at day 14 post antimicrobial therapy. If *N. gonorrhoeae* is not detected at these timepoints, *N. gonorrhoeae* challenge strain eradication has been confirmed. If *N. gonorrhoeae* is detected at these timepoints, the following additional testing will be performed to confirm persistent oropharyngeal carriage prior to the administration of additional antimicrobial therapy.

If *N. gonorrhoeae* is identified by culture of a combined oropharyngeal swab on day 7 after antimicrobial therapy, phenotypic antimicrobial susceptibility testing and whole genome sequencing will be performed to assess the cause of potential treatment failure (i.e., *in vivo* development of resistance to the challenge strain or infection with an alternative *N. gonorrhoeae* strain), and repeat *N. gonorrhoeae* culture and NAAT will be performed on a combined swab collected on day 14 days after antimicrobial therapy to confirm persistent infection. If *N. gonorrhoeae* is identified by culture at this time, individuals will be managed as a suspected case of persistent oropharyngeal *N. gonorrhoeae* infection and further antimicrobial therapy will be provided as below.

It is expected that *N. gonorrhoeae* will not be detected on a combined oropharyngeal swab via NAAT for the majority of participants at day 14. However, as *N. gonorrhoeae* DNA

Table 7. Indications for antimicrobial therapy for participants during the oropharyngeal gonorrhoea CHIM.

Indications for Antimicrobial Therapy	Criteria	Description
Study Participants		
Clinical symptoms/signs due to *N. gonorrhoeae* pharyngitis	As soon as practicable and within 24 hours of onset *of N. gonorrhoeae* pharyngitis	*N. gonorrhoeae* pharyngitis defined as: i) sore throat, examination score ≥2 (Figure 2) and microbiologically confirmed *N. gonorrhoeae* infection OR ii) examination score ≥3 (Figure 2) and microbiologically confirmed *N. gonorrhoeae* infection
End of experimental infection phase	At day 5 for all study participants, regardless of *N. gonorrhoeae* infection status, unless treated earlier due to symptomatic *N. gonorrhoeae* infection or participant request	Treatment provided at end of 5-day experimental infection phase, regardless of *N. gonorrhoeae* infection status
Participant request	As soon as practicable and within 24 hours of request by the participant, regardless of symptoms or signs	Treatment provided upon participant request at any time

can be detected by NAAT in up to 8% of cases 14 days after treatment[64], if *N. gonorrhoeae* is detected by NAAT at this timepoint, repeat sampling will be performed on day 21 after treatment, when detection of persistent *N. gonorrhoeae* by NAAT after successful treatment is less likely[65]. Phenotypic antimicrobial susceptibility testing and whole genome sequencing will be performed on any available culture isolates from the participant to assess the cause of potential treatment failure (e.g. development of AMR), assess if there has been a new infection (e.g. infection with a different strain) and to guide appropriate management. Repeat NAAT and culture will be performed at day 21 after treatment to assist determination of whether persistent *N. gonorrhoeae* detection by NAAT at the previous timepoint represented treatment failure, detection of non-viable DNA or new infection. If *N. gonorrhoeae* is not detected at this timepoint, *N. gonorrhoeae* challenge strain eradication has been confirmed. If *N. gonorrhoeae* remains detectable at this time, the case will be managed as a suspected case of persistent oropharyngeal *N. gonorrhoeae* infection and further antimicrobial treatment will be administered.

In the unlikely event of persistent *N. gonorrhoeae* infection, treatment will be guided by results of antimicrobial susceptibility testing, whole genome sequencing, and clinical judgement in consultation with the SMC. Options for treatment recommended by various international bodies will be considered: i) single-dose intramuscular ceftriaxone 1g in 3.5mL of 1% lignocaine plus one dose of oral azithromycin 2g, ii) daily intravenous ceftriaxone 1g administered for three days; and iii) daily intravenous ertapenem 1g administered for three days[23].

Challenge strain

A novel *N. gonorrhoeae* challenge strain, AUSMDU00053933, collected from a 27 year-old male with symptomatic urethritis attending Melbourne Sexual Health Centre in 2017 was selected for this study. The strain's multi-locus sequence type (MLST) is 1596 and its *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) is 4332. It is susceptible to all clinically-relevant antimicrobials (including ceftriaxone, azithromycin, ciprofloxacin, and tetracycline)[27]. As previously described, a genomics-based selection strategy was used to shortlist this strain from 5,881 clinical isolates of *N. gonorrhoeae* collected from adult patients in Victoria, Australia between January 2017 and June 2021[27]. This strategy utilized clinical, phenotypic and genomic characteristics to shortlist strains of global clinical relevance that met stringent safety criteria to reduce the risk of disseminated gonococcal infection and clinically significant AMR[27]. The results of *in vitro* assays and cell bank manufacture pilot studies, which will be published separately, indicated this strain is a suitable challenge agent for an initial oropharyngeal gonorrhoea CHIM, given its characteristics (attachment to epithelial cell lines, minimal epithelial cell invasion, absence of induction of a cytotoxic or inflammatory response, susceptibility to killing by normal human serum, and stability in cell bank manufacture).

Dose selection

The dose required to cause oropharyngeal infection in 60 to 80% (ID_{60}-ID_{80}) of participants with AUSMDU00053933 is unknown. However, the estimated dose resulting in infection in 50% of participants (ID_{50}) in the gonorrhoea urethritis model using alternative challenge strains has been calculated as 1.8 x 10^3 colony forming units (CFU) for *N. gonorrhoeae* MS11mkC and 1.0 x 10^5 CFU for *N. gonorrhoeae* FA1090[61]. As this study is aiming to define the ID_{60} to ID_{80}, dosing will commence at approximately 10^4 CFU, which approximates the estimated ID_{60} to ID_{80} of MS11mkC. This starting dose also approximates the predicted ID_{70} based on continual reassessment method (CRM) dose prediction simulations (CURE-NG Continual Reassessment Model Design (Extended Data)). This dose is approximately 10-fold lower than the median gonococcal bacterial DNA load detected per throat swab and per millilitre of saliva of individuals with oropharyngeal gonorrhoea, respectively[52]; approximately 10-fold lower than the median gonococcal bacterial DNA load detected per urethral swab of individuals with asymptomatic gonococcal urethritis, and approximately 100-fold lower than those with symptomatic gonococcal urethritis[67]. *In vitro* studies suggest that the estimated volume of uptake and concentration of bacteria released from Dacron swabs is approximately 10-fold lower than the concentration of the single-use vial they are dipped in prior to

inoculation[68]. As such, to obtain a starting dose of approximately 10^4 CFU, the swab will be inoculated in a single-use vial containing a concentration of $10^5 \pm 0.5$ $\log_{10}$/mL Due to the uncertainties involved in calculating the actual delivered dose, this study will refer to the dose level as the concentration of the dose vial rather than the estimated dose delivered (i.e., starting dose, $10^5 \pm 0.5$ $\log_{10}$ CFU/ml). This study has been designed based on a dose-escalation algorithm using a CRM as described below. Up to five dose levels are planned for testing if required ($10^4 \pm 0.5$ $\log_{10}$ CFU/mL to $10^8 \pm 0.5$ $\log_{10}$ CFU/mL).

Continual Reassessment Model study design

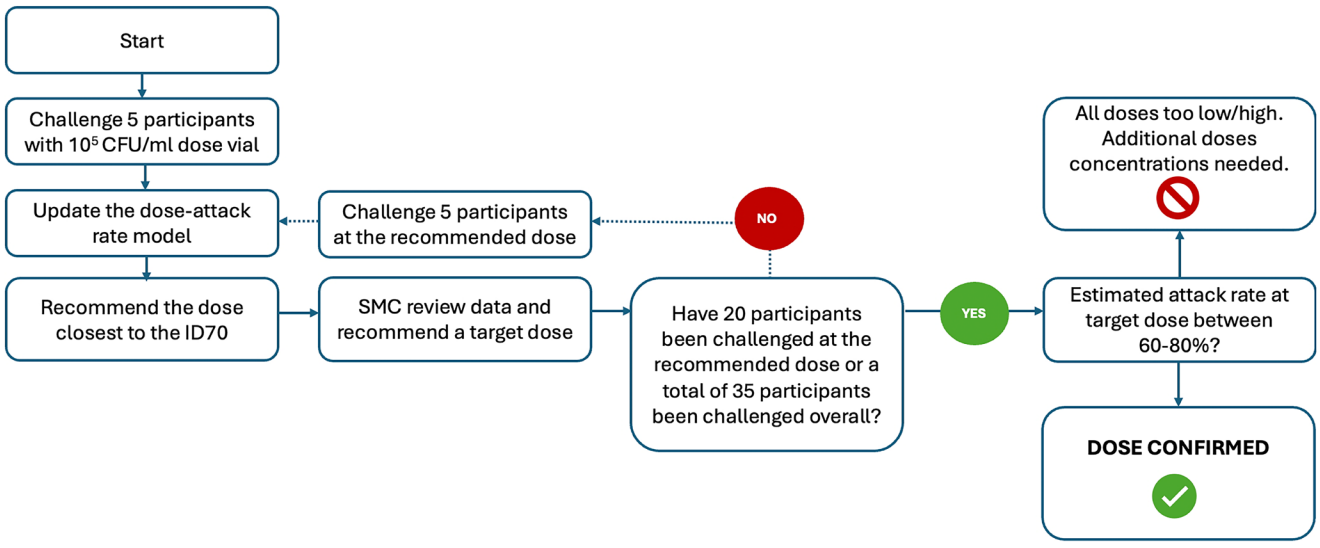
A CRM will be used to identify the AUSMDU00053933 dose that can establish oropharyngeal *N. gonorrhoeae* infection in 60–80% of participants (Figure 3). Initially implemented in Phase I trials to identify the maximum tolerated dose of a new drug or treatment, this method has recently been employed in the design of dose-finding CHIMs[39]. CRMs have been shown to more accurately identify the true maximum tolerated dose of a new therapy compared to traditional rule-based designs in Phase I trials[69].

The CRM study design was evaluated using a two-parameter dose-response model, within a Bayesian framework (described further in *Statistical Analysis*). Starting dose was specified as 10^4 CFU (dose vial $10^5 \pm 0.5$ $\log_{10}$ CFU/ml), and five individuals were allocated per group. Individual trials were simulated according to the following CRM rules:

1. Inoculate group at current dose.
2. Dose-response model is fit to observed data (i.e., number of infected individuals at current dose).
   - If the model-based estimate of the ID_{70} is ≥ 2 $\log_{10}$ CFU above the current dose, escalate two $\log_{10}$ doses.

   Otherwise,
   - if <75% chance that posterior probability of infection at that dose >0.6, then escalate one $\log_{10}$ dose.
   - if >95% chance that posterior probability of infection at that dose >0.8, then de-escalate one $\log_{10}$ dose.
   - if >75% chance that posterior probability of infection at that dose >0.6, and <95% chance that posterior probability of infection at that dose > 0.8, maintain current dose.
3. Repeat steps 2–3 until 20 individuals have been inoculated at the target dose or 35 individuals have been inoculated in total.

Given the sparse information on the dose-response properties of AUSMDU00053933, prior distributions on model parameters were specified such that there was approximately uniform probability of the ID_{70} falling between 10^2 and 10^7 ((CURE-NG Continual Reassessment Model Design (Extended Data); Figure S1). This range was informed based on existing CHIMs for similar pathogens (Table 1). The starting dose vial of AUSMDU00053933 used in this study will be $10^5 \pm 0.5$ $\log_{10}$ CFU/ml, and may increase to a maximum of $10^8 \pm 0.5$ $\log_{10}$ CFU/ml and de-escalate to a dose of $10^4 \pm 0.5$ $\log_{10}$ CFU/ml according to the CRM. The CRM



The first cohort of 5 participants will be divided into 3 groups (1, 2, 2 participants) and challenged within an interval of at least 7 days with safety review after each group. Subsequent participants will be challenged 5 participants simultaneously subject to SMC approval.

Figure 3. Overall study design and dose escalation/de-escalation procedures for the oropharyngeal gonorrhoea controlled human infection model. CFU, colony forming units; ID70, infectious dose 70%; ml, millilitre; SMC, Safety Monitoring Committee.

dictates that the model is updated after each cohort of five participants completes the experimental infection phase, and subsequent dose allocation is informed by the model-based estimate. The dose closest to the target attack rate of 70% (ID_{70}) will be identified using the CRM and presented to the SMC, who will recommend the dose for the subsequent cohort to the Trial Steering Committee (TSC) for approval. The next cohort of five participants will be challenged with the recommended dose until 20 participants have been inoculated at the dose predicted to achieve the target attack rate of oropharyngeal *N. gonorrhoeae* infection in 60–80% of participants or 35 participants have been inoculated in total (Figure 3). Once the dose closest to the target attack rate of 70% (ID_{70}) is identified using the CRM, results will be presented to the SMC, who will make a recommendation to the TSC for approval to close the study.

The operating characteristics of the CRM for this oropharyngeal *N. gonorrhoeae* CHIM are detailed in the extended data (CURE-NG Continual Reassessment Model Design (Extended Data); Table S1-S2 and Figures S1-S5). In brief, 1000 prior simulations indicated that an average of 27.8 participants will be expected to be required to achieve stopping criteria, and 96.7% of simulated trials will have 20 participants inoculated at the target dose. The 90% credible intervals associated with the estimated ID_{70} for these simulated trials had an average width of 1.74 $\log_{10}$ CFU. Individual scenarios were evaluated where the underlying ID_{70} sits at the extremes, ranging from approximately $10^{2.5}$ – $10^{6.8}$ CFU (100 simulations at each) (CURE-NG Continual Reassessment Model Design (Extended Data); Figures S1, S2). As expected, given the starting dose of 10^4 CFU (dose vial 10^5 ± 0.5 $\log_{10}$ CFU/ml), the average sample sizes were higher where the ID_{70} was expected to be higher, ranging from 23.35 for $10^{3.27}$ CFU to 30.15 for $10^{6.8}$ CFU (CURE-NG Continual Reassessment Model Design (Extended Data); Table S1; Figures S3-5). Similarly, the average width of the credible intervals of the ID_{70} estimate were wider where the underlying ID_{70} is expected to be higher, due to the shape of the dose-response curve (CURE-NG Continual Reassessment Model Design (Extended Data); Table S2) .

Statistical analysis

After each group of 5 participants has completed the experimental infection phase at the dose designated by the CRM, the primary endpoint infection data will be analysed. Data analysis will be done in a Bayesian framework using Markov Chain Monte Carlo to characterise the posterior distributions of the model parameters. The dose-response relationship will be represented by a two-parameter, independent-action dose-response model. Specifically, the probability of infection, P_{inf}, at dose D, is given by:

$$pinf(D) = 1 - \left(1 + \frac{D}{b}\right)^{-a}$$

Where b and a are the dose-response model parameters. The number of individuals infected at a given dose then follows a binomial distribution, with number of trials given by the group size and probability of 'success' given by $P_{inf}(D)$. After each group is inoculated and the analysis conducted, results will be reported to the SMC to determine appropriate course of action for subsequent groups, or stopping.

Safety measures

Serious Adverse Events (SAE) will be monitored as per standard definitions throughout the study. Medically Significant Events specific to this study include: i) disseminated gonococcal infection, including (a) purulent arthritis, tenosynovitis-dermatitis-polyarthritis syndrome, (b) bacteraemia, (c) meningitis, (d) osteomyelitis or (e) endocarditis; ii) gonococcal infection at other sites; iii) persistence of *N. gonorrhoeae* infection despite treatment (due to *in vivo* development of AMR or unknown host factor)s; iv) re-infection with the *N. gonorrhoeae* challenge strain or alternative *N. gonorrhoeae* strain during the study period; and v), adverse reactions to antimicrobial therapy, constituents in the inoculation media or inoculation procedure. Potential risks have been carefully considered, with risk mitigation strategies implemented to minimise risks to participants and the broader community, particularly to close contacts and clinical trial staff[17]. Oversight of conduct of the clinical trial and decisions during the study will be undertaken by the Medical Monitor and SMC, comprising medically-qualified personnel and supported by specific subject matter experts in STI care and statistical analysis. The SMC will review all SAEs during the trial.

Regulation, governance, ethics and dissemination

Infectious agents used in CHIM are not considered therapeutic goods requiring regulation by the Therapeutic Goods Administration in Australia. However, this trial has been designed to meet the high clinical and manufacturing standards expected of a contemporary CHIM. The challenge strain used in this study has been thoroughly characterized with dose manufacture processes subject to stringent quality control and release testing criteria. The procedures outlined in this provisional study protocol align with the international consensus criteria for gonorrhoea CHIM studies proposed by international experts at the inaugural Gonococcal Challenge Network Meeting in Oxford in March, 2025, that was funded by the Academy of Medical Sciences. It included consultation with community and independent experts and review by regulatory bodies including the United States Food and Drug Association (FDA) through feedback from a Pre-Investigational New Drug meeting. Publication of this provisional protocol is intended to facilitate transparent scientific peer-review prior to study commencement.

All dose analysis data and SAEs will be reviewed by the SMC, chaired by an independent clinician-scientist with appropriate experience. All dose-escalation and de-escalation recommendations will be made by the SMC. These recommendations will be reviewed by the TSC, who will approve all dose-escalation and de-escalation decisions. A broader Clinical Trial Reference Committee including community representatives, independent public health/sexual health clinicians and a bioethicist will provide advice prior to the trial to optimise acceptability of the study.

The study protocol accords with the standards outlined in the SPIRIT 2025 Statement[70] and the Guideline for Good Clinical Practice[71]. It also addresses the 10 key criteria recommended by the World Health Organization (WHO) for assessment of the ethical appropriateness of a CHIM[25] and proposed reporting standards for CHIMs[72]. This study protocol will be reviewed and approved by an authorised Human Research Ethics Committee (HREC), and be registered on the Australian New Zealand Clinical Trials Register (ANZCTR). Indemnity insurance for this study will be covered by a comprehensive institutional policy. Results of this study will be published in peer-reviewed journals and presented at scientific conferences to ensure dissemination of the findings to the scientific community.

Discussion

The WHO Global Health Sector Strategy on STIs has set an ambitious target of reducing worldwide *N. gonorrhoeae* incidence by 90% by 2030[73]. In the absence of an effective vaccine, it is unlikely this target will be met. This oropharyngeal gonorrhoea CHIM is designed to significantly advance development of prevention and treatment strategies - including vaccines and anatomical site-specific treatment protocols - by providing a platform for early-stage evaluation and selection of promising candidates for future clinical trials. This model will simultaneously generate new insights into early oropharyngeal *N. gonorrhoeae* infection, improving our understanding of pathogenesis.

As a first-in-human CHIM of *N. gonorrhoeae* at the oropharyngeal site, with a novel challenge strain, this study design prioritises safety and acceptability, and will recruit individuals from the MSM population who are at increased risk of *N. gonorrhoeae* infection. Safety has been enhanced through rational challenge strain selection[27], modernization of the dose manufacture process, careful participant selection, and implementation of risk mitigation strategies to minimise adverse outcomes and the risk of transmission to close contacts and the community[17]. The study has been informed by qualitative research and community consultation, which explored key ethical and acceptability concerns raised by both experts and potential participants, and informed study design, advertising materials, recruitment strategies, and governance[26].

This study protocol incorporates the outcomes of several parallel projects with the ambitious aim of modernising *N. gonorrhoeae* CHIM design. These include i) selection and characterization of a contemporary *N. gonorrhoeae* challenge strain, optimized for safety, generalizability and stability in modern cell bank manufacture ; ii) development of a *N. gonorrhoeae* cell bank manufacture protocol to enable dosing at bedside following thawing of a single-dose vial on the day of inoculation, simplifying the pre-inoculation dose preparation process, and enabling formal release testing and dose determination prior to participant inoculation; iii) use of a CRM for dose escalation, de-escalation and statistical analysis, improving the accuracy and efficiency of identifying the target challenge dose in this dose-finding study; and iv), incorporation of meaningful community consultation into study design and recruitment strategies; a key element of optimizing acceptability and cultural safety of a CHIM with a potentially stigmatising sexually-transmitted pathogen. Each element has been intentionally included to ensure alignment with international best practices for safety and scientific rigour in CHIM research.

There are inherent limitations to using gonorrhoea CHIMs for early-stage evaluation of prevention and treatment strategies. *N. gonorrhoeae* is characterized by antigenic variation and phase variability, meaning findings from a single-strain CHIM may not be generalizable to all circulating strains. To address this, we selected a challenge strain with MLST and NG-MAST sequence types representing diverse geographic regions across at least three continents between 2015 and 2021, improving generalisability[27]. This CHIM, which uses a different strain and anatomical site from the only other active gonorrhoea CHIM (the University of North Carolina urethritis model using *N. gonorrhoeae* FA1090), can complement existing models and provide broader data to inform early-phase testing of treatment and prevention strategies before proceeding to larger, more costly field studies.

Another potential limitation relates to the generalisability of the findings arising from the oropharyngeal site to urogenital sites of infection that cause greatest morbidity. However, there are several reasons to believe the oropharynx is a key site to test novel gonorrhoea treatment and prevention strategies. Firstly, it likely plays a significant role in transmission of gonorrhoea in the community[9], including bridging transmission of *N. gonorrhoeae* infection from MSM to women[74], who are at highest risk of long-term morbidity impacts of *N. gonorrhoeae* infection[74]. Secondly, it is a high-risk site for the development of AMR due to prolonged colonization[13] and horizontal transfer of AMR from commensal oropharyngeal microorganisms[12]. Thirdly, for any prevention strategy to have long-term public health impacts, it is critical that is has efficacy at extragenital as well as genital sites. Modelling studies have estimated that a vaccine without efficacy at the oropharynx is unlikely to have a significant impact on overall population rates of *N. gonorrhoeae*, and that prevalence may actually increase if a vaccine prevents symptoms but not infection or transmission[24]. Finally, population groups who bear the highest burden of morbidity from *N. gonorrhoeae* infection have been purposively excluded from this study, including individuals assigned female at birth, individuals who have sex with people assigned female at birth, and PLHIV. The impacts of gonorrhoea on people with a female reproductive system can be profound, and due to current limitations in understanding of *N. gonorrhoeae* transmission (including the role of autoinoculation) and female urogenital gonorrhoea pathogenesis, this group has been excluded from participation in this model for safety and ethical reasons[17]. PLHIV have also been excluded from this study due to increased reports of disseminated gonococcal infection among PLHIV[40,50,51]. Insufficient data are available regarding the differential risk of disseminated gonococcal infection among PLHIV with well-controlled HIV infection to enable risk stratification at this time. As has been planned for the pneumococcal CHIM[76], it is feasible that people with well-controlled HIV could be considered for inclusion in future studies if evidence suggested that such an approach would be safe and likely to yield important results for this population.

This protocol describes a first-in-human, dose-finding oropharyngeal gonorrhoea CHIM using *N. gonorrhoeae* strain AUSMDU00053933. Establishing a safe and reproducible dose required to achieve oropharyngeal infection will support the use of this model in translational research of novel treatment and prevention strategies. It will also generate novel insights into early infection dynamics and host immune responses at the oropharyngeal site. Ultimately, we hope this CHIM may contribute meaningfully to reducing the global health burden of *N. gonorrhoeae*.

Data availability

Figshare: CURE-NG Continual Reassessment Model Design (Extended Data); doi.org/10.26188/30285286 (Price DJ, Williams E, McCarthy JS, 2025).

CURE-NG Participant Questionnaire; doi.org/10.26188/30285433 (Williams E, Hocking JS, McCarthy JS, 2025).

CURE-NG Provisional Protocol SPIRIT 2025 Checklist; doi.org/10.26188/30413080 (Williams E)

This project contains the following underlying data:

- CURE-NG Continual Reassessment Model Design (Extended Data).pdf
- CURE-NG Participant Questionnaire.pdf
- CURE-NG Provisional Protocol SPIRIT 2025 Checklist.pdf

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Open Peer Review

Current Peer Review Status: ? ✓ ?

Version 1

Reviewer Report 12 February 2026

https://doi.org/10.21956/wellcomeopenres.27675.r142325

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Summary of the report: Controlled challenge studies for *N. gonorrhoeae* pharyngeal infection are currently lacking; this protocol fills a much needed gap that will enable further research into how *N. gonorrhoeae* establishes pharyngeal infection and other contexts that the authors identified. The research protocol provided seems mostly thorough and robust, but I have some comments.

Strengths: The authors have gone in a great deal of effort in involving 'lay' persons in the design of the research which should be commended. There also seems to be plans to continue the relationship throughout the duration of the study. Further, the authors seems to have thought about key definitions in detail, for example what they consider 'infection', considering

1. I presume that since this is a small study assessing primarily safety and tolerability, there will be a larger subsequent study. I am interested in whether the authors have considered monitoring drop out rates - especially as this is a 90 day follow up period - and implementing a questionnaire to understand why participants may be dropping out? This data will also be useful in calculating sample sizes in future studies. This is something perhaps that the 'lay' collaborators may be able to lead on.

2. The authors have excluded women (or AFAB) - a patient group often excluded from research - based on the risk of auto inoculation and fertility risks. Is there any evidence that auto inoculation actually occurs from the pharynx to urogenital sites and if so, how often? The authors have proposed stringent infection control measures in MSM that presumable can be applied to any group.

3. I am wondering if there are any plans to perform metagenomic studies or even better isolate any commensal *Neisseria* species from participant pharyngeal swabs pre Ng inoculation? There is some evidence that *N. lactamica* may provide protection against *N. meningitidis* and some in vitro studies showing some commensal *Neisseria* species can kill *N. gonorrhoeae*. Having this data may

be informative for participants who do not become 'infected'. I appreciate that the sample size in this study will be too small to extrapolate anything concrete but may provide a basis for a larger study.

4. Regarding the estimated inoculum, the authors state that the dacron swab deposits 10-fold lower cfu that the initial suspension. They have referenced a study that states this; I am wondering if they have validated this in-house? And can the authors explain why a swab inoculation method was chosen instead of a sprayed inoculum?

5. The participants will be receiving treatment during the study - after 5 days if asymptomatic and before that if symptomatic. Have the authors considered performing molecular typing on the isolate to ensure the strain they are treating is in fact the strain inoculated and not one acquired through oral sex? I am aware that participants will be coached on proper conduct for the duration of the study but they are human after all. Having this proof key, considering one of the outcome measure is calculating colonisation rate (ID_{60} - ID_{80}). Molecular typing should be possible even without a gonococcal isolate.

6. How will the results of this study feed into subsequent larger research studies? I am particularly interested in the collected serum and salivary samples for the immunological response work. Not a lot of information is given on that aspect of the protocol. Can the authors specify the exact non-microbiological testing that will be performed on these samples?

Stats: I am not a stats expert so will not be able to comment on the statistical testing proposed.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a clinical academic with expertise in microbiological diagnostics/antimicrobial susceptibility testing and have developed a niche research interest in pharyngeal gonorrhoea and HGT with commensal Neisseria species.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 21 January 2026

https://doi.org/10.21956/wellcomeopenres.27675.r142984

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The global threat of gonorrhea and its antimicrobial resistance continues to pose significant public health challenges, and currently, no effective vaccine exists. This study describes a protocol for an oropharyngeal gonorrhea controlled human infection model, designed with a strong focus on maximizing participant safety. The primary aim is to develop this model as a platform to accelerate the development of prevention and treatment strategies. The study design incorporates critical considerations for safety and tolerability, along with the determination of the minimum infectious dose of the isolate. The protocol also includes early evaluation of clinical, immunological, microbiological, and pharmacometric responses, which are pivotal for advancing future prevention and treatment strategies, including vaccine development and treatments targeting specific anatomical sites. The data generated from this study could serve as a valuable foundation for future clinical trials and offer a promising candidate model. Furthermore, this model provides new insights into oropharyngeal gonococcal infections, enhancing our understanding of the pathogenesis of the disease. However, there are some limitations to consider. The recruitment of participants has been limited to men who have sex with men (MSM). While the authors have provided reasons for this choice, the broader applicability of the model should be carefully considered, especially in the context of different populations. Additionally, the relatively small sample size of recruited participants could be a limitation. If feasible, increasing the sample size in future studies would help validate the model's robustness and generalizability.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious diseases and antimicrobial resistance in Neisseria gonorrhoeae.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 08 January 2026

https://doi.org/10.21956/wellcomeopenres.27675.r142982

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Brief introductory note
Firstly, I thank Wellcome Open Research for their invitation to peer review this study protocol. To be entirely transparent from the beginning, I am not medically trained, nor do I have expertise in the legal/regulatory space regarding human infection challenge studies. I have reviewed this *Neisseria gonorrhoeae* (*N. gonorrhoeae*) oropharyngeal human infection study protocol mainly based upon its scientific, epidemiological, and public health qualities. It is reassuring to see that a plethora of parties of interest have been involved in this study protocol design: community-based organisation representatives; subject matter experts; public health physicians; industry representatives; and individuals who would be eligible to participate in such a study. I defer the peer review of matters concerning bedside management, clinical decision-making, bioethics, community acceptability, and any other areas outside of my scientific expertise, to those who are authoritative sources of valid peer review in these areas.

Overview
N. gonorrhoeae is a bacterial causative agent of gonorrhoea, a sexually transmissible infection (STI) which, in higher-income nations, disproportionately infects gay, bi, and other men who have sex with men (GBMSM).[1] The authors describe their stipulated study protocol for an oropharyngeal controlled human infection study using a genomically-informed and epidemiologically-relevant *N. gonorrhoeae* strain. The rationale for, and objectives of, the study have been clearly described by the authors. The overall study design is generally appropriate, alongside the detailed descriptions of methodologies for reproducibility purposes. I have some comments and suggestions, which I have outlined below, regarding the study design and reproducibility of methods.

Review of the inoculation methodology study design
The authors state that the starting dose is '$10^5 \pm 0.5\ log_{10}\ CFU/ml$', and that this is referring to the concentration of the dose in the vial rather than the estimated dose delivered. While the authors have cited that Dacron swabs typically release a 10-fold lower concentration of bacteria than the dose in the starting vial (therefore, here, the dose given in this study would be typically around 10^4 CFU/ml), there are steps that could be employed within the protocol itself to make the actual dose inoculated more likely to be consistent.
The authors note that '*the vial will be prepared by performing gentle inversion ten times to mix the inoculum and the sterile swab will be dipped in the vial for ten seconds*' and that '*...the operator will apply the inoculum by rolling the swab back and forth over the tonsillar arches and posterior*

oropharynx.' To make this application procedure more reproducibly robust, the authors could consider incorporating rigid operator training regarding many factors: the thawing procedure and thawing time-keeping of the vial; a robust methodology for the homogenisation of the contents of the vial (though ten gentle inversions may be sufficient if there is no excessive clumping or settling present); pressure of the swab on the relevant anatomical areas; the number of passes with the swab; what constitutes as a '*pass*'; the surface area needed to be touched by the swab; the time of contact between the swab and the participant; the exact anatomical placement of the swab, and what exactly constitutes as adequately '*rolling the swab back and forth*'; and finally perhaps also highlighting the need to record these swabbing event details for each participant.
If feasible, a record of the mass of the Dacron swab before and after dipping into the vial could be taken to investigate variability among operators (if there are indeed multiple operators performing the inoculations) and/or variability among batches of swabs/vials following uptake of liquid into/onto the swab.
I believe it to be fundamentally essential for this study to have a standardised inoculation checklist, a comprehensive training and competency plan for the operator(s) inoculating the participants, and a clear outline of the level of detail required to be recorded by the operator(s) of events before, during, and following the inoculation process (those being the numbers that could be collected at each stage outlined above, such as the number of passes with the swab, contact time, etc.) and any instances of the gag reflex. The authors have indicated that an '*exaggerated*' gag reflex is an exclusionary criterion, however all gag reflexes (and indeed even coughs and sneezes) – even those not considered to be '*exaggerated*' – should at least be recorded, descriptively at a minimum, as this may impact the deposition of the *N. gonorrhoeae* in the anatomical region and perhaps account for variability in the minimum inoculum dose of *N. gonorrhoeae* among the participants.
One cofounding factor in the minimum inoculation dose of the *N. gonorrhoeae* in the participants may be variability in the amount of saliva produced among the participants, and this could be systematically investigated (via a salivary flow rate test) at the inoculation stage and be pre-emptively recorded for ease of interpretation in the future if unexplainable variations do indeed occur.
The authors may also wish to also consider, following the development of a robust swab-liquid uptake and inoculation protocol, quantitatively deducing the likely dose given to each participant by following the protocol at the bench and inoculating onto agar to then determine the colony forming unit (CFU) count and evaluate the reproducibility of the liquid-uptake and inoculation techniques employed among swab/vial batches and technique of the operator(s).

Review of the eligibility and exclusion criteria study design
The authors note that as part of their eligibility criteria, '*drug/alcohol misuse*' is listed for exclusion, specifically '*...illicit drug use, or any prior intravenous usage of an illicit substance*', as per Table 3 in the study protocol. Also, that during the study '*...participants must not use illicit drugs or alcohol*' (Table 4). I think that it may be worth the authors clearly outlining what are considered 'illicit' drugs/substances for the purposes of this study protocol, given the behaviourally disinhibiting effects of some substances that may not be technically 'illicit' (if one interprets 'illicit' to simply mean 'illegal') in Melbourne, Australia. For example, the 'chemsex' party-drug group of alkyl nitrites (colloquially known as 'poppers') which are typically legally sold as commercial products such as leather cleaners, Video Home System (VHS) head cleaners, solvents, air fresheners, among many others.
While specific sexual activities with others are clearly prohibited while being a participant of this study (Table 4), alkyl nitrites are not solely used for their analgesic vasodilatory muscle-relaxing

properties during 'chemsex', and may also be taken in social gathering settings simply for their euphoric and 'warming' effects, which could inadvertently lead to disinhibition of sexual behaviours and therefore potential non-compliance with the study participation guidelines. In addition, as alkyl nitrites are taken via inhalation through the nose and/or mouth (leading to the vapours being exposed to the oropharyngeal region and nasopharynx), this could have unintended consequences for the localised microbiota and any *N. gonorrhoeae* inoculated in the region.

Indeed, it may be that historical use of poppers should also be considered for eligibility purposes due to the caustic and irritating nature of the substance(s) which may have caused persistent anatomical changes in the oro/nasopharyngeal region (lesions, burns, irritation, even potentially a changed microbiota) which could confound the results of this human infection challenge study. I note that the authors are employing urine-based drug-screening in their study, which would typically be unable to identify the presence of alkyl nitrites due to their rapid metabolisation and excretion through urine very soon after inhalation.

I note that the authors have stated that exclusion criteria also include '*history of any clinically important cardiac, endocrinologic, haematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, renal or other major disease, as determined by the Investigator*'. It may be worth the authors explicitly outlining, at this early stage, a comprehensive list of specific associated conditions which would undoubtedly come under these exclusionary criteria due to their anatomical and immunological relevance. The authors have, understandably, explicitly excluded those with a history of tonsillectomy and adenoidectomy, though the authors may also wish to consider those who have had uvulopalatopharyngoplasty (UPPP), uvulectomy, palatoplasty, among many other procedures, particularly as participants may not be immediately aware of having had these surgical procedures if they were undertaken at a young age and may not be readily available in their medical history. A record of anatomical variations in the participants, resulting in the need to deviate from the prescribed inoculation protocol by the operator(s), should be recorded.

The authors have stated that '*use of vitamin supplements taken at standard doses is allowed*', in the consideration of exclusionary criteria (Table 3). The authors may wish to reconsider their allowance of the use of vitamin supplements (and also mineral supplements) during the study period, even at '*standard*' dosage (and if the authors elect to continue to allow supplement use, '*standard doses*' should be explicitly defined, and a record taken from each participant for which supplements, at what dose(s), and whether or not the participant has been determined to be deficient in the associated supplement previously). The reasoning for this is that some vitamin/mineral supplements may impact the gastrointestinal tract microbiome (including the oropharyngeal cavity), particularly in the case of iron supplementation when taken with no known deficiency.[2]

Also, *N. gonorrhoeae* requires iron to proliferate (as do most bacteria) which may inadvertently impact the minimum inoculum dose of the challenge study whereby those who take iron supplements may be inadvertently more susceptible to colonisation by *N. gonorrhoeae*, or have a changed salivary microbiome which may influence the results of this study. Likewise, iron deficiency may also impact the oral microbiome which could inadvertently affect the results of the study.

Another exclusionary criterion stipulated by the authors is being a '*current active smoker defined as having smoked a cigarette or cigar in the four weeks prior to challenge*'. The authors may wish to consider also explicitly extending this to include active 'vapers' (e-cigarette users) who have 'vaped' in the four weeks prior to the challenge. It has been previously established that e-cigarette use alters the oral/salivary microbiome.[3]

The authors may wish to edit '*participants must not engage in sexual activity including contact with the mouth (including deep kissing and saliva use in sexual activity), or contact with the penis, urine, semen or anorectal region with any other individual*' in Table 4 to also include contact with the vagina. While the participant requirement is indeed '*male volunteers who exclusively have sex with people assigned male (assigned at birth)*', there may be participants who have sexual partner(s) that were assigned male at birth who now have a vagina via genital surgery. It may also be prudent to expand the guideline to be '*must not engage in any and all sexualised contact and activities, including but not limited to…*', as the participants may theoretically (though unlikely) be able to transmit their inoculated *N. gonorrhoeae* to sexual partner(s) through the handling of sex toys (not necessarily '*sharing*' the use of, which the authors have already prohibited in the participant guidelines in Table 4), as the risk of even handling sex toys to use on their partner may not be immediately apparent to the participant(s) to be contrary to the participant guidelines (due to residual self-derived saliva/semen/rectal mucus left on their hands, etc.). The handling/sharing of non-sexual fomites (towels, etc., beyond just cutlery and drinking vessels) may also be worth indicating as contrary to the guidelines. There are also other sexualised activities involving bodily fluids beyond saliva/semen/urine, such as faecal ('scat')-play, which may not be immediately apparent to be contrary to the participant guidelines, but could theoretically facilitate onward transmission of the *N. gonorrhoeae* from the oral cavity if endogenous transmission within the host occurs.

Advising participants regarding the risk of endogenous infection spread from their orally inoculated *N. gonorrhoeae* to their own penile and anorectal region may also be prudent. Such an event may occur if a participant uses their saliva as lubricant during masturbation, oral-anal sex toy use, auto-anal-digital contact (self-'fingering'/'fisting'), autofellatio (self-oral-penile masturbation), self-use of urethral sounding rods, among other activities, as Table 4 currently only seems to guide against sexual activities '*with any other individual*' and not necessarily oneself.

Review of the challenge strain antimicrobial treatment study design

The authors note that in the scenario of treatment failure of the challenge strain with ceftriaxone (less likely, due to the initial genomic content of the strain used indicating susceptibility to a wide range of antimicrobials, confirmed via antimicrobial susceptibility testing), the *N. gonorrhoeae* cultured from the participant will undergo whole genome sequencing (WGS) and phenotypic antimicrobial susceptibility testing '*to assess the cause of potential treatment failure*'. The authors may wish to require all participants to keep a record of consumption of food, drink, vitamin and mineral supplements (if the authors elect to continue to allow their use), and any non-antibiotic medications (NAMs) that they may have taken during the study period. There is an increasing awareness among the scientific community that microbial exposure to NAMs may indeed facilitate an environment of selective pressure for the evolution of antimicrobial resistance, via multiple molecular mechanisms previously outlined elsewhere,[4,5] and also facilitate changes in the abundance and diversity of microbes within the gastrointestinal tract microbiome.

As the authors have elected to have '*sporadic use of non-steroidal anti-inflammatory drugs*' (e.g. ibuprofen, aspirin, etc.) not be an exclusionary criterion for participating in the study, it is important that the participants record their use of NAMs – particularly if needed in the near future to assess potential causes of treatment failure and/or variability in the colonisation and minimum inoculum doses of *N. gonorrhoeae* among the participants. The authors may wish to consider excluding those who have used NAMs during the study, due to this phenomenon of NAM-mediated selection pressure for the evolution of antimicrobial resistance in exposed microbes,

and disruption of the microbiome, which is currently being extensively investigated by the scientific community.

I also express tentative concern regarding the decision to treat '*all participants*' with ceftriaxone, particularly in cases where *N. gonorrhoeae* infection (true colonisation) is not confirmed to have been established in the participant. While understandable from one viewpoint of participant safety (where preventing unnoticed colonising and proliferation of *N. gonorrhoeae* during the initial study period is concerned), treatment with ceftriaxone is not without its risks – also including in regards to the gut microbiome of the participant,[6] considering the impact on the diversity and abundance of specific microorganisms but also the potential impact on the resistome in the participant. Also, as the participants are being followed up for three months following their initial participation, there may be abundant opportunity to identify true microbiologically-confirmed *N. gonorrhoeae* colonisation and/or delayed symptomatic infection events, and hence appropriate treatment with ceftriaxone, beyond the maximum 'wait' period of only five days in the initial participation protocol.

The authors should also make the participants aware of specific NAMs that may be associated with poor clinical outcomes when taken in combination with ceftriaxone, the most relevant to immediately come to mind being proton pump inhibitors (PPI),[7] though there may be others. I defer the comprehensive review of this to clinically trained colleagues.

While the authors have stated that STI screening will be undertaken as part of the pre-screening for eligible participants, it is not clear whether carriage of any/all sexually transmissible pathogen(s) would also exclude an individual from participating in the study (beyond carriage of HIV and *N. gonorrhoeae,* which have been clearly outlined by the authors as exclusionary criteria). Sexually transmissible enteric pathogens, such as *Campylobacter* spp., *Shigella* spp., *Salmonella* spp., and certain *Escherichia coli* pathotypes, among many other species,[8] transmitted via the faecal-oral route during sexual activities involving anal-digital and anal-oral contact, are known to disproportionately infect GBMSM via this route – though are not usually routinely tested for with traditional, and even 'enhanced', STI screening. The potential for asymptomatic carriage of sexually transmissible enteric pathogens has also been previously established,[9] alongside patterns of bystander resistance[10] influenced by the changing treatment regimens of other sexually transmissible infections (such as *N. gonorrhoeae*) whereby these enteric pathogens are exposed to antimicrobials during the treatment of other sexually transmissible infections (gonorrhoea, syphilis, etc.) and are becoming increasingly multidrug resistant (MDR) and extensively drug resistant (XDR) themselves likely partly due to this exposure. This phenomenon must also be considered where there have been any previous instances of antimicrobial treatment in participants, particularly for the treatment of sexually transmissible infections that may predate the three-month exclusionary criteria for gonorrhoea in this study.

While the *N. gonorrhoeae* strain used in this challenge study is initially genomically susceptible to a plethora of antimicrobials, the current resistome of the participant must be considered due to the potential for treatment failure of the challenge strain via horizontal gene transfer (HGT) of antimicrobial resistant determinants to the challenge strain from the participant microbiome. I defer, to medically trained colleagues, the determination of the clinical assessment of balancing the risk between treatment/non-treatment with ceftriaxone in participants who do not have seem to have become infected (truly colonised) with the *N. gonorrhoeae* challenge strain – though it would seem essential to inform participants of the potential impact on their gastrointestinal

microbiome (including resistome) and the implications of this for themselves and their current/future sexual partner(s).

The authors may wish to consider including pre-screening the oral microbiomes of potential participants for antimicrobial resistance determinants (genes, plasmids, etc.) through 16S rRNA microbiome sequencing of the saliva and other oropharyngeal regions. This could inform exclusionary criteria based upon the presence of relevant antimicrobial resistance determinants (those likely to cause treatment failure with ceftriaxone) that could likely be transferred to the *N. gonorrhoeae* challenge strain from commensal oral bacteria. This could be followed by oral/faecal/urine 16S rRNA microbiome sequencing subsequent to treatment with ceftriaxone during the study, to evaluate the potential resistome burden induced by its use in this study.

The authors mention that if a participant makes the study staff aware of '*infection control breaches*' during the study period, that the study staff will facilitate contract tracing. The authors should make explicitly clear what events would constitute as an '*infection control breach*' worthy of this response – does this include any and all behaviours contrary to the participant guidelines in Table 4, including the sharing of cutlery/drinking vessels? How would this be communicated in a way that remains anonymous, while sufficiently informing the traced individual of the identified behaviour (since the infection risk between sexual activity and sharing of fomites may not necessarily be equal, and is the traced individual required to be informed of the route of transmission)?

Review of the study design of microbiologically-confirmed *N. gonorrhoeae* being via nucleic acid amplification testing (NAAT)

The authors have stated that the case definition of microbiologically-confirmed *N. gonorrhoeae* for this study protocol, via nucleic acid amplification testing (NAAT), is the '*detection of N. gonorrhoeae by NAAT with two different N. gonorrhoeae genomic targets from a combined oropharyngeal swab collected from the posterior oropharynx, palatine tonsils and saliva.*' This approach requires great caution, and is the major methodological concern with the study protocol as it currently stands, as the detection of *N. gonorrhoeae* DNA in the oropharyngeal cavity >48 hours post-inoculation may not necessarily equate to colonisation of the oropharyngeal region by *N. gonorrhoeae*, and could simply be remnant *N. gonorrhoeae* DNA in the oral cavity following the inoculation procedure – particularly if the inoculum dose remains inconspicuously trapped in the oropharyngeal tonsillar crypts. This is further confounded by the decision to use a combined swab of multiple sites and the saliva of the participant, which could further result in residual *N. gonorrhoeae* DNA – not associated with actual colonisation – to be inadvertently recorded as being microbiological confirmation of infection (particularly as saliva is being included).

I note that in the text, the authors state that '*the case definition… can be established via microbiological criteria alone, or combined clinical and microbiological criteria*.', contrary to Figure 2 in the study protocol which appears to illustrate that both microbiologically-confirmed *N. gonorrhoea* infection and indicative symptoms or examination signs are findings that are needed at the same time in order to define a case occurrence. The authors may wish to consider making this clearer in the protocol.

Due to the tendency of *N. gonorrhoeae* infection in the oropharyngeal cavity to be asymptomatic, it is an understandable approach by the authors to not solely rely on symptoms for confirmation of infection – though it would seem prudent to increase the NAAT-defined infection case definition as

being where two consecutive days of positive-NAAT detection of *N. gonorrhoeae* >48 hours post-inoculation, and/or requiring both microbiological and symptom/examination based indications to be present for a case definition as it seems to be presented in Figure 2 of the study protocol. Though, I defer this to the authors' discretion for how to better approach case definition.

Conclusion

To conclude, this *N. gonorrhoeae* oropharyngeal human infection study protocol has clearly been designed carefully with the involvement of a range of parties of interest and their expertise. I also look forward to reading the forthcoming publication regarding the specifics of the community engagement undertaken in the design of this study protocol, as this is undoubtedly essential for determining the community acceptability of this study. The authors have clearly stated the rationale and objectives of their study, and, with further considerations and clarifications regarding the study design and efforts to improve reproducibility, this study would appear to be highly impactful for the scientific and public health epidemiological understanding of *N. gonorrhoeae* transmission among populations at a higher risk of transmission in a higher-income nation, and the development of community-acceptable interventions in reducing the burden of *N. gonorrhoeae* infection through vaccination and other holistic means.

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Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My areas of expertise are predominantly microbial genomic epidemiology, antimicrobial resistance, microbial bioinformatics, phylogenetic analyses, and general bacteriology. I have worked extensively to investigate the evolution and spread of multidrug resistant (MDR) and extensively drug-resistant (XDR), sexually transmissible, Shigella species. I have experience undertaking patient and public involvement and engagement (PPIE) to evaluate community acceptability of my research, which focusses on sexually transmissible enteric infections among men who have sex with men (MSM).

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.