

FULL TITLE:

Acceptability and feasibility of longitudinal sample collection to better understand the epidemiology of enteric infections in men who have sex with men (MSM): a pilot study

SHORT STUDY TITLE:

Sexually Transmitted Enteric Infections in MSM: STEIM Study v1.0

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KEY WORDS

Men who have sex with men; Public Health; Epidemiology; Enteric pathogens

LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
BBV	Blood-Borne Virus
BEP	Bacterial Enteric Pathogen
CRN	Clinical Research Network
DNA	Deoxyribonucleic Acid
GBRU	Gastrointestinal Bacteria Reference Unit
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
HTA	Human Tissue Act
ISO	International Organisation for Standardisation
LGV	Lymphogranuloma Venereum
LSOA	Lower Layer Super Output
MSM	Men who have sex with men
NIHR HPRU	National Institute for Health Research Health Protection Research Unit
PCR	Polymerase Chain Reaction
PIS	Participant Information Sheet
PPIE	Patient and Public Involvement and Engagement
PrEP	Pre-Exposure Prophylaxis
R&D	Research and Development
REC	Research Ethics Committee
RiiSH	Reducing inequalities in Sexual Health
SHAC	Sexual Health and Contraception Clinic
SHHAPT	Sexual Health and HIV Activity Property Type
SHC	Sexual Health Clinic
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
UKHSA	UK Health Security Agency (formerly Public Health England)
WGS	Whole Genome Sequencing

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1 INTRODUCTION

Within the last two decades, there have been an increasing number of enteric pathogen outbreaks among men who have sex with men (MSM) globally, primarily *Shigella* spp., often associated with antimicrobial resistance (AMR). Recent evidence has suggested that asymptomatic carriage might play an important role in sustaining transmission in specific sexual networks of MSM.

The overall aim of our research is to better understand the epidemiology of enteric pathogens in MSM to inform the targeting, development and delivery of interventions to control transmission and maximise patient and public health benefit. The research is being conducted by researchers through the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in collaboration with University Hospitals Sussex NHS Foundation Trust.

We are planning to conduct a non-interventional longitudinal study to explore the drivers of sustained transmission of, and development of AMR in, enteric infections among MSM. However, such a study will require participants to return faecal samples and/or rectal swabs over a period of several months and we require better understanding about the acceptability and feasibility of this in a sexual health clinic (SHC) population in order to develop a larger study. This protocol is therefore for a pilot study, which aims to assess the feasibility and acceptability of longitudinal sample collection and analysis. The pilot study will provide preliminary findings on the duration of carriage and will compare the use of rectal swabs with faecal samples for enteric pathogen detection and typing.

In this pilot study, we will recruit 200 MSM attending a sexual health and HIV clinic for routine sexually transmitted infection (STI) screening and care. Participants will be asked to complete a baseline questionnaire and to provide an initial faecal sample and rectal swab. Follow-up questionnaires and rectal swabs will be collected at weekly intervals for 3 months. The faecal samples and rectal swabs will be tested for a range of enteric pathogens and a comparison will be made between faecal samples and rectal swabs. Test results will not be returned to participants. The test results and questionnaire responses will be linked to disaggregate patient-level socio-demographic, clinical and behavioural data extracted from the national GUMCAD STI Surveillance System (herein GUMCAD). Qualitative interviews will be held with selected participants to explore the facilitators and barriers to participation and longitudinal sample collection including an exploration of time commitments.

2 BACKGROUND AND RATIONALE

2.1 Enteric infections

Enteric pathogens can cause serious ill-health and disease in the form of gastroenteritis that is characterised by the sudden onset of diarrhoea and/or dysentery, with additional symptoms including abdominal pain, fever and/or vomiting. In severe cases, significant morbidity and mortality may be associated with bloody diarrhoea, severe dehydration, bacteraemia, haemolytic uraemic syndrome and Guillain-Barre Syndrome (1-3). Transmission occurs via the faecal-oral route and, in the UK, is usually caused by the consumption of contaminated food or water, which is often associated with travel to low-income countries with poor food and water hygiene where the incidence of enteric pathogens tends to be high.

2.2 Sexual transmission of enteric infections

Sexual transmission of enteric pathogens occurs through the ingestion of faecal matter during or after sexual activity. Transmission occurs through direct oro-anal practices, indirectly through oral sex after anal sex or via fingers or fomites. MSM are particularly at risk as they may engage in sexual behaviour that increases the likelihood of faecal-oral transmission. Within the last two decades there have been

an increasing number of enteric pathogen outbreaks among MSM globally, including *Shigella* spp. hepatitis A virus, *Campylobacter* spp. Shiga toxin-producing *Escherichia coli* (STEC) and *Entamoeba histolytica*.

Much of our understanding about the epidemiology of enteric infections in MSM is based on analyses of laboratory surveillance data, clinical case reports and information collected during public health follow-up of outbreaks. In England, a national outbreak of domestically-acquired *Shigella flexneri* 3a, which started in 2009, was later shown to be associated with sexual transmission among MSM and was disseminated internationally (4, 5). Since 2010, there have also been increases in cases of *S. flexneri* 2a and *S. sonnei* through separate introductions to the MSM population, including sustained transmission of specific strains that have persisted over long periods of time (>2 years) (6, 7). During 2013 to 2014, a small cluster of Shiga toxin-producing *Escherichia coli* (STEC) O117 was described in MSM associated with sexual transmission, and in 2017, a large international outbreak of hepatitis A was reported across MSM in Europe (including the UK), and parts of North and South America, Australia and Asia (8, 9). The behavioural profile of cases in these outbreaks was similar, including specific reported sexual practices and drug-use behaviours predominantly among MSM living with human immunodeficiency virus (HIV) (10). The increased incidence of enteric pathogens in MSM has also occurred alongside rapid increases in STIs including gonorrhoea, syphilis and lymphogranuloma venereum (LGV), consistent with overlapping sexual networks (11, 12).

2.3 Asymptomatic and sub-clinical infection

There is currently no routine screening or treatment for asymptomatic carriage of enteric pathogens, not least because the clinical implications and onward transmission risk of carriage are not well understood and there is limited evidence that it is beneficial at an individual or population level. However, previous studies have shown that asymptomatic carriage might play a key role in sustaining transmission of these pathogens among specific sexual networks of MSM. These studies have used residual rectal swabs collected from MSM attending SHCs. In 2012, a UK-based feasibility study found that among 444 MSM diagnosed with rectal chlamydial infection at selected SHCs, 8.6% (95% confidence interval: 6.3% to 11.6%) had a bacterial enteric pathogen (BEP) detected (13). About half of the specimens that had a pathogen detected were from cases that did not report symptoms. We subsequently conducted a cross-sectional study at the UK's largest SHC to explore the prevalence and risk factors of BEPs in MSM. Rectal swabs were collected from all MSM attending the SHC for routine STI testing and anonymously screened for a range of BEPs without return of results (n=2,116) (14). The results obtained from BEP detection were linked to clinical, socio-demographic and behavioural data extracted from the clinic database and from GUMCAD. The study found that 1 in 10 MSM attending the clinic had a BEP detected, and most did not have symptoms of gastroenteritis or diarrhoeal illness. BEP detection was associated with a suite of higher-risk sexual behaviours providing strong evidence of sexual transmission.

2.4 Antimicrobial resistance

In addition to the increasing number of outbreaks reported in MSM, the emergence and spread of resistance to first or second-line antimicrobials, including fluoroquinolones and macrolides, in some BEPs is also of concern (15, 16). Whole genome sequencing (WGS) of clinical isolates of *S. flexneri* 3a from the UK, contextualised using samples from a global dataset, identified a new lineage which had spread intercontinentally via sexual transmission in MSM (15). The pathogen had acquired resistance to first- or second-line antimicrobials, including the macrolide antimicrobial azithromycin. The same genetic determinants of azithromycin resistance were subsequently detected in different *S. flexneri* serotypes and *S. sonnei* circulating in MSM across Europe, North America, Asia and Australia. Shigellosis is often managed with supportive care but, where clinically indicated, the recommended antimicrobial is ciprofloxacin (a fluoroquinolone). It is hypothesised that resistance to azithromycin in MSM-associated *Shigella* spp. is linked to selection pressure from high levels of antimicrobial use for bacterial STIs (15).

Our cross-sectional study conducted at a large SHC (see section 2.3 above) explored the prevalence of *mphA*, a genotypic marker of azithromycin resistance that has previously been detected within MSM-associated strains of *Shigella* spp. Among MSM with a BEP detected, *mphA* was strongly associated with a bacterial STI diagnosis in the past year, which might indicate that previous antimicrobial exposure acts as a selective pressure on gut organisms and results in the accumulation of AMR determinants (14).

2.5 Research in context

Our previous work provided robust estimates of BEP prevalence among MSM attending a SHC in England and contributed towards the wider understanding of BEP spread and persistence in MSM. However, the use of routinely collected data in this study meant that it was not possible to collect specific information about gastrointestinal symptoms, recent antimicrobial treatment, recent travel history or more specific information about sexual practices that may facilitate faecal oral transmission, such as oral-anal contact (rimming) or recent chemsex drug-use, all of which could have helped interpretation.

In addition, there is currently limited understanding about the duration of carriage or infectiousness, particularly among asymptomatic carriers, and how this might contribute to sustained transmission of BEPs in sexual networks of MSM. An analysis of *Shigella* spp. isolates from symptomatic adult men from the general population reported a median time interval of 23.5 days (range 6–176 days; interquartile range 8–70) between isolates recovered from the same individual and showing persistent carriage (17), but there are no comparable data for asymptomatic individuals or in the MSM population or among those living with and without HIV infection.

Finally, given the importance of controlling AMR, there is a need to better understand the mechanisms through which BEPs acquire genetic elements that encode AMR determinants, and how this relates to prior STI infection, antimicrobial exposure and patient characteristics. It is hypothesised that the gut microbiota could play a role in the development and severity of gastrointestinal disease and the transmission of AMR genes.

From a public health perspective, data on the above knowledge gaps will deepen our understanding of the epidemiology of, and inform the design, development, evaluation and potential delivery of appropriately tailored interventions that seek to control, enteric pathogens, including guidelines on BEP screening in MSM and appropriate management of asymptomatic carriage.

3 OVERALL AIM AND OBJECTIVES

The overall aim of this research is to better understand the epidemiology of enteric pathogens in MSM to inform the design, development, evaluation, and potential delivery of appropriately targeted clinical and public health interventions that seek to control enteric pathogens. We aim to improve our understanding of factors that might be sustaining transmission of, and the development of AMR in, enteric pathogens in sexual networks of MSM through the collection of information on symptoms and longitudinal sampling.

The principal objectives for the study are to:

- Investigate the duration (mean, median, range) that enteric pathogens can be detected in the gut, and how this varies by symptom status, type or duration of reported symptoms, bacterial species, patient characteristics, sexual behaviour and HIV status

- To determine whether genetic determinants of AMR differ by patient characteristics, sexual behaviour, HIV status and recent antimicrobial usage
- To determine whether the gut microbiome and resistome differs among MSM and how this correlates with enteric pathogen infection, disease severity and, behavioural and clinical history (pending additional funding)

The study will require participants to return faecal samples and/or rectal swabs over a period of months. However, we require better understanding about the acceptability and feasibility of this approach. In addition, preliminary data on the prevalence of enteric pathogens in the SHC population will help to inform sample size calculations and the overall design of a larger study at multiple SHCs. Therefore, the study will be conducted in two phases; this protocol covers the pilot phase (phase 1) of the study:

- Phase 1: we plan to conduct a longitudinal, observational pilot study. The aim of the pilot is to i) optimise the methodology for conducting a longitudinal study involving repeated collection of biological samples and the collection of epidemiological, behavioural and clinical data and ensure the acceptability to participants and feasibility of our approach, ii) compare rectal swabs with faecal samples for enteric pathogen detection, and iii) provide preliminary data on our scientific objectives
- Phase 2: based on evidence from the pilot phase, we will implement the optimal methodology to conduct a larger, longitudinal study that will address our scientific objectives

4 PHASE 1 PILOT STUDY

4.1 AIM

The aim of phase 1 is to assess the feasibility and acceptability of the study methodology, including the collection of rectal swabs and/or faecal samples and willingness to return rectal swabs at multiple time points throughout the study period. We also aim to compare rectal swabs and faecal samples for the detection of enteric pathogens (including pathogen quantification) to determine whether our scientific objectives can be met using only rectal swabs. Stool specimens are the gold standard specimen for the clinical diagnosis of enteric infections. However, previous studies have shown that rectal swabs provide a practical and cheaper method of detecting such infections for research purposes, and rectal swabs are routinely collected in MSM for STI testing. A like-for-like comparison of rectal swabs and faecal samples will provide information about the sensitivity of rectal swabs for pathogen detection, typing and sequencing compared to the gold standard.

4.2 OBJECTIVES

The phase 1 pilot study will address the following objectives:

- Of those approached about the study, determine the proportion that agree to participate
- Evaluate the characteristics and representativeness of the respondent sample compared to all MSM attending the SHC during the study period
- Determine the proportion of participants who return samples at different time points
- Explore the differences in the characteristics of participants who return samples and those who do not
- Determine the acceptability of different communication types (e.g. text messages reminders)
- Explore the facilitators and barriers to longitudinal sample collection

- Compare rectal swabs with faecal samples for enteric pathogen and AMR detection, and pathogen typing
- Explore the prevalence of enteric pathogens in the SHC population
- Explore the duration of carriage
- Explore the genetic determinants of AMR

4.3 Study design

A longitudinal, observational study design will be used whereby MSM attending the SHC during the study period will be approached for recruitment to the phase 1 pilot study. Individuals will be asked to complete a short questionnaire to obtain clinical and behavioural information and will be asked to provide an initial rectal swab and a faecal sample (both self-collected). Participants will be provided with sample collection kits (including pre-paid envelopes) and can either take the initial samples in the SHC or at home. Participants will be advised on the Participant Information Sheet (PIS) that they can opt not to provide a faecal sample and still participate in the rest of the study. Individuals will also be asked to provide additional follow-up rectal swabs for up to 3 months at weekly intervals. The samples will be tested for a range of enteric pathogens. Consent will be sought to link the questionnaire responses and test results to data routinely collected from GUMCAD. Consent will also be sought from participants to store residual study samples for future ethically approved studies.

4.4 Study setting

The study will take place within Brighton and Sussex. Sexual health and HIV services are provided by the Brighton & Hove Sexual Health and Contraception Service (SHAC), and Sexual Health West Sussex. SHAC includes the Claude Nicol Centre (SHAC East) at The Royal Sussex County Hospital, which provides sexual health services to a large MSM population and has previously reported an outbreak of shigellosis associated with significant morbidity in some patients. There is a separate HIV outpatient's clinic, the Lawson Unit, which offers treatment, support and ongoing care for individuals living with HIV, but it is common for patients receiving treatment for HIV to use the Claude Nicol Centre for other STI-related services. There is a specific SHAC clinic for MSM. Sexual Health West Sussex provides services across Worthing and Chichester.

4.5 Study population

The study will aim to recruit a representative sample of MSM attending the sexual health or HIV services for routine STI screening or care, regardless of symptoms.

4.5.1 Inclusion criteria

- The study will include all men (cis/transgender), trans women or gender-diverse people, aged 16 years or older, who attend the clinics during the study period, report sex with another man (cis/transgender) or non-binary person assigned male at birth in the past three months and have not participated in the study already.

4.5.2 Exclusion criteria

- The study will exclude people who are unable to consent and/or complete the study procedures due to English language or literacy. We have decided not to include these people for the following reasons:
 - The majority of MSM attending the clinics are able to read and write in English. Therefore, we are unlikely to exclude many persons due to this criterion.
 - It is not possible within the time and financial constraints of this study to translate materials into different languages.
 - If some respondents answer survey questions via a translator, it may introduce social desirability bias.

- Participants who cannot use tablets, a computer or a smartphone and the Internet will also be excluded from the study because the data collection will be done using an online questionnaire. Other UK Health Security Agency (UKHSA)-facilitated surveys conducted among persons attending SHCs have used similar data collection methods.
- We will exclude participants who report that they have not had any male sexual partners in the past three months because we wish to explore the association between various recent sexual behaviours and enteric pathogens. We anticipate that the proportion of MSM attending SHCs who have not been sexually active in the past three months will be very low.

4.6 Sample size

No formal sample size calculation will be performed for the phase 1 pilot study. We aim to recruit 200 MSM to the study.

Previous research that involved the recruitment of MSM attending a central London SHC with completion of a questionnaire and the collection of biological samples had a high acceptance rate (81.4%) (18). Recruitment rates for other research studies involving the completion of questionnaires among selected groups of attendees at SHCs have varied. In Positive Voices, the national survey of people living with HIV, 90% of individuals accepted the survey initiation, of which 23% completed it (19). In the Reducing Inequalities in Sexual Health (RiiSH) Survey, 85.3% of participants agreed to participate, of which 73.0% completed the survey (20). However, none of these studies have involved longitudinal sample collection so the proportion of people likely to return samples over time is not known.

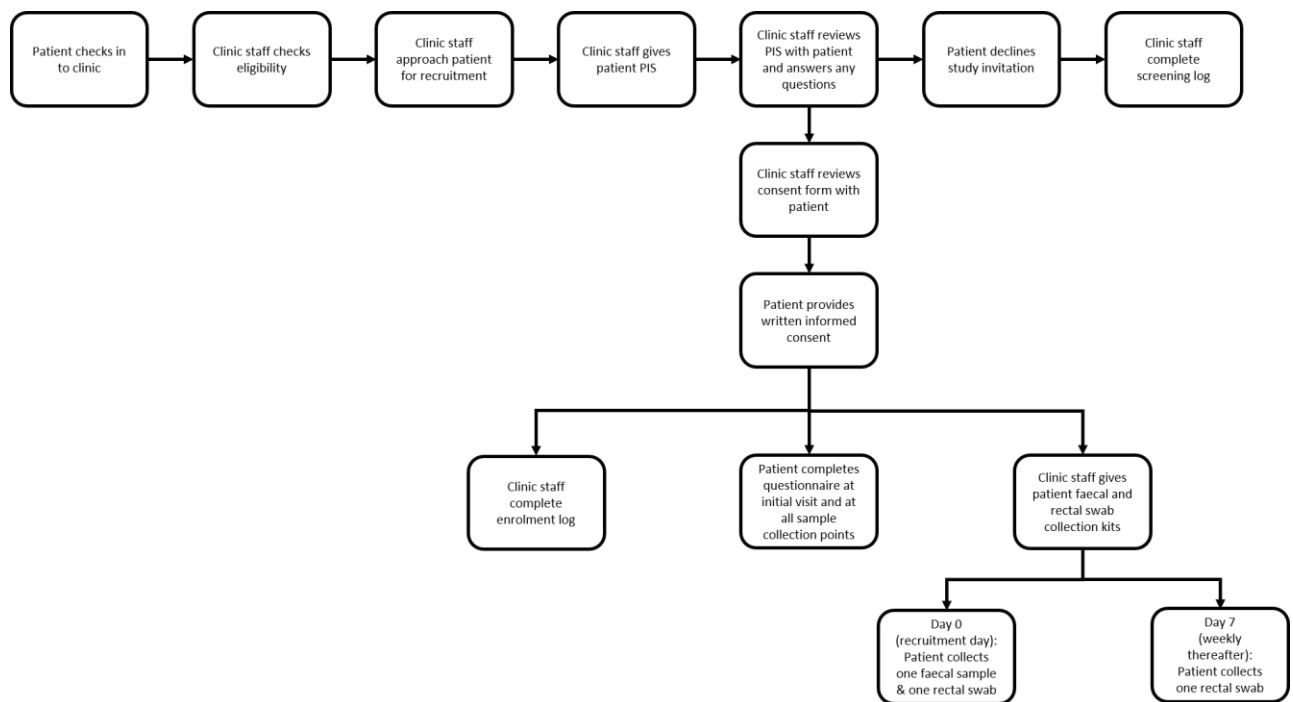
Approximately 100 MSM attend the SHAC east service every week for face-to-face appointments, the majority of whom are asymptomatic MSM accessing HIV pre-exposure prophylaxis (PrEP). An additional 100 asymptomatic MSM living with HIV attend the Lawson Unit for routine care every week. We estimate that 10% of MSM approached about the study will agree to participate. Therefore, we anticipate that it will take approximately 12 weeks to recruit our target sample size of 200 MSM (Dr Daniel Richardson, personal communication). With 10 to 30% of participants returning longitudinal samples, we would obtain follow-up samples for between 20 and 60 participants.

We also require data on the prevalence of enteric pathogens in the study population to understand how many positive samples to expect. Based on previous studies, we might expect approximately 10% (20 MSM) to have an enteric pathogen detected. However, the exact prevalence of enteric pathogens in this SHC population is not known. We will keep recruitment to the pilot study open until we obtain over 20 positive samples (to allow comparison of rectal swabs and faecal samples). Preliminary data on the prevalence of enteric pathogens will help to inform future sample size calculations.

5 STUDY PROCEDURES

5.1 Overview

Figure 1 provides an overview of the study procedures carried out by the clinic and participant. All follow-up procedures will be conducted remotely.



5.2 Recruitment and informed consent

Individuals attending for routine sexual health care who meet the inclusion criteria will be invited to take part in the research study. It is the responsibility of the local clinic principal investigator to nominate the clinic staff members who are appropriately trained and will be responsible for recruitment. The research study team will discuss the best way of implementing the study with the local clinic staff to ensure that eligible persons attending the clinic are approached to participate and to avoid persons being approached twice. We will apply for Clinical Research Network (CRN) support. All research/clinic staff involved in recruitment will be trained in the protocol and will have completed Good Clinical Practice (GCP) training.

Recruitment and informed consent will take place during the clinic visit or remotely (online). For those recruited during their clinic visit, the clinic staff members will discuss the study with the patient, check eligibility and give them the PIS to read. This will outline why the study is being conducted and what will happen if they choose to take part. No study procedure will be undertaken until written informed consent is given by the patient and the inclusion/exclusion criteria verified. If the patient agrees to take part, the clinic staff member will witness provision of written consent during their clinic visit. Interested and eligible participants who have an upcoming clinic appointment can also be recruited online. Prior to their appointment, potential participants will be directed to a study webpage where they can read the PIS and complete an online consent form to join the study. On completion of the online consent form, participants join the study and will collect their sample kits at their clinic visit.

Some of the items on the consent form are a requirement for participations whereas other items are optional. Consent to the following items will be a requirement for the study:

- Participation is voluntary and participants can withdraw at any time. However, any data or samples already collected will be retained in the study.
- Samples provided may be tested for enteric pathogens and antimicrobial resistance markers. Participants will not be given any results from the tests performed on their samples
- Information will be treated as strictly confidential

Consent to the following is optional for the study:

- Linkage of biological samples and questionnaire responses to GUMCAD data
- Contacted with reminders for the submission of longitudinal samples
- Contacted about participating in a 1 to 1 interview
- Long-term storage of samples and data for future ethically approved studies

Participants will be asked to initial the appropriate boxes to indicate that they have read PIS and to confirm that they would like to participate in the study, and whether or not they agree to linkage of their questionnaire responses. One original signed and dated completed consent form will be stored in the Investigator Site File. A copy will be given to the study participant (along with the PIS) and a separate copy will be stored in the patient medical notes. If a participant would like more information about the study, they can contact a member of the research team using the contact details provided on the PIS. Participants will be able to withdraw from the study at any time, but any data or samples already provided will be retained in the study. The research team will be unable to link the data/samples to a unique individual.

Participants who agree to be contacted will be asked for their contact details (email address and/or phone number for text messages). The consent form will note that contact details will only be used for the purposes of the study and will be held securely at the clinic.

A study log (see section 7 below) will be maintained by the clinic to record the details of each person approached and asked to participate in the study, regardless of whether they accept or decline the invite. There will be two parts to the study log: the enrolment log (for participants who agree to participate) and the screening log (for those who decline to participate). Selected information (see section 7.2 below) from the study log will be transferred on a regular basis to the study management team at UKHSA.

5.3 Sample collection

Participants will be provided with bacterial rectal swab and faecal sample collection kits (including pre-paid envelopes). All kits (including those required for longitudinal follow-up) will be provided at the initial visit so participants do not have to return to the clinic. The self-collection of rectal swabs is a standard procedure among MSM attending SHCs for routine STI testing (e.g. gonorrhoea and chlamydia). The collection of rectal swabs is highly acceptable to most MSM, both in the clinic and for home-based STI testing.

The initial rectal swab and faecal sample should be collected during the clinic visit if possible, or as soon as possible after the clinic visit. Participants will be encouraged to collect both samples on the same day. The samples will be returned directly to the laboratory by post using the pre-paid envelopes that fit easily into a standard post box and with appropriate labelling and packaging.

For longitudinal follow-up samples, participants will be asked to collect a rectal swab every week for 3 months. There is no need for the participants to return to the clinic; they will be asked to send the samples directly to the laboratory by post. Participants will receive a weekly email or text message reminder if they agree to this on the consent form. The reminder message will be sent on the day of recruitment, i.e. if recruited at the clinic on a Monday, the reminder will be sent every Monday. Participants can continue to take part in the study even if they do not provide all the requested samples (e.g. they miss a sample due to being on holiday).

All samples will be pre-labelled with a unique study ID and the sample number. Participants will need to add the date of collection before posting. No identifiable or personal details will be recorded on the samples.

5.4 Questionnaire completion

Participants will be asked to complete a short online questionnaire every time they collect a sample (i.e. every week). The initial baseline questionnaire, which can be completed in clinic or at home, will be slightly longer to accommodate more detailed questions on sexual behaviour and HIV status. All questionnaires (initial and follow-up) will collect information on sexual risk behaviours, drug use, gastrointestinal symptoms, antimicrobial use and travel history.

Participants will complete the questionnaire online using a smartphone or other personal device with internet access. Once the patient goes to the questionnaire website, they will be asked to log-in to the survey using their unique study ID. We will use SnapSurvey Inc. software to administer the questionnaire. This approach has been used successfully in questionnaire-based studies among persons attending SHCs. (19, 21)

5.5 Qualitative interview

To understand how best to minimise barriers and better facilitate participation and retention in the study in addition to study time commitments, a 1 to 1 interview will be conducted with selected participants. Potential participants for the interviews will be drawn solely from MSM participating in the pilot study who provide consent for follow-up discussion. The consent form for a 1 to 1 interview will be completed upon signing up to study in clinic or online. Their permission will be sought for recording the discussion, but no identifiable data or names of participants will be recorded. Participants will also be informed that if during the discussion they share information that suggests that they are at risk of serious self-harm or harm others or disclose about sexual abuse/violence, the researcher may need to speak to the appropriate clinic staff/authority to ensure their safety.

A member of the clinic staff will contact potential participants by email and/or telephone using the information provided at recruitment to arrange their further involvement.

We will conduct up to 20 interviews. Participants for interviews will be purposefully sampled to ensure representation across age range, and whether they have, or have not, provided a faecal sample and longitudinal samples. This is to achieve the goal of understanding the different factors which motivated or prevented sample collection. The interviews will take place via telephone or video call (e.g. Microsoft Teams or Zoom). However, face-to-face interviews may also take place where appropriate. These would take place in a quiet and private space in the clinic, or dedicated space provided by a community-based organisation (Terrence Higgins Trust). If any interviews take place face-to-face, the participants will be reimbursed for their travel costs.

A topic guide will be used during the interviews. Trained members of the research team who have substantial experience of conducting qualitative research on sensitive topics will facilitate the discussion. It is anticipated that each interview will last for approximately 60-90 minutes.

6 DATA ANALYSIS

6.1 Sample testing

Rectal swabs and faecal samples received at UKHSA will undergo Deoxyribonucleic Acid (DNA) or Ribonucleic acid (RNA) extraction and amplification via real-time Polymerase Chain Reaction (PCR) to detect a range of bacterial, viral and parasitic causes of gastrointestinal infection including *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., *E. coli*, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* spp. and Hepatitis A virus. This will be done within the Gastrointestinal Bacteria Reference Unit (GBRU) at UKHSA.

Faecal samples are the preferred material for detection of enteric pathogens. Culture is required for speciation, molecular typing by WGS and antimicrobial susceptibility testing. Cultured isolates from faecal samples are the preferred material for WGS of pathogens. However, rectal swabs may offer a more acceptable and pragmatic method for longitudinal sample collection. In our pilot study, we aim to compare faecal samples with rectal swabs for enteric pathogen and WGS analysis.

Metagenomics may be performed to detect the complete diversity of pathogens and genetic determinants of AMR found in participant samples.

6.2 Data analysis

The final dataset will be managed and analysed by the study management team using STATA v15 available on the UKHSA server. Data will be cleaned using consistency checks. Data generated through WGS and metagenomics will be analysed using bioinformatic software tools designed for genomic and phylogenetic analysis.

Descriptive analysis will be conducted to examine:

- the number of people who decline to take part and the reasons
- the characteristics of the study population
- whether the study population is representative of all MSM attending the clinic during the study period (using the complete GUMCAD data submitted to UKHSA)
- the number and proportion of participants providing samples at various time points
- the characteristics of participants who return samples compared to those who do not
- the prevalence of enteric pathogens (with 95% confidence intervals)
- sub-types and relatedness of different enteric pathogens
- duration of carriage
- AMR characteristics

6.3 Qualitative data analysis

All qualitative data will be transcribed and analysed using qualitative data analysis software NVivo. A matrix based approach for ordering and synthesizing qualitative data known as the 'Framework approach' will be used for analysis (22). The analysis of the data will follow the key features of this approach like familiarisation with the data and developing a thematic framework and subsequently applying this framework to the entire dataset. Themes will be charted using a matrix and within- and between-case analysis will be conducted to develop descriptive and explanatory accounts of the data, identify emerging concepts and typologies, and to find associations and explanations for the patterns observed in the data.

7 DATA HANDLING AND MANAGEMENT

7.1 Maintenance of study log

Clinic staff or the study research nurse will be responsible for maintaining the electronic study log (Excel spreadsheet). The main purpose of the study log is to record the details of each person approached and asked to participate in the study.

There will be two parts to the study log: the enrolment log (for participants who agree to participate) and the screening log (for those who decline to participate).

The enrolment log will be maintained by the clinic to record the unique study ID (anonymised identifier assigned to each participant), the clinic patient number (specific to the clinic), clinic attended, date of attendance and contact information (Table 1). Clinic staff may wish to complete a hard copy of the log during recruitment. However, the electronic version of the study log should be updated after each day of recruitment and should be kept as a long-term record of the study.

The screening log will be completed by the clinic for those who decline to participate in the study. The screening log will be used to understand the level of participation and reasons for not participating (Table 2).

Clinic staff or the study research nurse will extract data from study log on a monthly basis. The study logs will be password protected.

Table 1. Enrolment log fields

Field	Description	Notes
Study ID	Unique study identifier (anonymous)	Pre-assigned unique identifier
Clinic patient number	Clinic patient number	Unique identifier at the clinic. Also included in routine GUMCAD data. This is a pseudonymised number (as defined by the Information Commissioner's Office)
Date	Date of attendance	
Clinic attended	e.g. SHAC East, Lawson, Brighton PrEP clinic	
Agree to data linkage	Y/N	Whether participant agreed to have their test results and questionnaire responses linked to GUMCAD data
Agree to contact for follow-up sample reminders	Y/N	Whether participant agreed to reminders to collect & send samples
Agree to contact for one to one interview	Y/N	Whether participant agreed to contact to discuss experiences, barriers and facilitators

Contact details 1	Email address	For those who agree to any contact
Contact details 2	Telephone number	For those who agree to any contact
Agree to long-term storage of samples & data	Y/N	Whether participant agreed to long-term storage
Would like to receive study results	Y/N	Whether participant would like to receive a summary of the study results at the end of the study
Baseline procedures conducted at clinic or home	Clinic/home	Whether participant collects samples (and completes questionnaire) during clinic visit or takes home

Table 2. Screening log fields

Field	Description	Notes
Date	Date of attendance	
Reason for not participating	Dropdown list of reasons for not participating to include: Time, Does not want to provide (extra) samples, Anxious/upset, Concerned about longitudinal collection, Declined for other reasons, No reason given	Select all that apply
Further details	Free text option to provide further details if declined for other reason	

7.2 Data storage and transfer

7.2.1 Study log

The electronic study log will be stored on a secure server at the clinic. Any hard copies of this log will be stored in a locked filing cabinet. Selected fields from the study log will be extracted, password-protected and emailed over a secure connection (via NHS.net email) to UKHSA monthly. The research study team will not have access to any identifiable data or contact information – this will remain with the clinic staff.

The following parts of the log should be sent to the study team at UKHSA:

1. Enrolment log: study ID, clinic patient number, clinic attended, attendance date and information on who has agreed to data linkage, contact about participating in the qualitative interview, long-term storage of samples and whether participant has collected samples in clinic or at home. No contact information will be sent to the study research team as part of the main study. A surveillance scientist at UKHSA will use the study ID and clinic patient

number to extract and anonymise GUMCAD data. Linkage will only be performed for those who provide consent. The log will be kept secure and separate from other study files, including the analysis dataset and will be deleted at the end of the study.

2. Screening log: date and reason for not participating

7.2.2 Questionnaire data

The online questionnaire is hosted on a secure (HTTPS) connection and data are encrypted at the point of transmission and stored on a secure, dedicated virtual server hosted at UKHSA. Online questionnaire responses will be extracted in an electronic .CSV file and imported into Stata v15, available on the UKHSA server.

7.3 Sample storage and transfer

All samples will be posted directly to the national reference laboratory at UKHSA GBRU by the participant using pre-paid envelopes. The samples will be transported following UN 3373 regulations for the transport of biological material. All samples will be sent to UKHSA labelled with the study ID, sample number and date of collection only.

When samples are received by UKHSA, the barcodes will be scanned so that an electronic record of biological samples is created. This record will contain the study ID, date of collection and sample number. Once testing has been completed, the results will also be added to this record. Laboratory staff will send this record to the surveillance scientist on a monthly basis.

In the study, rectal swabs and faecal samples will be collected from participants in accordance with the PIS and only after written informed consent has been obtained. Samples will be processed and stored at GBRU in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act (HTA) 2004 and any amendments thereafter. All rectal swabs and faecal samples will be kept until DNA/RNA extraction, testing and culture are complete (see Section 6.1). The swabs and samples will then be disposed of. The extracted DNA/RNA will be stored in an anonymised form at UKHSA for a maximum of 10 years and may be used for future ethically approved studies.

The custodian of the samples will be Dr Claire Jenkins, Head of E. coli, Shigella, Yersinia and Vibrio Reference Services and Deputy Head of GBRU, UKHSA.

7.4 Data linkage

Participants will be asked whether they consent to their questionnaire responses and test results being linked to socio-demographic and clinical data from GUMCAD, including STI and HIV tests and diagnoses (Table 3). Linkage will only take place for those who agree to linkage on the consent form. The enrolment log look-up (see section 7.2.1) will be used to extract GUMCAD data for all participants and the clinic patient numbers will subsequently be removed from the dataset.

Linkage of online questionnaire responses from SHC attendees to GUMCAD data was used successfully in the RiSH Survey with 95.5% of MSM taking part consenting to data linkage.(21)

Data from GUMCAD will be used to describe the characteristics of the study population, and to describe clinical outcomes (prevalence of enteric pathogens, genetic determinants of AMR, pathogen sub-types, duration of carriage) by patient characteristic.

Table 3. GUMCAD surveillance system data items

Data item	Date item description
Clinic patient number	Pseudonymised local patient identifier. This number is unique within a clinic and cannot be used to track patients across different SHCs or other NHS services
Attendance date	Date of attendance
Sexual Health and HIV Activity Property Type (SHHAPT) code	STI surveillance code– STI diagnoses and sexual health services provided
Gender	Male (including trans man), Female (including trans woman), non-binary, other
Age	Age at attendance date (years)
Sexual orientation	Heterosexual, homosexual, bisexual, not known
Ethnicity	Ethnic category code as defined in the 2001 census
Country of birth	International Organisation of Standardisation (ISO) standard list for countries
Lower Layer Super Output (LSOA) Area of Residence	LSOA of patient residence (approximately 32,000 in England). The mean population size is 1500.
Attendance Type	New or follow-up episode

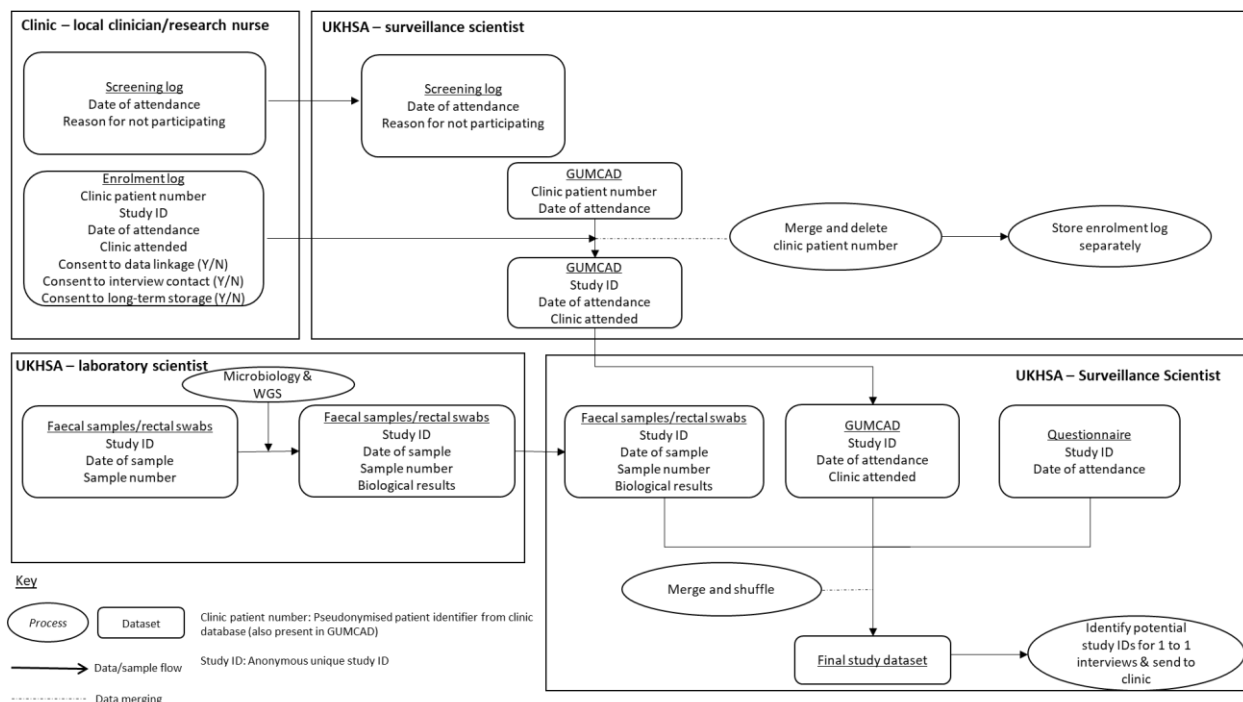
7.5 Identification of participants for 1 to 1 interview

The surveillance scientist will identify potential participants for the interviews. Participants will be selected to ensure representation across age groups, and whether participants have, or have not, returned faecal and longitudinal samples. The list of potential Study IDs will be sent to clinic so that they can contact the participants directly about taking part in the interviews, if they have provided consent to this previously. The clinic will not receive any information about the number of samples collected or the test results.

If those contacted are interested to participate in the interview, clinic/research staff will inform them that a member of the research team will contact them subsequently to organise the interview.

7.6 Data management algorithm

Figure 2 provides an overview of the proposed data handling and management algorithm for the study.



8 ETHICAL CONSIDERATIONS

8.1 Assessment and management of risk

This study does not involve any intervention and poses very low risk to individual patients. Eligible patients will be asked to read the PIS and will have the opportunity to ask questions before consenting. All data analyses will be performed using a unique study ID (anonymous). All contact details will only be used for the specified purpose (reminders to send samples and/or to discuss experiences of the study in a 1 to 1 interview) and will be deleted at the end of the study.

The transmission dynamics and contextual factors associated with enteric pathogens are not very well understood. The potential benefit here is substantial because the data will provide valuable information which can be used to inform the development of improved future public health and clinical control measures for enteric pathogens.

8.2 Return of test results

The test results from the research study will not be reported back to the individual participants and clinic/research staff. The rationale for the non-return of test results is as follows:

1. Participants will provide consent for the study and will be informed about what samples are collected, the tests performed and that their results will not be returned or used to inform their care or treatment at the clinic.
2. Some of the pathogens being detected are statutorily notifiable which would require adherence to standards for clinical testing, including timely return of results and follow-up (if necessary). It was not considered practical to try to achieve these standards within the resources of the study. Furthermore, the participants are not required to return to the clinic during the study and may choose not to be contacted.

3. The methodology using rectal swabs, although useful for research purposes, has not been validated for clinical diagnostic use.
4. The tests undertaken in this study do not have specific clinical implications in the absence of symptoms. Microbiological testing is recommended when individuals present with symptoms of gastroenteritis.
5. Individuals attending the clinic will be asked about symptoms by their clinician as part of routine care (including about anal/rectal symptoms and symptoms of enteric infection) and these will be managed appropriately, and faecal samples collected where necessary for NHS-based diagnostic testing. Individuals will be informed that if they have any symptoms during the study follow-up period, they should seek appropriate care from the clinic or their GP.
6. The samples and test results will be associated with the unique study ID only. The research study team will not have any personal identifiers or contact information as part of the main study to link the results to a unique individual.
7. While we recognise the importance of patient autonomy in research, in this case, the study is very unlikely to lead to any harm (or benefit) to individual patients.

The pilot study will enable us to explore the acceptability of this approach with study participants.

8.3 Withdrawing from the study

Participants will be informed that they can withdraw from the study at any time, without giving a reason and it will not affect the standard of care they receive or their legal rights. Participants can withdraw consent by writing to the clinic or research study nurse (as indicated in the PIS). Any samples or data already provided will be retained and used in the study. The samples and the final dataset will be associated with a unique study ID only. The research study team will be unable to link the results to a unique individual as they will not have any personal identifiers or contact information as part of the main study.

8.4 Confidentiality

All information will be treated as completely confidential. The questionnaires and samples will contain the unique study ID only, with no identifying information. The patient's name and clinic patient number will not be recorded on the questionnaire or samples. Participants will be informed that their questionnaire responses will not be seen by clinical staff or recorded in their clinical notes. The test results from the study will not be reported back to the individual participants or clinic staff. All analyses will be conducted using only the unique study ID. No individual patient will be identifiable in any results or publications.

The study log will be the only document linking the study ID with the clinic patient number and will be password protected and kept separate from the study datasets. Any personal identifiable information from the study logs (contact details) will only be used for the consented purposes (reminders to send samples and/or to discuss experiences of the study in an interview) and will only be accessible to clinic staff.

The data from the questionnaires and samples will be kept securely and separately away from the study log information and will be identified by the unique study ID only.

If a participant agrees to take part in an interview, their name and contact details will be shared with the research team member who is conducting the interview only. Their name and contact details are required to arrange the interview time, and to set up the interview over MS Teams or Zoom. The study team member conducting the interviews will not have access to the unique study ID, clinic patient number or study datasets; their involvement is limited to the qualitative interviews only. Participants'

details will be deleted after the interview and will not be linked with the transcripts. The transcripts will be given a separate anonymous number e.g. ID_101.

8.5 Data security

At the clinic, the electronic study log will be stored on a secure server. Any hard copies of this log will be stored in a locked filing cabinet. The relevant fields from the study log will be password-protected and emailed over a secure connection to the study team at UKHSA on a monthly basis.

All electronic data will be securely held in the Blood Safety, Hepatitis, STI and HIV Division, UKHSA. All data will be stored on a secure encrypted drive at UKHSA and the dataset will be in a restricted access folder.

The study logs will be password protected and only the study team will have access to these. The logs will be stored separately from the dataset and samples. The study log containing the clinic patient number and unique study ID (enrolment log) will be deleted at the end of the study.

Anonymisation of GUMCAD data will be done by replacing clinic patient number with the study ID. This will be performed by the UKHSA surveillance scientist team member who already has access to the clinic patient number and the national surveillance dataset.

The questionnaire will be administered and stored on a secure remote encrypted UK-based UKHSA server. Online questionnaire responses will be extracted in an electronic .CSV file and imported into Stata v15. Clinic patient numbers will not be recorded in the questionnaire responses and all data analyses will be conducted using only the unique study ID.

All interviews will be recorded for transcription and analysis. Interviews which occur via MS Teams will be recorded using built-in functions on MS Teams. Interviews which occur in person will be recorded using an encrypted Dictaphone. All recordings will be stored on a secure, encrypted drive at University College London or UKHSA and the recordings will be in a restricted access folder. The recordings will be transcribed by an external agency that will be bound by a confidentiality agreement and has substantial experience of doing transcription of sensitive data. The electronic recordings will be transferred to the external agency via an online secure system. Recordings will be destroyed immediately following transcription. All qualitative data will be analysed using qualitative data analysis software NVivo. Qualitative data will be kept separate to all other study files and datasets.

The study requires regulatory approval from the following bodies: Health Research Authority (HRA Approval), NHS Research Ethics Committee (REC) and NHS Research and Development (R&D). Each approval will be obtained before the study commences.

9 PATIENT AND PUBLIC INVOLVEMENT AND ENGAGEMENT (PPIE)

Cognitive interviews were used to pilot the PIS, consent forms (main study and qualitative aspect) and study questionnaire (baseline and follow-up). Individual cognitive interviews were chosen because this method enables testing of the acceptability, comprehension and clarity of the questions and information. The interviews also explored general interest in the research study proposal and methodology.

Volunteers were recruited through the Brighton and Hove SHAC or through existing MSM groups at Terrence Higgins Trust (local community-based organisation). Volunteers received a £25 e-voucher as

a token of appreciation for their contribution and time. In total, 5 volunteers took part in the individual interviews.

Interviews were conducted via a 45 to 60-minute video call using MS Teams or Zoom. All volunteers were shown the PIS, consent forms and questionnaire over MS Teams or Zoom. Specifically, participants were asked to read the information/questions in one section at a time and highlight the information/questions they did not understand, had concerns about, or had difficulty in deriving responses. A think aloud technique was explained and used with prompting and questioning for explanations which related to acceptability of language, recall, tone, wording and timing.

The researcher sought participant suggestions to improve comprehension and acceptability on the PIS, consent forms and questionnaire (baseline and follow up) as required. Adjustments were made to the protocol and study documents based on feedback received from the volunteers.

10 TIMELINE

	2021					2022					
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr-Jun	Jul-Sep	Oct-Dec
Public and Patient Involvement and Engagement	X	X									
Sponsorship, Ethics, Research and Development applications			X	X							
Recruitment					X	X	X	X			
Collection of samples					X	X	X	X	X		
DNA extraction/testing of samples					X	X	X	X	X		
Qualitative work						X	X	X	X		
Data analysis							X	X	X	X	X

11 PUBLICATION AND DISSEMINATION POLICY

The results will be disseminated through peer-reviewed publications and conference presentations. The NIHR HPRU has a Public and Patient Involvement strategy, which includes engagement through social media and lay press. Results will be disseminated through public engagement seminars.

12 FUNDING

The study will be supported by The National Institute for Health Research Health Protection Research Unit in Blood Borne and Sexually Transmitted Infections at University College London in partnership with UKHSA, and the NIHR HPRU in Gastrointestinal Infections at the University of Liverpool in partnership with UKHSA and the University of Warwick.

The NIHR HPRU in STIs and Blood-borne viruses (BBVs) have allocated funds to support study implementation, data collection and analysis.

The NIHR HPRU have allocated funds to the GBRU to cover the cost of laboratory consumables. All laboratory testing, culture and sequencing will take place at the GBRU.

13 MONITORING AND AUDITING

The study team will ensure there are adequate quality and monitoring activities conducted. This will include adherence to the protocol, procedures for consenting and ensuring adequate data quality.

The clinic will be responsible for communicating with the study team regarding progress and any problems identified.

The study team will inform the sponsor should they have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

14 ARCHIVING

UKHSA and each collaborating site recognise that there is an obligation to archive study-related documents at the end of the study (as such end is defined within this protocol). The Chief Investigator confirms that they will archive the study files at UKHSA or UCL for the period stipulated in the protocol and in line with all relevant legal and statutory requirements. The principal investigator at the participating site agrees to archive their respective site's study documents in line with all relevant legal and statutory requirements.

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