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STUDY PROTOCOL

Experimental Human Pneumococcal Carriage using *Streptococcus pneumoniae* serotype 3 in Malawi: a dose ranging and reproducibility human infection study [version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract

Background

Streptococcus pneumoniae is a major cause of morbidity and mortality from respiratory tract infections, pneumonia, meningitis, and sepsis. Nasopharyngeal carriage of pneumococcus is a prerequisite for pneumococcal disease and transmission. Since the global introduction of pneumococcal conjugated vaccines, rates of pneumococcal disease have declined for many vaccine type serotypes but serotype 3 (SPN3) continues to cause significant disease. The Experimental Human Pneumococcal Challenge (EHPC) model is a unique method of determining pneumococcal colonisation, understanding the impact of colonisation on acquired immunity and for testing pneumococcal vaccines. This study will develop a serotype 3 EHPC model to address some pertinent questions on the burden of pneumococcal disease in

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	1	2	3
version 1 13 Aug 2024	✓ view	? view	✓ view

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Methods

Healthy adults aged 18-50 years will be recruited, with a maximum target of 83 participants to complete all study visits. The study will consist of a dose ranging and safety study, followed by a reproducibility study. Sequential cohorts of 10 healthy participants will be challenged with escalating doses of SPN3 in the dose ranging study. Samples will be collected before inoculation and on days 2, 7, 13, 16, 21 and 28 following inoculations, for determination of carriage. A total of 33 participants will be enrolled in the reproducibility part and will use a dose that established ≥60% of carriage, and with a high safety profile. Samples will be collected for determination of both local and systemic immunological responses to pneumococcal challenge. Upon completion of study visits, participants will complete a questionnaire establish acceptability.

Interpretations

We expect to establish an optimal SPN3 dose required to establish nasopharyngeal colonisation in healthy adults in an EHPC model. The model can then be used to evaluate pneumococcal vaccines in both healthy and at-risk populations.

Plain Language Summary

Healthy adults and children commonly have pneumococci (the bacteria that causes the infection) living in their nose without any symptoms. In most adults these bacteria are present at least once per year. The presence of these bacteria, called “carriage”, may help to develop natural protection against infection. However, there is also a risk that these bacteria can cause illness for some vulnerable people.

Infections can develop from carriage in vulnerable populations. Certain infections with pneumococci are very common, e.g. ear infections in children. Infections of the lung (pneumonia) or the brain (meningitis) or the blood (sepsis) may develop in very young children or in elderly adults or those who are weak with other illnesses. These severe infections are very uncommon in healthy adults.

The PCV-13 pneumococcal vaccine has been given to children under the age of one in Malawi since 2011. This vaccine has been only partially successful in Malawi; it reduces the risk of diseases such as pneumonia for vaccinated children, but it does not stop them from carrying the bacteria in their noses. Children can pass on the bacteria to vulnerable people who have not been vaccinated, increasing their risk of developing severe infections. Malawi needs better vaccines that prevents nasal carriage to protect both those who are vaccinated and vulnerable people around them.

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

We will use this experiment to determine a dose of *Streptococcus pneumoniae* serotype 3 that can be carried in the nose and check if by repeating the dose, bacteria will be found as previously, and check the body's response to presence of the bacteria. Pneumococcus bacterium will be put into participants' nose and participants will be closely monitored to make sure they are safe. Samples will be taken to see how participants' bodies develop protection against the bacteria.

Keywords
Pneumococcal carriage, Streptococcus pneumoniae Serotype 3, Experimental Human Pneumococcal Challenge, Human Infection Study, Controlled Human Infection Model, vaccine

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Introduction

Streptococcus pneumoniae (SPN), also known as the pneumococcus, is a major cause of vaccine-preventable disease world-wide[1,2]. Nasopharyngeal (NP) carriage of pneumococcus is a prerequisite for pneumococcal disease and transmission of the bacterium and thus represents a risk to carriers themselves and to the wider community[3].

In sub-Saharan African settings, pneumococcal carriage remains high despite good conjugate vaccine coverage. Pneumococcal conjugate vaccines (PCVs) confer direct protection to those vaccinated and indirect protection to unvaccinated individuals through lower carriage rates and reduced *S. pneumoniae* transmission (herd effect). PCVs are highly effective against invasive pneumococcal disease (IPD)[4]. However, they are limited in serotype coverage and may be associated with serotype replacement and high residual carriage of vaccine serotypes in low and medium income countries (LMIC)[5–7]. Next generation PCVs with expanded valency are urgently needed[8]. Further, the herd effect of childhood immunisation programmes for reducing carriage and disease in the wider community has not been replicated in children and adults in low income countries as expected following success in high-income countries[6,9].

The 13-valent pneumococcal conjugate vaccine (PCV-13), whose formulation contains polysaccharides from 13 serotypes, is the most commonly used conjugate vaccine worldwide[10]. IPD has significantly reduced among all age groups since the introduction of PCV-13 into national paediatric immunisation programmes[11] although rates of disease attributable to serotype 3 (SPN3) have shown no or limited decline. Current conjugated vaccines do not elicit the level of antibodies required to confer protection against SPN3[12,13]. This ”vaccine escape” is thought to be due to specific characteristics of the SPN3 capsule, which is shed by the bacteria, potentially as a defence against phagocytosis[14]. The profuse production and release of serotype 3 capsule may overwhelm the protective capacity of antibodies that are produced in response to the vaccine[14], especially at the mucosal surface.

SPN3 is characterized by its high virulence, invasiveness, and mortality in vulnerable populations. In many settings, SPN3 is highly susceptible to clinical antibiotics (low antimicrobial resistance)[15] but in Malawi there is a higher rate of antimicrobial resistance in SPN3 than other serotypes (R Heyderman, m/s submitted). In UK, observational data analysis from 2014 to 2018 showed an increase in the proportion of IPD cases due to SPN3 and that the contribution of PCV13 serotypes to IPD increased with age[16]. In addition, there was no significant reduction in NP acquisition of SPN3 post PCV-13 in a randomised controlled trial (RCT) by Dagan *et al.*[7]. These data suggest that the immune response to SPN3 elicited by PCV-13 is insufficient to lead to the large, sustained indirect protective effects although there is some degree of direct protection[7,17]. This has led to great interests in novel vaccine technologies that could provide higher levels of protection against SPN3 acquisition, colonisation, and disease.

This study, therefore, aims to determine an optimal dose for SPN3 to establish NP colonisation and to improve knowledge of immune responses to SPN3 colonisation in Malawian healthy adults using an antibiotic sensitive strain previously isolated in the country. Findings from this study will be used to test novel vaccines and to inform designs of future pneumococcal vaccine RCTs.

The Experimental Human Pneumococcal Challenge (EHPC) model provides an effective tool to test vaccines for their effect and answer mechanistic questions on experimental colonisation. In vaccine studies, the EHPC model requires fewer participants than phase III clinical trials as such it is more cost-effective[18–20]. The EHPC model has been established in Malawi using type 6B pneumococcus (SPN6B)[21] and used in our PCV13 randomised controlled trial[22]. A decade of experience in our team using the EHPC model in the UK, along with our Malawi feasibility study and the PCV13 RCT at Malawi-Liverpool Wellcome programme (MLW) is used to inform this current study and further EHPC work in Malawi. In the EHPC model, participants are intranasally inoculated with SPN and about half of them develop a stable colonisation episode for about 1–3 weeks, at a density typical of natural colonisation. Experimental carriage offers the opportunity to obtain both mucosal and peripheral blood samples to assess colonisation, density and duration as well as host immune responses in a setting where the onset and termination of carriage are known[18]. The model has been well developed in the UK for SPN6B and SPN15B with over 1500 volunteers challenged in 25 independent studies, showing the model is safe and has reproducible carriage rates. The model has been used to demonstrate a reduction in pneumococcal carriage, density, and duration in healthy adults with conjugate vaccine exposure[23,24] and has been used to demonstrate the importance of the nasal route for both local and systemic pneumococcal immunity[25]. More recently 96 participants were challenged with three different SPN3 strains by the LSTM team in a series of different doses. Colonisation rates varied between 30% and 70%[26]. This data shows that a model with SPN3 is feasible and safe.

We have demonstrated that nasal cells can be sampled using non-invasive and well-tolerated techniques[21,27]. The collected nasal cells and nasal fluid have successfully been used to describe immune cell activation using the methods proposed in this study, including immunophenotyping, cytokine assay, and single-cell sequencing[27,28].

To increase the relevance of the EHPC model to the Malawi context and its use for assessing future vaccines, we propose to set up an EHPC model with a serotype 3 strain that was previously isolated from Malawi. The safety and dose-ranging study will inform the optimum SPN3 dose for colonisation acquisition while the reproducibility study will confirm the dose in a subsequent larger cohort. Mucosal and systemic immune responses to SPN3 and their association with protection against colonisation acquisition and clearance will also be studied.

Study protocol

Study objectives

1. To determine the optimal SPN3 dose to establish colonisation of the nasopharynx in healthy Adults.

2. To understand local and systemic immunological responses to SPN3 colonisation

Study design

This is a non-randomised, safety and dose escalation human infection study of healthy adults aged 18–50. Participants will be nasally inoculated with pure culture of well-characterised, fully sequenced amoxicillin sensitive SPN3. They will be observed for safety and development of pneumococcal colonisation and immune responses with samples collected at different time points (Figure 1).

Phase A - Dose ranging: Sequential cohorts of 10 healthy participants will be challenged with escalating doses of SPN3 until a cohort experiences 6 or more colonised participants (≥ 60%).

Phase B - Reproducibility: 33 participants will be inoculated with SPN3 using the dose that results in ≥60% colonisation acquisition rate to check for reproducibility of carriage rate and immunological responses.

Primary outcomes

Study objective 1:

- Detection of SPN3 in nasal wash by classical microbiology at day 2, 7, 13, 16, 21 and 28 post inoculation
- Establishment of a dose of SPN3 carriage following experimental pneumococcal inoculation
- Ascertain the rate of experimental colonisation acquisition of SPN3 in healthy adults post inoculation, by microbiological and molecular methods.
- Establishment of the rate of adverse events recorded following experimental pneumococcal inoculation.
- The density and duration of experimental SPN3 colonisation of the nasopharynx are determined.

Study objective 2:

- Characterisation of mucosal immune cell populations and dynamics in response to SPN3 experimental inoculation in nasal cell samples
- Levels of mucosal and systemic SPN3 polysaccharide-specific antibodies determined at baseline and after SPN3 experimental inoculation.

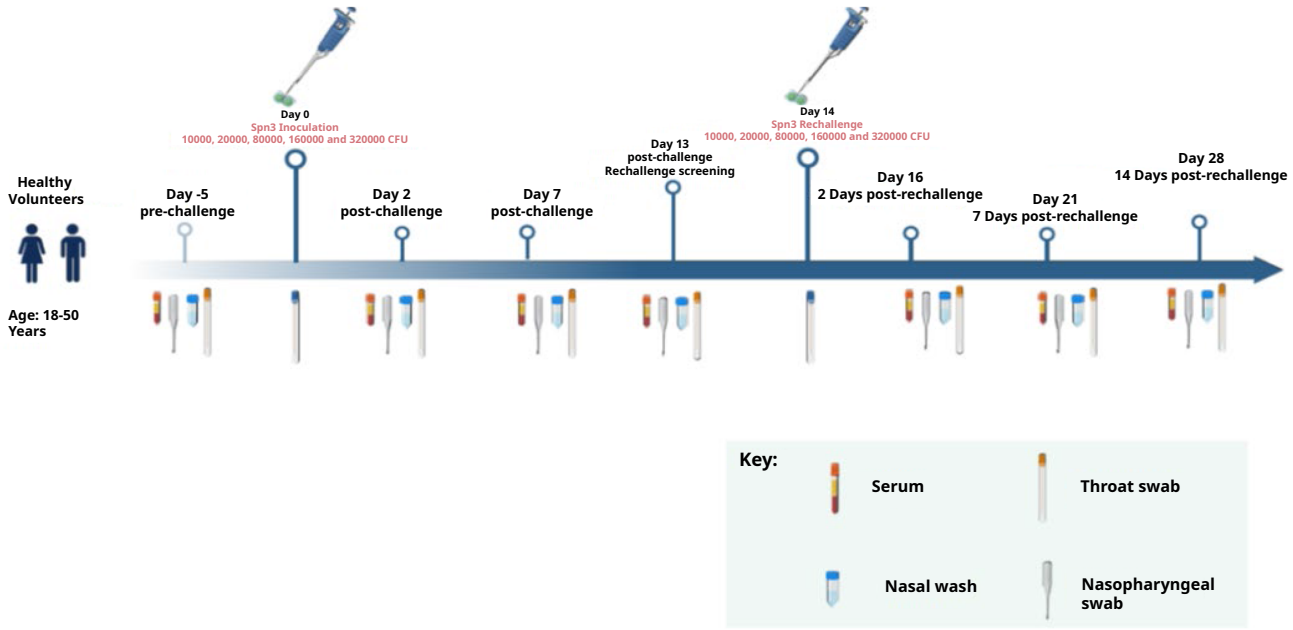

Figure 1. Study design and sample visit schedule. Showing participants' visit schedules including screening, inoculation and follow up visits on days 2, 7, 13, 16, 21 and 28. Day 14 is for rechallenge for participants not colonised on days 2 and 7. Samples include but not limited to throat swab, nasopharyngeal swab nasosorption, nasal cells, nasal wash, saliva, and blood for serum, and peripheral blood mononuclear cells. Safety monitoring post-inoculation includes a residential stay for three nights for both initial inoculation and rechallenge, health-checks at each study visit, 24/7on-call study doctor, mobile phone contact with study team, and a safety pack containing a thermometer, antibiotics, and safety information leaflet.

- Levels of polysaccharide specific SPN3 memory B cells determined at baseline and after SPN3 experimental inoculation.
- Nasal inflammatory kinetics induced by SPN3 experimental inoculation described (samples obtained in reproducibility study).
- The effect of natural SPN (non-SPN3) colonisation on SPN3 colonisation is determined (samples obtained in reproducibility study).
- Carriage rates, density and duration of SPN3 colonisation in saliva versus nasopharyngeal samples are established and compared (samples obtained in reproducibility study).

Study setting
This is a single centre study to be conducted at the Malawi-Liverpool Wellcome Programme, using a MARVELS clinic that is located at Ward 3A of Queen Elizabeth Central Hospital (QECH).

Recruitment target
Eighty-three HIV-negative adults aged 18 to 50 years old.

Study duration
Recruitment commenced October 2023 and expected to finish in June 2024.

Participant schedule
Prior to inoculation, participants will attend two visits (information and screening) in order to evaluate participant eligibility and safety to participate and to establish fully informed and voluntary consent.

Study procedures (Methods)
Participant recruitment
This human challenge study protocol has been reported following the SPIRIT reporting guidelines[29].

Participant identification and recruitment will be done in several ways including but not limited to public engagement events, posters/leaflets in public and private areas, social media, local press, television, and radio, including articles/advertisements about the study and passive recruitment. The above is not exhaustive, other recruitment methods may be utilised if required. Interested persons will be asked to contact the research team via phone or email. Potential participants will be given a copy of the participant Information Leaflet (PIL) and invited to contact a member of the team if they would like to participate.

Inclusion criteria
- Healthy adults aged 18–50 years.
- Fluent spoken English and/or Chichewa.
- Capacity to give informed consent.
- HIV negative
- Access to their own mobile telephone

Exclusion criteria
- Currently involved in another study unless observational or non-interventional.
- Participant in any previous EHPC trial in past year
- Previous pneumococcal vaccination. This can be self-reported or confirmed from the health passport if deemed necessary at clinician discretion.
- HIV positive
- Allergy to penicillin/amoxicillin
- Chronic ill health including immunosuppressive history, diabetes, asthma (on regular medication), recurrent otitis media or other respiratory disease.
- Medication that may affect the immune system e.g., steroids, inflammation altering or disease-modifying anti-rheumatoid drugs.
- Long term use of antibiotics for chronic infection.
- Major pneumococcal illness requiring hospitalisation in the last 10 years.
- Other conditions considered by the clinical team as a concern for participant safety or integrity of the study.
- Significant mental health problems (uncontrolled condition or previous admission to a psychiatric unit)..
- Direct caring role or close contact with individuals at higher risk of infection
    - Children under 5 years age
    - Adults with chronic ill health or immunosuppression
    - Hospital patients
- Smoker:
    - Current or ex-smoker (daily cigarettes, daily e-cigarettes/vaping and daily smoking of recreational drugs) in the last 6 months. Participants who smoke <5 cigarettes per week may be included.
    - Previous significant smoking history (>20 cigarettes per day for 20 years or equivalent [>20 pack years].
- Pregnancy:
    - Currently pregnant/lactating/ Intending on becoming pregnant during the study period – minimise the risk of pneumococcal disease

- **History of or current drug or alcohol abuse:**
    - Men should not drink >3 units/day regularly
    - Women should not drink >2 units/day regularly
- **Overseas travel planned** in follow up period of study visits
- **Natural SPN3 colonisation in baseline nasal wash** – if a participant is colonised with non-SPN3 pneumococcus, they can be included as part of exploratory analyses, but would not be included in the primary analysis
- **STOP criteria** – participants who meet the STOP criteria at time of screening as previously described by Hazenberg *et al.*[30] (Table 1)

Information visit
Study information will be given verbally and in written form in English and/or Chichewa to potential participants which include but not limited to description of the study and its objectives, and an outline of participation and its associated benefits and risks. Interested individuals will be given an unrestricted amount of time to decide on participating in the study if the recruitment capacity has not been exceeded.

Consent
Written consent will be obtained at this visit. A questionnaire will be conducted before written consent is obtained to ensure understanding of the study and participation before written consent is obtained. Candidates will be invited to ask questions and they will be asked how long they would like to consider the information and whether they require further time to make an informed decision. The participant will be invited to provide written informed consent if the study team are satisfied that they meet the eligibility criteria and that they have voluntarily agreed to take part in the research.

Screening
Participants will be asked questions about medical history by one of the research doctors/nurses to ensure that they meet the inclusion criteria and do not meet any of the exclusion criteria. Clinical examination includes vital signs including blood pressure, heart rate, temperature, peripheral oxygen saturations and auscultation of lung fields and heart sounds. Height and weight will also be measured. Urinary pregnancy test will be done in all biologically female participants of child-bearing potential. Nasal wash samples will be collected at this screening visit to determine existing natural pneumococcal carriage. Participants naturally colonised with *Streptococcus pneumoniae* serotype 3 (SPN3) will not be eligible to continue in the study. If a participant is colonised with non-SPN3 pneumococcus, they will continue in the study and be included as part of exploratory analyses.

Inoculation visits
All participants will be inoculated at day 0 in both dose ranging and reproducibility studies. Using a P200 micropipette, 0.1ml of pneumococcus containing fluid will be instilled into their nose. After inoculation, the participant will remain in a semi-recumbent position for up to 15 minutes. They will be given a post-inoculation advice sheet (including emergency contact details), thermometer, a course of amoxicillin and a daily symptom log to complete. Participants are asked to contact the team daily (before 1200hrs) for the first 5 days with their temperature recording and any symptoms. If they do not make contact, a member of the research team will contact the participant. If no contact is made, a defined 'secondary contact' will be telephoned. During the post-inoculation period, participants will have access to a 24/7 on-call telephone service until the end of the study. Participants reporting symptoms consistent with pneumococcal disease (e.g. [but not limited to] ear pain, sore throat, cough, fever) will be reviewed in person by clinicians within the research team.

An additional targeted repeat inoculation of the same inoculum dose will be given to participants on Day 14, if they have

Table 1. Baseline assessment and STOP criteria.

Clinical history and examination	STOP if unexplained or concerning findings on history or examination
Severe adverse event (SAE) or research related injury (RRI)	STOP if related SAE or RRI reported
Engagement with research team	STOP if research team have concerns about participant's ability to commit to frequent communication and safety checks
Full blood count (FBC)	STOP if Hb <10g/l STOP if total WCC <1.5 x10^9/l STOP if total WCC >12 x10^9/l STOP if platelets <75 x10^9/l
Resting SpO2	STOP if <94%
Illness during study	STOP if participant develops a medical condition or commences medication while on study that would meet exclusion criteria

Outlining baseline assessment and stop criteria with regard to clinical history and examination, laboratory results and non-commitment to engage study team.

tested negative for SPN3 on Day 2 and Day 7 samples. By using double inoculation, we hope to improve our carriage rate. A higher carriage rate would allow future vaccine studies to require a reduced sample size to reach statistical significance, in turn reducing time and cost of the study. Based on results of the dose-ranging study, the Chief Investigator (CI) will decide if double inoculation should be applied in the reproducibility study. If a second inoculation is included in the reproducibility study, it will be only given to those participants who are carriage negative at Day 2 and Day 7.

Dose ranging study (Phase A)

We will start at 10,000 CFU/naris and after n=10, escalate to 20,000 CFU/naris (n=10), 80,000 CFU/naris (n=10), 160,000CFU/naris and then 320,000 CFU/naris if safe to do so. We may increase the dose further depending on colonisation rates after consultation with the Data, Safety and Monitoring Board (DSMB). Targeted repeat inoculations will be given to participants on Day 14, if they have tested negative for SPN3 on Day 2 and Day 7 samples. If optimum colonisation rates are achieved (≥60%) in the lower doses, the higher dose escalation groups may not be completed – this will be discussed with the Trial Steering Committee (TSC) before a decision to omit the higher doses is made. Dose escalation can occur once 10 participants have been inoculated with the previous dose, provided there are no safety concerns.

The first challenge cohort at 10,000CFU/naris will start slowly with a smaller group (n=1-4) inoculated per week for safety before completing the group (n=10) (these participants are expected not to carry). If there are no safety concerns, the dose will then be escalated to 20,000CFU/naris with an additional 10 participants and so on. During the initial safety phase, additional participants will be "on-standby" to receive inoculation in case selected participants are unable to attend due to illness/other problems/ineligibility. These "on-standby" participants will be screened prior to the inoculation visit and will be asked to keep the inoculation day free in case they are needed. If they are not needed, they will be included in a subsequent group in the study.

Reproducibility study (Phase B)

Thirty-three (33) participants will be challenged with an appropriate dose that resulted in optimal colonisation during the dose escalation study. Additional specific immune parameters will be assessed during this phase to describe human immunity to SPN3 and compare the results with previous data obtained for SPN6B.

Follow-up visits

Participants in Phase A will be followed up at day 2, 7 and 13 post inoculation. A repeat inoculation may be performed at day 14 and further follow up visits at day 16, 21 and 28 will occur.

Participants in Phase B will be followed up at day 2, 7, 13, 16, 21 and 28. Additional inoculation may be carried out at day 14 at the discretion of the CI. If a second inoculation is included, only those who are negative for SPN3 at days 2 and 7 will receive the second dose.

Samples taken during these visits are detailed below.

Visit windows

This schedule has been developed with a flexible sample window to ensure that appointments can be made with sufficient accuracy for safety and immunogenicity assessment whilst remaining practicable for volunteers. Whilst the scheduled visits will be adhered to as closely as possible, follow up visits may be arranged within the number of days specified in Figure 2.

Safety monitoring

Due to inoculating participants with pneumococcus, there is a low risk of otitis media (OM) and sinusitis, and very low risk of pneumonia, bacteraemia and meningitis. While the risk to individuals of developing any infection is very low (40%), Malawian adults experience natural colonisation at any time, and the incidence of invasive disease is 20/100 000 patient years[31]. The study is designed to ensure any risk is minimal by rigorous safety procedures including daily monitoring and providing participants with a thermometer and a course of amoxicillin tablets in case of emergency, 24-hour emergency telephone contact with researchers, including close individual daily monitoring, and access to hospital facilities and prompt treatment if required. To further mitigate risk, participants will be provided with accommodation for three (3) nights immediately following nasal challenge. Twice daily checks could replace the resident period if a participant determines that it is not possible to stay at study accommodation for three nights. Participants reporting symptoms consistent with pneumococcal disease (e.g. [but not limited to] ear pain, sore throat, cough, fever) will be reviewed in person by clinicians within the research team.

Sampling methods

Sampling methods used in this study have been successfully used during the PCV13 study at MLW, and are well-tolerated by participants[21,27]. All samples will be collected by members of the team who are trained and delegated to appropriately do so.

Oropharyngeal swab: Simple posterior pharyngeal swabbing on a dry swab (throat swabs) and placed into culture media will be obtained for detection of bacterial and viral pathogens by microbiological and molecular techniques.

Nasopharyngeal and throat swab for COVID-19: Simple posterior pharyngeal swabbing on a dry swab for viral co-infection testing.

Nasosorption: Strips collect concentrated nasal lining fluid to measure inflammatory responses induced by infection that may be associated with increased colonisation density and acquisition. The strips will be held inside the nares for a minimum of 2 minutes, removed and placed in the micro-centrifuge tube for storage.

Nasal wash: Five millilitres of saline is instilled into the nostril and held for a few seconds in the nares before being allowed to drip into a sterile Galli pot. This is repeated twice in each naris using 20ml saline in total.

Figure 2. Flowchart of schedule study visits.

Ideally, a minimum 10ml of wash should be obtained in the Gallipot.

Nasal cells: Nasal cells (cup-shaped end of a plastic) will be collected using a nanosampling method in which cells are obtained through minimally invasive superficial nasal scrape biopsies (rhinoprobe). A maximum of 4 samples (2 per nostril) will be obtained at each sampling visit. The rhinoprobes are placed in transport media immediately after collection.

Blood sampling: Blood samples will be collected using venepuncture at screening visit, Day 13 and Day 28 of the study for analysis including FBC (for

safety) and other laboratory measures including, but not limited to, serum immunoglobulins, Peripheral blood mononuclear cell (PBMC) populations and host RNA expression.

Saliva samples: The participant will provide a saliva sample by spitting 1ml of saliva into 1ml of skim milk-tryptone-glucose-glycerine (STGG) medium in a falcon tube. These will be transported to the lab on ice and stored at -80ºC pending molecular testing.

Urine samples: Clean catch urine (~20 ml) will be collected from each participant at every post-inoculation visit for pneumococcal polysaccharide antigen screening using the Luminex-based extended-range antigen capture assay.

Determination and monitoring of colonisation

Colonisation will be defined as result of nasal washes taken at 2, 7, 13, 16, 21 and 28-days post-inoculation. Nasal washes will be plated onto culture media and incubated overnight at 37°C in 5% carbon dioxide (CO_2) as per SOP. Colonies will be confirmed as SPN using classical microbiological techniques such as (i) typical draughtsman-like colony morphology, (ii) the presence of α-haemolysis, (iii) optochin sensitivity, (iv) solubility in bile salts and (v) Gram-positive diplococci. Typing by latex agglutination will be done using Immulex Pneumotest reagents (Statens Serum Institute, Copenhagen, Denmark) to confirm pneumococcal serogroup. Isolates will be frozen at -80^{0}C for storage. Results from the cultured nasal wash will also be confirmed using a multiplex quantitative Polymerase Chain Reaction (qPCR) based methods of bacterial detection.

Molecular methods of detection

DNA will be extracted from bacterial pellet post-nasal wash sample centrifugation. SPN3 detection will be done by multiplex qPCR. This technique will enable us to detect individuals who are potential carriers with very low bacterial density.

Viral detection and quantification

Viral multiplex qPCR for detection and quantification will be performed on DNA and RNA of stored swab and/or nasal wash to detect all common respiratory viruses.

Immune measurements

Serotype-specific responses and their association with both acquisition and clearance of colonisation (density and duration) will be measured. We will compare antibody levels and function between those colonised and those protected against colonisation. Levels of immunoglobulin to the capsular polysaccharides SPN3 in serum and nasal washes before and after inoculation will be determined. Levels of SPN3-specific memory B cells will be assessed using Peripheral blood mononuclear cells (PBMCs) collected pre and post inoculation.

Cellular responses

Flow cytometry will be used to examine the induction of antigen-specific cellular responses in blood including B cells and T cells. Mucosal cellular responses will also be measured by flow cytometry. Additionally, the mucosal inflammatory response associated with inoculation will be evaluated using 30-plex Luminex or other efficient method to detect cytokines and chemokines in nasal filters.

Termination of colonisation

Participants who have been positive for *Streptococcus pneumoniae* colonisation at any time point, and who have not subsequently had two consecutive negative nasal wash samples, will be asked to take oral amoxicillin 500mg three times daily for 5 days at the end of the study in order to clear colonisation.

Withdrawal criteria

Participants may withdraw from the study at any time and that this will not have any effect on them. Samples already collected prior to withdrawal will continue to be used in the research.

Participants may be withdrawn from the study for the following reasons:

- If they become pregnant
- If they develop a condition that forms part of exclusion criteria
- If a medication is started that would affect the study results (e.g., antibiotics)
- Any concern about participant safety including that posed by new medication prescriptions or medical issues, or compliance with monitoring appointments (at the discretion of the study physician).

Sample size and justification

We have adopted a stepwise approach to escalating the inoculation dose as previously described by Hazenberg *et al.*[30]. In brief, the protocol is designed to minimise the possibility of repeatedly trying to attain colonisation at a particular dose. Small groups of participants will be inoculated then move to larger groups and escalating to higher doses. This will maximise the safety of study participants and give a reasonable precision of colonisation rates.

We have given lower and upper bounds for the number of participants depending on which dose finally works. We expect 43 participants to complete the study with a single inoculation dose and a colonisation rate of 45%, with 95% confidence level, and a margin of error of 15%. We will recruit a maximum of 104 participants with an estimated rate of 20% for drop out/screening failure. This would mean having a maximum of 83 volunteers for both study arms (50 in Phase A and 33 in Phase B).

Statistical analysis plan

Statistical Analysis will be performed by statisticians within MLW following pre-defined Statistical Analysis Plan. Given

the design nature of this study, descriptive analysis will be the main analysis, unless otherwise stated.

The binary endpoints (presence/absence of pneumococcus at each time point) will be summarised using number (%) with 95% confidence interval of participants with an event at each time point. Density of pneumococcal colonisation at different time points will be available for those who have a recorded density (positive for colonisation) and will be analysed in two ways.

a. The density will be summarised using number, mean, geometric mean, standard deviation, median, minimum, maximum, and 95% confidence at each time point.

b. The area under the curve of log density of pneumococcal colonisation over time will be derived and summarised using the above descriptive statistics.

The duration of pneumococcal colonisation in nasal washes collected following experimental pneumococcal inoculation at the end of study will be determined by the presence of pneumococcus in the most recent visit.

All statistical analyses will be performed using appropriate software.

Participants who are natural carriers of non-SPN3 pneumococcus at screening will be excluded from the primary analysis. They will be included in analysis of exploratory outcomes.

Adverse event and serious adverse event monitoring

Non-serious adverse events will be collected systematically during the research and recorded in the case report form. Participants will keep a log of symptoms, and this will be summarised and reported to the DSMB. Any SAE as defined in ICH-GCP occurring to a research participant will be reported to the DSMB, NHSRC and study sponsor within 24 hours of the study team becoming aware of the event. In this event, the research will be stopped temporarily for investigation and any further work deferred until DSMB and NHSRC advice has been provided to the SSC for consideration. All SAEs will be followed until resolution/stabilisation or until the end of the participants last study visit.

Study auditing

The Clinical Research Support Unit (CRSU) at MLW developed a trial monitoring plan based on the trial risk assessment. CRSU monitors will verify that the trial is conducted in compliance with the protocol and its associated written standard operating procedures (SOPs) and that data are generated, documented and reported in compliance with good clinical practice (GCP) and the applicable regulatory requirements.

Ethical approvals

The study protocol has been approved by the National Health Sciences Research Committee (NHSRC) of Malawi (22/12/3106) (5 May 2023), and by the Liverpool School of Tropical Medicine Research Ethics Committee (22-088) (15 Sep 2023). Amendments to the protocol will require approval by the NHSRC and the Sponsor.

Study sponsorship and funding

The study is supported from the Malawi Accelerated Research in Vaccines, Experimental and Laboratory Systems (MARVELS) grant under Liverpool School of Tropical Medicine (LSTM) as sponsor. It is funded by the Wellcome Trust (211433). The funder and the sponsor had no role in the study design nor in the decision to submit the report for publication.

Study registration

This trial is registered with the Pan African Clinical Trial Registry, registry number PACTR202309751708712 (13 Sep 2023).

Compensation

Participants will be compensated for travel, communication, time spent and burden during each study visit (20,000 Malawian Kwacha per visit, 180 000 Malawian Kwacha if all study-visits are completed). The sums offered in this study are consistent with compensation guidelines published in Malawi, paid pro-rata (per activity and not dependent on study completion)[32].

Confidentially and anonymity

Only information of direct relevance to the study will be collected. Only authorised members of the research team will have access to any personal information. All electronic records containing personal information will be stored in a password protected database on a password protected server at MLW. Electronic case report form data will be collected on encrypted study-specific tablet devices and synchronised daily onto the MLW server. This approach is well established at MLW, and the policy governing MLW data management policy is available upon request. Paper documentation containing personal information will be kept in a locked filing cabinet in a locked room in the Queen Elizabeth Central Hospital research clinic at ward 3A.

Each participant will be assigned a unique non-identifiable study number by a member of the clinical research team at recruitment. Unlinked non-identifiable clinical data will be stored and analysed at the MLW-laboratories and collaborating laboratories.

Sample and data storage

MLW will act as custodian for all data and samples collected during the study. With consent from participants, samples will be sent to international collaborating laboratories to utilise specialist expertise. All samples will be labelled with the anonymised study number. Samples may be stored by MLW for up to 5 years. After this time, the remaining samples may be destroyed or gifted for future use in research with consent obtained at recruitment from participants (optional).

Dissemination of findings

We intend to disseminate findings from this study amongst the scientific community, publish in peer reviewed scientific journals, and present data at appropriate local, national and international conferences. We will produce a lay report of our findings which will be made available to all participants. A close-out report will be produced and shared to the NHSRC and LSTM (sponsors) at the end of the study and a final report once data are published.

Study status

The study started on 19th September 2023 with 4 participants undergoing screening visit (including data collection). To date, the study is still recruiting participants, currently in the 160,000CFU cohort.

Discussion

Since conjugated vaccines do not elicit the level of antibodies required to confer protection against SPN3[12,13], a CHIM using *Streptococcus pneumoniae* serotype 3 is relevant for Malawi and the sub-Saharan Africa region. This study will establish a dose of SPN3 that can experimentally colonise healthy adults in an EHPC in Malawi and demonstrate if with appropriate safety precautions in place, an SPN3 human challenge model can be safely established for efficient vaccine-efficacy trials. Furthermore, the study will identify the immune correlates of protection against SPN3 pneumococcal carriage to guide more effective vaccine immunogenicity evaluations considering that there are concerns that pneumococcal conjugate vaccines in sub-Saharan Africa sub-optimally interrupt *Streptococcus pneumoniae* vaccine-serotype carriage and transmission[6,33]. As previously demonstrated by other studies, pneumococcal colonization using SPN6B is asymptomatic[34]. However, a recent study using SPN3 in Liverpool showed that colonized participants frequently reported symptoms, particularly sore throats within the first 7 days post inoculation with data suggesting that the events were related to inoculum dose used. Furthermore, antibiotic usage was greater in the higher dose inoculum groups[26]. We have previously confirmed that the experimental human pneumococcal challenge SPN6B model can be used in Malawi to test vaccines, and to explore the differences that underpin the observed differences in epidemiology[21,24].

Conclusion

This model will provide an important platform for investigating vaccine efficacy against SPN3 acquisition and impact on colonization density for this clinically important disease-causing serotype.

Ethics and consent

The study protocol has been approved by the National Health Sciences Research Committee of Malawi (22/12/3106) (5 May 2023), and by the Liverpool School of Tropical Medicine Research Ethics Committee (22-088) (15 Sep 2023). The participant will be invited to provide written informed consent if the study team are satisfied that they meet the eligibility criteria and that they have voluntarily agreed to take part in the research.

Data availability

Underlying data

No data are associated with this article.

Reporting guidelines

SPIRIT checklist for Experimental Human Pneumococcal Carriage using Streptococcus pneumoniae serotype 3 in Malawi: a dose ranging and reproducibility human infection study. https://zenodo.org/doi/10.5281/zenodo.11025417[35].

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgements

We are thankful to Queen Elizabeth Central Hospital and Mwaiwathu Private Hospital for their support and agreeing to facilitate inpatient clinical care should it be required. We are grateful to the Clinical Research Support Unit (CRSU) at MLW for their invaluable dedicated work, support, and guidance in preparing for this study.

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Open Peer Review

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Version 1

Reviewer Report 05 September 2024

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✔ **David W Cleary**
University of Birmingham, Birmingham, UK

The authors present a study protocol of a controlled human infection model (CHIM) using a serotype 3 expressing strain of *Streptococcus pneumoniae*. The CHIM will evaluate establishment of carriage in a safety/dose ranging exercise in an adult Malawian cohort. The study will recruit a maximum of 104 volunteers in total, all healthy adults, in two phases. The first will determine dose to establish carriage in at least 60% of participants, and the second will examine carriage reproducibility at that dosing level in addition to examining immunological response.

The challenge model is well established for other serotypes, and has been done using the serotype 3 strain proposed here, albeit it in a UK setting. The value of this particular study extends beyond the evaluation of EHPCs as a tool for vaccine studies, also focussing on a serotype to which current vaccines struggle to produce effective protective responses.

I have only minor comments.

Introduction

There seems limited detail on the strain of the serotype 3 pnuemococcus. Was this isolated from an adult or child? Do you have information on ST/GPSC that can be included?

Background detail on carriage prevalence in Malawi would be useful, including frequency of serotype 3 in carriage and disease.

Study Protocol

What do the authors envisage as exploratory analyses for those colonised with non-SPN3 pneumococci?

Study Procedures

Nasal cells: What does "(cup-shaped end of a plastic)" mean?

Molecular Methods of Detection and Viral Detection: Can the authors specify the multiplex targets that will be used? The phrasing implies both generic pneumococcal targets and those specific to the serotype 3 strain. It might also be useful to state which viruses are being detected and how.

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?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Streptococcus pneumoniae; bacterial genomics; epidemiology

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 03 September 2024

https://doi.org/10.21956/wellcomeopenres.23220.r95163

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?

Paul Vincent Licciardi
Infection, Immunity and Global Health, Murdoch Children's Research Institute (Ringgold ID: 34361), Parkville, Victoria, Australia

This manuscript by Chikaonda and colleagues describes the clinical protocol for the establishment of a pneumococcal serotype 3 experimental human challenge model in healthy adults in Malawi. This builds on the investigators extensive experience with such challenge models, including a related serotype 3 model that was conducted in the UK. The rationale for a counterpart study in a setting such as Malawi has been clearly outlined by the authors given the higher and persistent pneumococcal carriage rates, even in the context of PCV use. Serotype 3 is a highly virulent strain of the bacteria causing IPD, and with current PCVs not having much of an impact on disease. In some settings, serotype 3 IPD is rising, giving cause for concern about how to reduce disease burden by this serotype. The authors suggest that differences in serotype 3 characteristics and

biology are a contributor to the inefficient host response. The challenge model would be uniquely placed to address these questions by examining bacterial and host immune factors during experimental infection.

I have a few comments that the authors may wish to consider:

1. There are several differences with this model compared to the previously published study by the same authors. This includes the requirement to achieve ≥60% colonisation (compared to ≥50% previously) and the dose ranging studies going as high as 320,000 cfu (compared to 160,000 cfu). Some additional details around the justification for this would be helpful for readers to understand. For example, was this based on the higher serotype 3 force of infection in Malawi?

2. The study design may require volunteers to be re-challenged. How many are likely to need this, based on the previous paper? The requirement for re-challenge as I understand is a negative NP swab result but could positive serology be also used or would the time frame be too short to see any response. This relates to the fact that serotype 3 is rarely found in carriage (is that true also in Malawi) so there may be missed opportunities to detect a carriage episode. Some comments about this would be useful to include.

3. Would a high serotype 3 antibody response (or B cell level) be a reason for exclusion into the study?

4. The introduction should include some information on what the carriage rate of serotype 3 is in Malawi.

5. Will there be equal males and females included in the study?

6. The study timelines were confusing. It says that study duration started in September 2023 and is expected to finish in June 2024 but then later it says as of September 2023, 4 participants were undergoing screening. Have any participants been recruited since September 2023 and are there any initial safety issues? Some further information would be ideal.

7. In the safety monitoring section, it says that there is a 40% risk to individuals of developing any infection in this model which is stated as ‘very low’. This doesn’t sound very low so further explanation of this is warranted.

8. In the immune measurements section, please specify which samples will be used for which measurements.

9. In the sample size justification, it states that 45% colonisation is expected which is lower than what is stated earlier (≥60%). What is the reason for this?

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?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pneumococcal vaccines, immunology

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 01 September 2024

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Sophie Belman
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This protocol describes an experimental pneumococcal human challenge model applied to serotype 3. Serotype 3 is notorious for its poor response to pneumococcal conjugate vaccines. This is postulated to be a result of its overly sugary capsule. This study aims to establish the optimal dose for inoculation and colonization with serotype 3 and estimate the immune correlates of protection. The participants will be healthy adults between 18 and 50 years old in Malawi. The exclusion criteria are well defined. The protocol includes a clever sequential dose increase with 5 groups of 10 participants until reaching a dose at which 6 of the 10 individuals are colonized (60% colonization). The dose in the group with >60% uptake will then be included in a reproducibility arm with 33 participants. The sample size for the study is described and accounts for drop-outs. Recruitment of 104 individuals is estimated to allow 83 individuals in total to complete the study assuming a 20 percent drop out rate. Participants will be inoculated and followed up 2,7, and 13 days after inoculation and then with 3 additional follow ups to day 28. Phase B will also be followed from days 2-28 six times. Sampling methods have been employed previously and are standard. Colonization will be terminated using amoxicillin if there are not two consecutive negative nasal washes.

Statistical analyses, while largely descriptive, are outlined here. Adverse events and withdrawal

criteria are outlined and ethical considerations are addressed.

This protocol will establish a human challenge model for serotype 3 which will allow development of novel vaccines. This is important to limit pneumococcal disease caused by a vaccine evading, clinically important serotype (SPN3). This is a well detailed protocol which will advance our understanding of serotype 3 acquisition.

I include specific comments below:

Introduction:

- Are there numbers of carriage rates in Malawi? It would be nice to see these to determine how many of your recruited individuals may already be carrying SPN.

- There is a citation missing in the 6th paragraph of the introduction regarding the fact that half of EHPC participants develop stable colonization for 1-3 weeks. Are these numbers from the UK or Malawi? Where do these numbers come from?

- Inclusion of the 96 participants challenged by the LSTM team is important however it is unclear if this was in Malawi or elsewhere. Please clarify.

Study Protocol:

- In objective 1 “Adult” should not be capitalized.

Methods:

- It is unclear what exploratory analyses are being conducted for individuals already colonized with non-SPN3.

Consent:

- The second sentence of the Consent paragraph includes multiple duplicated words “before written consent is obtained”.

Dose Ranging Study (Phase A):

- It is unclear what will determine whether you include 1, 2, 3, or 4 individuals in the first challenge cohort. Please clarify.

- Will the on-standby group be remunerated for their time as they may have to take time of work to be on-standby?

Follow-up visits:

- Is the additional inoculation at day 14 at CI discretion only if the participant was not carrying in the prior three visits? What will inform the CIs decision? Please make this section more clear.

- Sampling Methods: Nasal Cells: What does the “cup-shaped end of a plastic” mean. It appears there is a typo.

Sample size and justification:

-

- This paragraph does not read easily. It is unclear how 43 participants became 104 to be recruited. I see the 83 participants resulting from a 20% dropout rate from 104 recruited. Is the 43 participants from both study arms and how was this estimated?

Statistical Analysis Plan:

- It would be preferable for you to include what software you'll be using for example, R, Stata, python, Excel. Will the analysis scripts be published alongside the results?

- Sample and data storage: I am unclear why you have included that after 5 years the samples will be destroyed or gifted for future research conset is "optional".

Study Status:

- I do not understand why 4 participants recruited in September 2023 are highlighted. Can this be updated to reflect the numbers in August 2024?

Discussion:

- CHIM is never defined.

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?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology, bioinformatics, Streptococcus pneumoniae, genomics.

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.