Lactamica 9: Defining upper respiratory colonisation and microbiome evolution in mother-infant pairs following *Neisseria lactamica* inoculation in late pregnancy

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Principal Investigator: Dr Anastasia A. Theodosiou

Chief Investigators: Dr Christine E. Jones, Professor Robert C. Read

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1.4	11/02/22	AA Theodosiou	Study amendment

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Abbreviations and acronyms

A&E	Accident and emergency
AE	Adverse event
API	Analytical profile index
AR	Adverse reaction
CES	Clinical and Experimental Sciences
CFU	Colony forming units
GMP	Current Good Manufacturing Practices
CHI	Controlled human infection
CI	Chief Investigator
CIR	Centre for Inflammation Research
CRF	Case report form
CTA	Clinical trials assistant
DNA	Deoxyribonucleic acid
ESC	External Safety Committee
GDPR	Data Protection Act 2018 General Data Protection Regulation
HRA	Health Research Authority
HTA	Human Tissue Authority
ICH GCP	International Conference on Harmonisation E6 Good Clinical Practice CPMP/ICH/135/95
LyoNlac	Lyophlised *N. lactamica*
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
MIC	Minimum inhibitory concentration
NHS	National Health Service
NIHR-CRF	National Institute for Health Research Clinical Research Facility
PAH	Princess Anne Hospital
PBS	Phosphate buffered saline

PCR	Polymerase chain reaction
PI	Principal Investigator
PIS	Participant information sheet
PPI	Patient and Public Involvement
QA	Quality Assurance
R&D	Research and Development
REC	Research Ethics Committee
rRNA	Ribosomal ribonucleic acid
RTI	Respiratory tract infection
SAE	Serious adverse event
SAR	Serious adverse reaction
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TOPS	The Over-volunteering Prevention System
UAR	Unexpected adverse reaction
UHS	University Hospital Southampton
UoE	University of Edinburgh
UoS	University of Southampton
URT	Upper respiratory tract
X-gal	5-bromo-4-chloro-3-indolyl-β-galactopyranoside
ISF	Investigator Site File

1.0 Investigator and study site details

1.1 Chief Investigators (CIs)

Dr Christine E Jones

Associate Professor & Honorary Consultant in Paediatric Infectious Diseases & Immunology

Faculty of Medicine and Institute for Life Sciences, University of Southampton

NIHR Southampton Clinical Research Facility and Southampton Biomedical Research Centre

Level F, South Academic Block, University Hospital Southampton

Tremona Road, Southampton SO16 6YD

Email: C.E.Jones@soton.ac.uk

Professor Robert C Read

Professor and Consultant in Infectious Diseases

Clinical and Experimental Sciences, University of Southampton

NIHR Southampton Clinical Research Facility and Southampton Biomedical Research Centre

Level C, South Academic Block, University Hospital Southampton

Tremona Road, Southampton SO16 6YD

Email: R.C.Read@soton.ac.uk

1.2 Principal Investigator (PI)

Dr Anastasia A Theodosiou

Medical Research Council Clinical Research Training Fellow

Specialty Registrar Infectious Diseases and Medical Microbiology

Clinical and Experimental Sciences, Level C, South Academic Block

University Hospital Southampton, Tremona Road, Southampton SO16 6YD

Email: A.Theodosiou@soton.ac.uk

1.3 Co-investigators

Dr David W Cleary

Senior Research Fellow in Microbial Sciences

Clinical and Experimental Sciences, Level C, South Academic Block

University Hospital Southampton, Tremona Road, Southampton SO16 6YD

Email: D.W.Cleary@soton.ac.uk

Dr Jay R Laver

Senior Research Fellow in Molecular Biology

Clinical and Experimental Sciences, Level C, South Academic Block

University Hospital Southampton, Tremona Road, Southampton SO16 6YD

Email: J.R.Laver@soton.ac.uk

Dr Adam P Dale

National Institute for Health Research Academic Clinical Lecturer

Specialty Registrar Infectious Diseases and Medical Microbiology

Clinical and Experimental Sciences, Level C, South Academic Block

University Hospital Southampton, Tremona Road, Southampton SO16 6YD

Email: A.P.Dale@soton.ac.uk

Mr Matthew Coleman

Consultant in Maternal and Fetal Medicine

Princess Anne Hospital

Coxford Road, Southampton SO16 5YA

Email: Matthew.Coleman@uhs.nhs.uk

1.4 Collaborators

Professor Debby Bogaert

Senior Scottish Clinical Fellow and Honorary Consultant in Paediatric Infectious Diseases

Centre for Inflammation Research, University of Edinburgh

Queen's Medical Research Institute, Edinburgh BioQuarter

47 Little France Crescent, Edinburgh, EH16 4TJ

Email: D.Bogaert@ed.ac.uk

1.5 Study sites and locations (Table 1)

Study activities will take place at the following research sites and locations (Table 1):

- University Hospital Southampton (UHS) National Institute for Health Research Clinical Research Facility (NIHR-CRF), Tremona Road, Southampton, SO16 6YD.
- University of Southampton (UoS) Clinical and Experimental Sciences (CES) department, Tremona Road, Southampton, SO16 6YD.
- The volunteer's home, with permission from the volunteer. The study doctor may be accompanied by a study nurse, clinical trials assistant (CTA) or another member of the clinical study team for any home visits.
- Princess Anne Hospital (PAH), Coxford Road, Southampton, SO16 5YA, with permission from responsible staff/authority.
- Any other clinical area where the volunteer is receiving care, with permission from responsible staff/authority.
- University of Edinburgh (UoE) Centre for Inflammation Research (CIR), 47 Little France Crescent, Edinburgh, EH16 4TJ.

Study visit / activity	**Study site / activity location**
Screening visit (Visit 1)	UHS NIHR-CRF; OR PAH; OR volunteer's home
Inoculation visit (Visit 2)	UHS NIHR-CRF
Birth visit (Visit 3)	UHS NIHR-CRF; OR volunteer's home; OR PAH / other clinical area if volunteer not yet discharged home
Follow-up visits (Visits 4, 5 and 6)	UHS NIHR-CRF; OR volunteer's home
Initial sample processing (sample aliquoting, storage)	UoS CES department
N. lactamica processing (culture, identification tests, PCR, DNA extraction, isolate storage)	UoS CES department
Microbiome sample processing (DNA extraction, 16S rRNA gene sequencing)	UoE CIR
Clinical, microbiological and genomic data analysis	UoS CES department; UoE CIR

Table 1: Study sites and locations.

2.0 Introduction

2.1 Upper respiratory pathobionts and infant disease

Upper respiratory tract (URT) pathobionts are common colonising bacteria that are capable of causing disease in immunocompetent individuals (e.g. *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*). Although harmless in many hosts, these organisms remain a leading cause of global childhood death and disease: respiratory tract infection (RTI) is the most common cause of mortality under 5 years, and meningitis is the ninth most common (15% and 3% of 5.4 million deaths in 2017)[1]. Infants (under 1 year) are most at risk of invasive disease, accounting for 75% (492,000/653,000) and 61% (89,000/146,000) of childhood deaths due to RTI and meningitis, respectively. Further, although childhood mortality has fallen considerably over the past 30 years, disease incidence remains high, with over 68 million annual episodes of childhood RTI[2], and meningitis incidence has increased from 2.5 million to 2.8 million in that time[3]. While many such infections are vaccine-preventable, global access to and acceptance of vaccinations remains incomplete: 20% of children globally are not fully vaccinated, and up to half of surveyed European adults mistrust vaccines[4], such that 1.5 million vaccine-preventable childhood deaths occur each year. Other infections of global importance are not currently preventable through maternal or childhood vaccination (e.g. group B streptococcus and respiratory syncytial virus), or are caused by bacterial serotypes not covered by existing vaccines (e.g. *S. pneumoniae*). These challenges are compounded by rising global antimicrobial use and resultant antimicrobial resistance, and it is estimated that 214,000 neonates (under 1 month) die of resistant infections each year[5].

In recent years, there has been increasing research into the relationship between URT pathobionts, childhood disease, and the evolving infant microbiome (the site-specific total microbial community). Taxonomic profiling (e.g. by pan-prokaryotic 16S rRNA gene sequencing) demonstrates infant URT over-representation of the genera *Streptococcus, Haemophilus* and *Moraxella* during acute RTI, and depletion of the genera *Corynebacterium, Dolosigranulum* and *Staphylococcus*[6]. Infant microbiome profiles also correlate with RTI severity, with a *Haemophilus*-dominant nasopharyngeal profile predicting intensive care admission[7]. Moreover, pathobiont-rich microbiota and recurrent RTI are associated with accelerated microbiome evolution (approximating that of older infants)[8], childhood chronic wheeze and early allergic sensitisation[6,9].

Infant RTIs are often preceded by microbiome changes, including enrichment for URT pathobionts and loss of topography (i.e. blurring of distinction between microbiomes at adjacent anatomical sites)[10], suggesting a causal role in developing infection. However, as up to 93% of infants are colonised with at least one URT pathobiont at any time[11], it appears that pathobiont colonisation is not sufficient to cause infection: rather, the presence of pathobionts may indicate a state of dysbiosis (microbial dysregulation), in which disease-causing bacteria and/or viruses can cause infection in a previously resilient ecological niche.

2.2 Manipulating upper respiratory microbiota to prevent infant disease

Given these findings, it is worth considering whether developing infant URT microbiota could be manipulated to encourage favourable commensalisation, limit pathobiont colonisation, and reduce disease. As early as 1963, nasal inoculation with low-virulence *Staphylococcus aureus* strain 502A was used to prevent neonatal colonisation by high-virulence strain 80/81 in an inpatient outbreak setting[12]: carriage of the high-virulence strain fell to 5% (4/76) compared with 41% (45/111) in uninoculated controls, with evidence of staphylococcal disease in only 9% (9/96) of 502A-colonised neonates and household members, compared with 73% (22/30) of 80/81-colonised. Pharyngeal inoculation with strain 215 alpha-haemolytic streptococci has also been shown to reduce pharyngeal pathobionts (*Escherichia coli, Klebsiella pneumoniae, S. aureus*) in neonatal intensive care inpatients, although no clinical outcomes were reported[13]. A variety of probiotics (living microorganisms administered to confer a health benefit) have been trialled in children and adults, with some reporting a reduction in upper RTI incidence and duration[14]. However, high quality evidence is lacking, with significant inter-study variation in the probiotic strain (e.g. lactobacilli, streptococci and bifidobacteria), preparation (e.g. capsules, dairy supplement), dose and regimen. That being said, there is evidence that many probiotics are safe in pregnancy, neonates and lactating mothers, with some already available over-the-counter to these groups[15].

To date, there has been only one interventional study reporting on the infant URT microbiome[16]: a nested factorial double-blind placebo-controlled randomised trial involving upper airway sampling (from 1 week to 3 months of life) from 695 infants following maternal administration of high-dose vitamin D3 (2400 IU per day), n-3 long chain fatty acids (LCFA), both or placebo (from 24 weeks gestation until 1 week post-partum). One month old infants whose mothers received LCFA had reduced upper airway *Gemella* and *Veillonella* abundance, and reduced putative *S. pneumoniae* for those receiving vitamin D3; these microbiota correlate

with increased risk of later asthma development, and the ability to reduce their relative abundance suggests a possible avenue for modifying airway colonisation.

2.3 Understanding infant upper respiratory microbial evolution

Despite growing research interest, the origins and evolution of the URT infant microbiome remain incompletely characterised. Broadly speaking, neonates become rapidly colonised at birth with a highly diverse pioneer microbiome of presumed maternal and environmental origin, with very little site-specific differentiation[17]. Within the first few days of life, and certainly by one week, differences appear between discrete anatomical niches (e.g. skin, gastrointestinal tract, mouth), with reduced species diversity and increased microbial density at each niche[18]. Indeed, microbiome samples from different 6-week-old infants at the same site are more homologous than samples from the same infant at different sites[19]. URT niche differentiation continues over at least the first year of life, with increasing biomass and alpha-diversity (indicating species richness and evenness) at each discrete site[8]. The rate of change slows progressively over the first year, although it remains unclear at what point the infant microbiomes approximate their stable adult-like counterparts.

Longitudinal cohort microbiome studies provide some evidence for horizontal transfer of URT flora between mothers and their infants: there is greater overlap between a neonate's oral flora and that of its own mother than unrelated mothers, and shared bacterial strains (detected by whole genome sequencing) are more long-lived in an infant's mouth than strains not present in its mother[17]. Moreover, a subset of maternal oral strains account for a disproportionately large share of their infants' oral flora. However, such mother-infant cohort studies are lacking for adjacent URT niches (nose and pharynx), so further research is needed.

2.4 Controlled human infection with *Neisseria lactamica*

Unlike probiotic studies (which often employ proprietary food supplements with uncontrolled concentrations of bacteria), controlled human infection (CHI) offers a robust model for studying the impact of a defined bacterial inoculum on a human host. To date, no studies of the maternal or infant URT microbiome in the context of CHI have been performed. However, there is scope to use CHI to improve understanding of infant microbiome acquisition and evolution, and even to manipulate the URT microbiome using controlled commensal inoculation.

Our research group has previously developed a safe and reliable CHI model using nasal inoculation with 10^4-10^5 colony-forming units (CFU) *Neisseria lactamica* (strain Y92-1009) in healthy adults[20]. They have used this CHI model in randomised blinded placebo-controlled studies to characterise *N. lactamica* colonisation density, duration, cellular and humoral immune responses, and genomic microevolution[21]. Our research group has observed colonisation rates of up to 85%, with no serious adverse reactions following over 400 inoculations.

N. lactamica is a common acapsulate pharyngeal commensal that does not cause invasive disease in immunocompotent hosts. To date, there have been 14 published cases of invasive *N. lactamica* in humans, although almost all were in individuals with significant risk factors (e.g. immunosuppresion, indwelling prosthetic material, severe co-morbidities)[22]. Of these, three cases in seemingly immunocompotent children were unconvincing for true *N. lactamica* invasive infection: microbiological diagnoses were based on subjective colorimetric tests with no confirmation by a reference laboratory, and there have been no further reports since 1978.

In addition to being a safe and well-characterised model, *N. lactamica* CHI may be particularly suited to infant URT microbiome research. Carriage studies reveal that natural *N. lactamica* colonisation peaks at over 40% in 1-2 year old children[23], before falling to less than 10% in adulthood[24]. Further, *N. lactamica* carriage correlates inversely with *N. meningitidis* colonisation[25] and invasive disease[24]. Indeed, *N. lactamica* CHI in healthy adults reduces naturally-acquired *N. meningitidis* carriage from 18% to 8% (both by displacing existing colonisation and preventing new acquisition), exceeding the impact of glycoconjugate ACWY vaccination[20]. This displacement effect is not specific to a particular serogroup, unlike available meningococcal vaccines. Further, vaccination with *N. lactamica* whole killed bacterial cells or outer membrane proteins protects mice from subsequent haematogenous challenge with *N. meningitidis*[26]. Thus, *N. lactamica* inoculation may offer a viable model for studying the impact of induced commensal acquisition on infant microbiome development, and even for reducing *N. meningitidis* colonisation and disease in the first year of life.

3.0 Study overview

We plan to perform nasal inoculation with *N. lactamica* (wild type strain Y92-1009) in healthy pregnant women, to establish whether horizontal *N. lactamica* transfer to their neonates occurs, and to characterise the impact on the developing neonatal URT microbiome. If successful, this study will provide a novel model for inducing and capturing a natural colonisation event in neonates. Unlike traditional CHI models, which capture inoculation-induced colonisation, this first-in-man CHI model would study person-to-person commensal transmission, allowing comparison of microbiome changes and adaptive commensal microevolution in mother-infant pairs. We have already conducted relevant Patient and Public Involvement (PPI) research, in which all 12 pregnant women interviewed reported approval for this proposed study, and 11 expressed that they would have been interested in taking part in such a study. Other infections of global importance are not currently preventable through maternal or childhood vaccination (e.g. group B streptococcus and respiratory syncytial virus), or are caused by bacterial serotypes not covered by existing vaccines (e.g. *S. pneumoniae*). These challenges are compounded by rising global antimicrobial use and resultant antimicrobial resistance, and it is estimated that 214,000 neonates (under 1 month) die of resistant infections each year[5].

We will approach healthy pregnant women in their second and third trimesters of pregnancy (Figure 1; Table 2). Eligibility screening and enrolment will take place at 34+0 to 36+6 weeks gestation, and 20 women (not already colonised with *N. lactamica*) will be inoculated nasally with 10^5 CFU *N. lactamica* Y92-1009 at 36+0 to 37+6 weeks gestation. Any natural *N. lactamica* carriers identified at screening will not be inoculated, but will be invited to complete all remaining follow-up study visits.

Samples will be obtained from new mothers (nasopharyngeal, oropharyngeal and saliva) and their neonates (nasopharyngeal and saliva) at 1 day, 1 week and 1 month post-partum. Volunteers may opt-in to collection of an umbilical cord blood sample at delivery and an infant venous blood sample at 1 month post-partum, for storage and use in future studies. Optional breast milk samples will be collected at post-partum study visits, which will be cultured for evidence of *N. lactamica* and stored for use in future microbiome analysis. Volunteers may also complete an optional visit 6 at 15 weeks post-partum, involving maternal and infant pharyngeal, saliva and venous blood sampling, as well as saliva and oropharyngeal swabs from household contacts under 5 years old.

Pharyngeal and saliva samples will be suspended in storage medium, aliquoted and stored at -80°C. *N. lactamica* colonisation will be confirmed using selective agar, Gram stain, microscopy, and analytical profile index (API) testing or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). *N. lactamica* colonisation density will be quantified, isolates will be stored at -80°C, and Y92-1009 strain identity will be confirmed using targeted polymerase chain reaction (PCR).

Microbiome analysis will be performed on thawed aliquots of paired mother-neonate samples, by DNA extraction, 16S rRNA gene PCR, and amplicon sequencing. Poor quality and chimeric sequence reads will be removed, and high quality reads will be trimmed, aligned and clustered for taxonomic classification and statistical analysis.

Paired maternal and neonatal isolates confirmed as *N. lactamica* Y92-1009 will be sequenced, and resulting genomes will be mapped to a complete *N. lactamica* Y92-1009 closed reference genome, to assess for evidence of distinct microevolution.

We will also compare paired microbiome profiles to identify candidate organisms that are present in mothers and their infants. Paired mother-neonate sample aliquots will be thawed and plated onto selective media, and isolates of candidate species will be identified using Gram stain, microscopy, and MALDI-TOF or other relevant microbiological tests. Resulting isolates will be sequenced and analysed for evidence of strain sharing between mothers and their neonates, suggesting horizontal transfer.

3.1 Study overview flowchart (Figure 1)

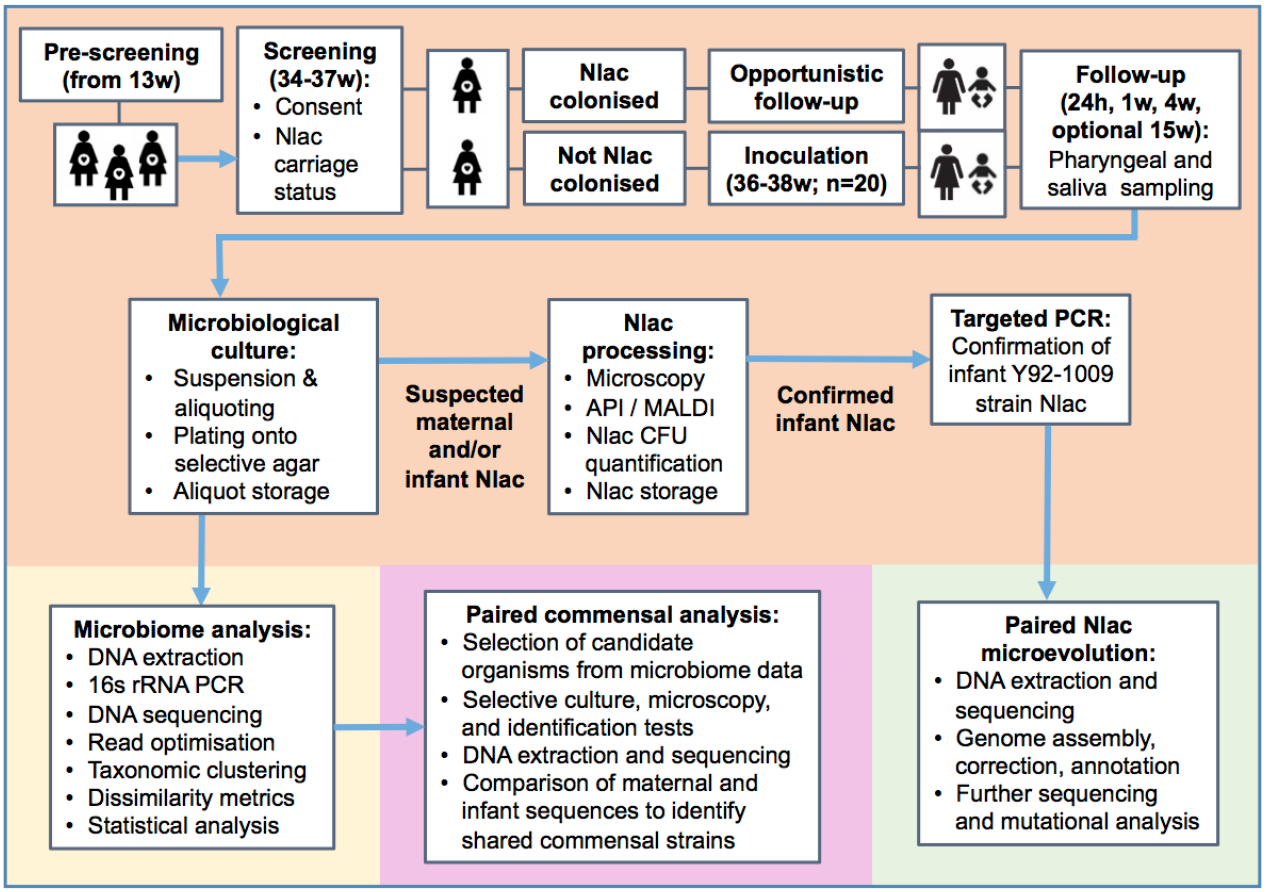

Figure 1: Study overview flowchart. Nlac: *Neisseria lactamica*; m: months; w: weeks; h: hours; API: analytical profile index; MALDI [MALDI-TOF]: matrix-assisted laser desorption/ionization time-of-flight; CFU: colony forming units; PCR: polymerase chain reaction; DNA: deoxyribonucleic acid

3.2 Study visits and activities (Table 2)

	Screening (Visit 1)	Inoculation (Visit 2)	Birth (Visit 3)	Follow-up (Visits 4 and 5)		Optional (Visit 6)
Timeline	**34+0 to 36+6 weeks gestation**	**36+0 to 37+6 weeks gestation**	**0-24 hours post-partum**	**7 +/- 3 days post-partum**	**28 +/- 3 days post-partum**	**15 +/- 2 weeks post-partum**
TOPS confirmation	+					
Participant Information Sheet and Consent Form	+					
Emergency contact card	+					
Volunteer compensation	£50 +/- parking	£50 +/- parking		+/- parking	+/- parking	+/- parking
Clinical review	**+**	**+**	**+**	**+**	**+**	**+**
***N. lactamica* inoculation (nose drops)**		**+** (Unless already colonised)				
Saliva swabs (mother)	**+**	**+**	**+**	**+**	**+**	**+**
Throat swabs (mother)	**+**	**+**	**+**	**+**	**+**	**+**
Nose swabs (mother)	**+**	**+**	**+**	**+**	**+**	**+**
Saliva swabs (baby)			**+**	**+**	**+**	**+**
Nose swabs (baby)			**+**	**+**	**+**	**+**
Breast milk (mother)			Optional	Optional	Optional	Optional
Umbilical cord blood			Optional			
Blood test (baby, 2ml)					Optional	Optional
Blood test (mother, 5ml)						Optional
Saliva +/- throat swabs (children <5)						Optional

Table 2: Study visits and activities. TOPS: The Over-Volunteering Prevention System.

4.0 Study hypotheses, aims, objectives and endpoints

4.1 Study hypotheses

1. Nasal inoculation of pregnant women with 10^5 CFU *N. lactamica* strain Y92-1009 will result in maternal upper respiratory *N. lactamica* colonisation; and
2. Horizontal transmission of upper respiratory *N. lactamica* will occur from experimentally-colonised women to their infants by 4 weeks post-partum.

4.2 Study objective

1. To establish whether horizontal *N. lactamica* transfer occurs from experimentally-colonised mothers to their neonates following nasal *N. lactamica* inoculation in late pregnancy, and to characterise the impact on the developing neonatal URT microbiome.

4.3 Study aims

1. To inoculate pregnant women with nasal *N. lactamica* to establish: *N. lactamica* colonisation kinetics in pregnancy and post-partum; and whether *N. lactamica* is transferred horizontally to their neonates.
2. To collect and analyse URT microbiome samples from mother-infant pairs to characterise longitudinal microbiome evolution following nasal *N. lactamica* CHI in pregnancy.
3. To study longitudinal genetic microevolution of *N. lactamica* in mother-infant pairs.
4. To investigate for horizontal transfer of naturally-occurring oral and pharyngeal commensals between mothers and their neonates.

4.4 Study endpoints

4.4.1 Primary endpoint

1. Confirmation of neonatal (aged 0-31 days) *N. lactamica* colonisation by selective culture of biological (nasopharyngeal and/or saliva) samples.

4.4.2 Safety endpoints

1. Percentage of volunteers with adverse reactions or serious adverse events within the study period.
2. Percentage of neonates with serious adverse events within the study period.

4.4.3 Secondary endpoints

1. Confirmation of neonatal *N. lactamica* Y92-1009 colonisation by targeted PCR of presumptive *N. lactamica* isolates obtained by selective culture of biological samples.
2. *N. lactamica* colonisation density in inoculated volunteers versus their infants across study time points and sample types.
3. Characterisation of microbiome composition, alpha diversity (within one biological sample) and beta diversity (between different biological samples) in inoculated volunteers compared with their infants across study time points and sample types.
4. If horizontal *N. lactamica* transfer is detected in at least some but not all infants: Characterisation of microbiome composition, alpha diversity and beta diversity in infants colonised with *N. lactamica* compared with uncolonised infants across study time points and sample types.
5. If horizontal *N. lactamica* transfer is detected in at least some infants: *N. lactamica* genome sequence for isolates derived from inoculated volunteers compared with their infants across study time points and sample types, and comparison with an *N. lactamica* Y92-1009 closed reference genome.

4.4.4 Exploratory endpoints

1. If natural *N. lactamica* carriers are identified at screening: Characterisation of *N. lactamica* colonisation density and microbiome profiles across study time points and sample types for mother-infant pairs following inoculation in pregnancy compared with natural colonisation.
2. Genome sequence of non-*N. lactamica* commensal species present in volunteers compared with their infants, to assess for evidence of shared commensal strains.
3. If *N. lactamica* is not detected by microbiological culture, but a signal corresponding to *N. lactamica* or genus *Neisseria* is identified by microbiome analysis: Attempted identification of *N. lactamica* Y92-1009 by targeted PCR of a thawed sample aliquot.
4. Collection of umbilical cord blood at birth (Visit 3); infant venous blood at 4 weeks (Visit 5) post-partum; infant and maternal venous blood at 15 weeks post-partum (Visit 6): storage of serum for use in future immunological analyses, to investigate association between *N. lactamica*-specific immunoglobulin and *N. lactamica* colonisation in volunteers and their infants.
5. Collection of maternal breast milk (Visits 3 to 6) for identification of *N. lactamica* by culture and future microbiome analysis.
6. Collection of saliva and oropharyngeal swabs (Visit 6) from household contacts under 5 years old, for identification of *N. lactamica* and future microbiome analysis.
7. Survey volunteers' personal experiences of research participation (including acceptability and tolerability of inoculation and maternal and infant sampling).

5.0 *N. lactamica* inoculum

5.1 *N. lactamica* inoculum source

10^5 CFU wild type *N. lactamica* strain Y92-1009, sequence type 3493, clonal complex 613 will be used for nasal inoculation. Previous inoculation studies performed by our group[20] used thawed frozen stocks of this strain, produced by the current Good Manufacturing Practices (GMP) pharmaceutical manufacturing facilities at Public Health England (Porton Down, United Kingdom). Our group have since optimised a method for preparing lyophilised (freeze-dried powdered) *N. lactamica* (LyoNlac) inoculum from these frozen stocks, adhering to GMP-like laboratory technical and reporting standards (unpublished data). Unlike frozen stocks, which are vulnerable to loss of viability in the event of cold chain (-80°C) disruption, lyophilised stocks can be stored at 4°C until required for inoculation, making this approach more amenable to downstream clinical application. Recovery of viable bacteria from lyophilised inoculum reconstituted in sterile water or phosphate buffered saline (PBS) is nearly 100% immediately following lyophilisation, and remains in excess of 10^7 CFU/ampoule for inoculum reconstituted over 3 months after lyophilisation. This approach thus offers reproducible inoculum dosing and reduces the potential variability associated with frozen stocks. *N. lactamica* cultured from reconstituted lyophilised inoculum is phenotypically identical to the frozen stocks from which it was produced. Quality assessment by microbiological culture has revealed no evidence of inoculum contamination to date.

5.2 *N. lactamica* inoculation safety

N. lactamica is a normal commensal of the human pharynx, and is not pathogenic in immunocompetent hosts. Our research group have previously inoculated over 400 adults with this strain of *N. lactamica* (of whom 30 received lyophilised inoculum, and 54 received 10^5 CFU), with no serious adverse reactions reported to date[20] (unpublished data).

Although invasive infection with *N. lactamica* is not expected, the inoculum strain is known to be sensitive to widely available antibiotics, with a minimum inhibitory concentration (MIC) of 0.003 micrograms/litre for ciprofloxacin, <0.02 micrograms/litre for ceftriaxone, and 0.38 micrograms/L for rifampicin (unpublished data).

5.3 *N. lactamica* inoculum management

All ampoules of lyophilised inoculum will be stored in a labelled refrigerator at 4°C in a secure laboratory. If possible, all inoculations will be performed using ampoules derived from a single batch of lyophilised *N. lactamica*, and a log will be kept of any ampoules removed from the refrigerator. A new ampoule will be used for each volunteer, and the inoculum will be reconstituted in PBS immediately prior to inoculation to achieve a concentration of 10^5 CFU/ml, following the study-specific **standard operating procedure (SOP): Preparation and monitoring of LyoNlac inoculum for nasal inoculation**. In our team's previous work, this dose resulted in *N. lactamica* colonisation in 100% of 10 adults using lyophilised inoculum, and 85% of 20 adults using frozen inoculum; i.e. 90% overall (unpublished data). A sample of residual inoculum will be diluted and cultured to confirm inoculum purity and dose by viability count.

6.0 Recruitment and pre-screening

6.1 Study advertisement and recruitment

The study will be advertised to potential participants using a variety of written and audiovisual media (including but not limited to posters, leaflets, presentations, social media posts, letters and emails), which will be distributed in the following ways:

- By direct mail or email to pregnant women. PAH midwives will identify and contact pregnant women who have had a normal dating ultrasound scan. The research study team will not have access to this list of patients, or to their contact details.
- In clinical and non-clinical healthcare settings (e.g. in clinics, waiting rooms, notice boards and/or corridors located in hospitals, general practice [GP] surgeries, midwife/birth centres, NHS antenatal classes), with prior permission from responsible staff / authority.
- In public places (e.g. public transport, parks), with prior permission from responsible staff / authority.
- In private businesses (e.g. gyms, coffee shops, cinemas, offices, private antenatal classes), with prior permission from responsible staff / authority.
- In newspapers, magazines or other literature for circulation.
- On radio or television.
- On social media platforms (e.g. Twitter, Facebook, Instagram, YouTube), using an account owned and operated by our research group or by UoS, UHS, PAH, or UoE.
- On websites operated by our research group or by UoS, UHS, PAH, or UoE, with prior permission from the website operator or equivalent authority.
- By email distribution to a group/list with prior permission from the network administrator or equivalent authority.
- By direct mail or email to individuals who have expressed an interest in participating in relevant research (e.g. the NIHR-CRF Database of Healthy Volunteers).
- On stalls / stands at exhibitions / fairs (e.g. university or hospital open days).
- By presentation to an audience by invitation (e.g. at lectures or seminars).

Distribution of written and audiovisual media may be accompanied by semi-structured verbal delivery of information (e.g. in person or over the phone) by members of the study team, or by NIHR-CRF and PAH staff. Women may be approached directly by these persons at any point during pregnancy, in healthcare settings, public places and private businesses (with prior permission from responsible staff / authority).

6.2 Pre-screening

Pre-screening will be conducted by members of the study team, PAH staff or NIHR-CRF staff, and may be performed in person, by phone or by email. Potential volunteers will be approached in the second and third trimesters of pregnancy, and will be asked to provide their full name and two forms of contact details (e.g. telephone, email address, home address). An abbreviated list of inclusion and exclusion criteria will be checked, and potentially eligible volunteers will be scheduled for a screening visit. Prior to screening, a participant information sheet (PIS) will be given (in person or by email), including potential risks and safety measures involved in the study. A log will be kept of any potential volunteers approached for pre-screening, including reasons for ineligibility.

7.0 Screening (Visit 1)

7.1 Timing, location and team members

The screening visit (Visit 1) will take place during from 34+0 to 36+6 weeks gestation. Screening will be conducted at the NIHR-CRF or in the volunteer's home (depending on volunteer preference and NIHR-CRF availability). The study doctor will explain the study details (including timeline, sampling procedures, risks and safety considerations, tissue and data handling, and volunteer compensation); confirm eligibility; address all of the volunteer's questions; seek informed consent; enrol the volunteer into the study; perform biological sampling; and schedule *N. lactamica* inoculation (Visit 2).

7.2 Inclusion criteria

All the following inclusion criteria must apply in order for the volunteer to be eligible for the study:

- Healthy adult aged 18 years or over on the day of enrolment.
- Singleton pregnancy, 34+0 to 36+6 weeks gestation on the day of enrolment.
- Documentation of a 20-week ultrasound scan with no life-limiting congenital anomalies, and no maxillofacial / otorhinolaryngological / neuroanatomical anomalies.
- Able and willing (in the study doctor's opinion) to comply with all study requirements.
- Able and willing to give written informed consent to participate in the study.
- Booked to receive antenatal care at UHS NHS Foundation Trust (including PAH, New Forest Birth Centre, and any community antenatal care facilities associated with UHS).

7.3 Exclusion criteria

The volunteer may not enter the study if any of the following exclusion criteria apply:

- Any confirmed or suspected immunosuppressive or immunocompromised state, including: HIV infection; asplenia; recurrent severe infections; or use of immunosuppressant medication (for more than 14 days within the past 6 months, excluding topical and inhaled steroids).
- Planned use of immunosuppressant medication in later pregnancy or post-partum.
- Occupational, household or intimate contact with any immunosuppressed persons.

- Participation within the last 12 weeks in a clinical trial involving receipt of an investigational product, or planned use of an investigational product during the study.
- Prior participation at any time in research studies involving inoculation with *N. lactamica.*
- Use of oral or intravenous antibiotics within 30 days prior to the *N. lactamica* inoculation visit.
- Planned use of oral or intravenous antibiotics at any time during the study period (e.g. for planned elective caesarean section or group B streptococcus colonisation).
- Allergy to soya or yeast.
- Previous stillbirth or neonatal death.
- Pre-pregnancy diabetes mellitus.
- Any other finding that may (in the study doctor's opinion): increase the risk to the volunteer (or their fetus/infant or close contacts) of participating in the study; affect the volunteer's ability to participate in the study and complete follow-up; or impair interpretation of study data.

7.4 Informed consent and enrolment

The PI is responsible for obtaining informed consent from volunteers (or for delegating this responsibility to a suitably trained study doctor), and for ensuring that any volunteers are not vulnerable or unduly coerced to participate. There is no minimum period between information provision and consent being given, or between screening and inoculation, provided that volunteers have sufficient time and information to make a decision.

Before seeking consent, the study doctor must ensure that:

- The aims and risks of the study have been fully explained.
- The volunteer has been given the opportunity to ask any questions, and has had time to consider whether or not to participate.
- The volunteer is aware that:
    - There is no direct benefit from participating.
    - Participation in the study is entirely voluntary.
    - Refusal to participate involves no penalty or loss of medical benefits.
    - They may withdraw from the study at any time without reason, but may be contacted by the study team for safety reasons following withdrawal.

- The volunteer understands and agrees that:
    - Their GP will be informed of their participation in the study, and their participation will be recorded in their NHS patient notes.
    - The study doctor may access their medical records, in order to identify any risks of participation and to capture relevant study metadata.
    - If they are lost to follow-up, the study doctor may contact their GP and/or access their NHS patient notes to check for a change in contact details.
    - They will be registered on TOPS (The Over-volunteering Prevention System; www.TOPS.org.uk). Proof of identification will be required for this purpose.
    - In the event that a follow-up study is planned, the study doctor may contact them in the future to ask if they are interested in participating.
    - Pharyngeal and saliva samples will be collected from them and from their infant, for analysis and storage (in link-anonymised format). The volunteer may decline or withdraw consent to sampling blood, breast milk, and other household contacts without affecting their eligibility to participate in the study (provided they consent to all other study visits and biological sampling).
    - Consent will be sought at screening (i.e. prior to delivery) from the volunteer on behalf of the infant. Following delivery, consent will be re-affirmed immediately prior to any sampling involving infants. Re-affirmation of consent will be documented in the relevant source form; a new consent form is not needed.
    - Samples will be retained and analysed even after the volunteer has completed or withdrawn from the study, unless they explicitly withdraw consent for this.
    - Biological samples (blood and swabs) will contain human tissue, and will be stored in accordance with the Human Tissue 2004. Although there is no intention to analyse human cells in this study, these samples may be used in future studies (with appropriate peer review and ethical approval), unless the volunteer explicitly withdraws consent for this.

The volunteer will be asked to sign and date the consent form, which will also be signed and dated by the study doctor. The original consent form will be stored in the case report form (CRF) in the NIHR-CRF, a photocopy will be given to the volunteer, and a scanned copy will be uploaded to the participant's electronic medical notes. Once the volunteer has signed the consent form, they are considered to be enrolled in the study.

7.5 Medical history

Once written informed consent is obtained, a brief clinical and personal history will be documented, including:

- Demographics (age, date of birth), gestation (in weeks and days) and contact details (two forms).
- History of any significant illnesses, surgical procedures, hospitalisations, or anatomical anomalies.
- Current medication (including prescribed, over-the-counter and recreational).
- Allergies (including medications).
- Household or occupational contact with any children (including ages) or animals.

Clinical examination will only be performed if the study doctor feels they are indicated based on the clinical history taken, and with the volunteer's consent.

If there is any uncertainty regarding the volunteer's history or eligibility status, the study doctor will review the electronic and/or paper-based NHS patient notes, and document any relevant information therein in the CRF.

7.6 Screening investigations

The study doctor (or a suitably trained study nurse or CTA) will obtain nasopharyngeal, oropharyngeal and saliva samples from the volunteer, according to the **SOP: Collection of adult biological samples**. These will be used to assess for baseline natural *N. lactamica* carriage, and may be processed for baseline microbiome analysis.

7.6.1 Baseline N. lactamica *carriage status*

If *N. lactamica* is present, the study doctor will contact the volunteer to inform them, providing reassurance that *N. lactamica* constitutes normal commensal flora, and that no clinical action is necessary. Volunteers already colonised with *N. lactamica* will not be inoculated, and so will not attend the inoculation visit (Visit 2). However, they will be invited to complete follow-up visits (Visits 3-5), which will be conducted in exactly the same manner as for inoculated volunteers (including all biological sampling of volunteers and their neonates). These natural *N. lactamica* carriers will serve as a convenience cohort for subsequent analysis and comparison with inoculated volunteers.

8.0 *N. lactamica* inoculation (Visit 2)

8.1 Timing, location and team members

Nasal inoculation with 10^5 CFU *N. lactamica* will take place in the NIHR-CRF at 36+0 to 37+6 weeks gestation. The study doctor will be responsible for performing the pre-inoculation and inoculation procedures, accompanied by a suitably trained study nurse, CTA, or another member of the clinical study team. The NIHR-CRF is situated in UHS, with access to a resuscitation team, intensive care facilities, and emergency paediatric services on-site (and to emergency obstetric services at PAH across the road). The study team will adhere to the UHS standard infection prevention and control precautions for all inoculations.

8.2 Pre-inoculation procedures

8.2.1 Confirmation of volunteer identity, consent and N. lactamica *carriage status*

The study doctor will confirm the volunteer's name and date of birth, and check these against the CRF and the inoculum label. The study doctor will answer any of the volunteer's questions, and re-affirm their consent to inoculation.

The study doctor will also confirm the volunteer's baseline *N. lactamica* carriage status, based on the biological samples obtained at screening (Visit 1). Only volunteers not already naturally colonised with *N. lactamica* will receive inoculation.

8.2.2 Clinical review

The volunteer will be asked:

- Have they been unwell or developed any problems with the pregnancy since the last visit?
- Have they taken any antibiotics or immunosuppressants since the last visit, or have these been planned for later in the study period?

If there is any uncertainty regarding the volunteer's history or eligibility status, the study doctor will review the electronic and/or paper-based NHS patient notes, and document any relevant information therein in the CRF.

Vital signs will be measured prior to inoculation, but further clinical examination will only be performed if the study doctor feels this is indicated based on the history taken, and with the volunteer's consent.

If there is evidence of inter-current infection (as defined by symptoms of infection AND: fever OR other abnormal vital signs or examination findings OR use of antibiotics), inoculation will not take place. If this infection resolves (as defined by normal vital signs and absent / significantly improved symptoms) and no antibiotics have been taken, inoculation may be rescheduled, provided it can be performed by 37+6 gestation; if not, the volunteer will be withdrawn from the study. Similarly, if the volunteer's eligibility status has otherwise changed, inoculation will not take place, and the volunteer may be withdrawn from the study.

8.2.3 Biological sampling

Prior to *N. lactamica* inoculation, the study doctor will obtain pharyngeal and saliva samples from the volunteer, according to the **SOP: Collection of adult biological samples**.

8.3 Inoculation procedure

8.3.1 Preparing the inoculum

The inoculum will be prepared in a decontaminated class 2 microbiological safety cabinet by members of the study team trained in the procedure, according to the **SOP: Preparation and monitoring of LyoNlac inoculum for nasal inoculation**.

8.3.2 Administering the inoculum

The inoculum will be administered by the study doctor according to the study-specific **SOP: Administration of *N. lactamica* inoculum**.

8.3.3 Post-inoculation observation

The volunteer will remain in the NIHR-CRF for 30 minutes following inoculation. The study doctor, nurse or CTA will ask the volunteer to report any problems or symptoms that developed during or since inoculation. If there are any concerns, the volunteer will be reviewed clinically by the study doctor. Volunteers will be encouraged to contact the study team at any time after inoculation to discuss any concerns, and they will have access to an emergency phone number to contact a study doctor out of hours.

9.0 Mother-infant pair birth visit (Visit 3)

9.1 Timing, location and team members

Visit 3 will be conducted within 24 hours of the volunteer giving birth. The volunteer, clinical staff, or any other nominated individual will contact the study team by phone or email as soon as is feasible following the birth. The study doctors may also check the Labour Ward admission log and ask PAH and birth-centre staff if any of the study volunteers have been admitted. If it is not possible to arrange a visit within 24 hours, the visit will be performed as soon as is feasible, and a protocol deviation form will be completed.

The visit will take place in the volunteer's home, or in the clinical area where the volunteer is located if they have not yet been discharged home (with permission from responsible staff / authority). If possible, the volunteer will be asked not to feed their infant for 30 minutes prior to collection of infant nasopharyngeal and saliva samples.

9.2 Review prior to biological sampling

The study doctor will confirm the volunteer's name and date of birth, and answer any of the volunteer's questions. The infant's identity and date of birth will be confirmed with the volunteer and documented in the CRF.

The volunteer will be asked:

- Have they been unwell or developed any problems with the pregnancy since the last visit?
- Have they taken any antibiotics or immunosuppressants since the last visit, or have these been planned for later in the study period?

Following discussion with the volunteer and (where necessary) review of the electronic and/or paper-based NHS patient notes, the study doctor will document relevant metadata in the CRF, including (but not limited to): mode of delivery; mode of feeding; peri-partum antibiotics, fever, or other features of infection; neonatal condition.

Clinical examination will only be performed if the study doctor feels they are indicated based on the clinical history taken, and with the volunteer's consent.

9.3 Biological sampling

The study doctor will obtain pharyngeal and saliva samples from the volunteer, according to the **SOP: Collection of adult biological samples**. After re-affirming the volunteer's consent, the study doctor will obtain nasopharyngeal and saliva samples from the neonate, according to the **SOP: Collection of infant biological samples**.

9.3.1 Umbilical cord blood sampling

Where the volunteer has given consent, umbilical cord blood will be collected by a suitably trained member of the clinical team immediately upon delivery (ideally at least 5 millilitres, although any volume obtained will be stored). The sample will be kept in the clinical area until the study doctor attends for the birth visit (Visit 3).

9.3.2 Breast milk sampling

Volunteers will be asked to provide a colostrum, whereby the volunteer will manually express colostrum (up to 5ml) into a sterile universal container.

10.0 Mother-infant pair follow-up visits (Visits 4, 5 and 6)

10.1 Timing, location and team members

Visits 4 and 5 will take place 7 +/- 3 days and 28 +/- 3 days post-partum, respectively, and an optional Visit 6 will take place at 15 +/- 2 weeks post-partum. These visits will be conducted by the study doctor, and will take place either in the NIHR-CRF or in the volunteer's home. A second member of the clinical study team may accompany the study doctor for any home visits. Suitably trained study nurses or CTAs may conduct Visits 4, 5 and 6 (including clinical review and biological sampling), provided a study doctor is available by telephone to address any clinical concerns that may arise during or after the study visit.

10.2 Review prior to biological sampling

The study doctor will confirm the volunteer's name and date of birth, and answer any of the volunteer's questions.

The volunteer will be asked:

- Have they or their neonate been unwell since the last visit?
- Have they or their neonate taken any antibiotics or immunosuppressants since the last visit, or have these been planned for later in the study period?

Following discussion with the volunteer and (where necessary) review of the electronic and/or paper-based NHS patient notes, the study doctor will document relevant metadata in the CRF (e.g. mode of feeding). Clinical examination will only be performed if the study doctor feels they are indicated based on the clinical history taken, and with the volunteer's consent.

10.3 Biological sampling

The study doctor will obtain pharyngeal and saliva samples from the volunteer, according to the **SOP: Collection of adult biological samples**. Volunteers will be asked to provide a breast milk sample at Visits 4, 5 and 6; a minimum of 5ml expressed milk will be collected, although any volume less than this will be accepted.

After re-affirming the volunteer's consent, the study doctor will obtain nasopharyngeal and saliva samples from the neonate, according to the **SOP: Collection of infant biological samples**.

10.3.1 Venous blood sampling

An optional infant venous blood sample will be collected at 4 weeks (Visit 5) and 15 weeks (Visit 6) post-partum. No more than 2ml blood will be taken at each visit, although any volume obtained will be stored. This volume of blood is feasible to collect at this age in a single venous sample, and is well within safety limits. If venepuncture is unsuccessful, capillary blood sampling ('heel prick') may be attempted.

An optional maternal venous blood sample will be collected 15 weeks post-partum (Visit 6). No more than 5ml blood will be taken, although any volume obtained will be stored.

10.3.2 Sampling household contacts under 5 years old

At 15 weeks post-partum (Visit 6), volunteers may opt-in to collection of saliva and oropharyngeal samples from any household contacts under 5 years old over whom they have parental responsibility. Saliva sampling will be performed first and, if the child is deemed (by the volunteer and the clinical study member) to be able to tolerate it, an oropharyngeal sample will be attempted.

11.0 Volunteer benefits and compensation

Volunteers will not benefit directly from participation in this study. However, it is hoped that this study will improve understanding of microbial acquisition and transfer during pregnancy and early post-partum, with the ultimate goal of developing safe and effective strategies of manipulating neonatal flora to improve health outcomes. Volunteers may also find contact with the Investigator and clinical study team comforting in the period leading up to and after their infant's birth.

Enrolled volunteers will be offered financial compensation for their time and for the inconvenience caused by study procedures, in the amount of £50 after completing the screening visit (Visit 1) and £50 after completing the inoculation visit (Visit 2). Compensation will not be offered for visits including neonate sampling (Visits 3-6), to ensure there is no inducement for volunteers to involve their infants in research, although reimbursement for on-site parking will be offered for visits conducted in the NIHR-CRF.

If a volunteer withdraws or is withdrawn from the study, they will receive compensation for the visits already completed, but not for any future visits. Compensation will be offered as a single payment following completion of the birth visit (Visit 3) or following withdrawal from the study (whichever is earlier). At the final study visit (Visit 6), the volunteer will be offered a small gift as a token of appreciation; e.g. an age-appropriate children's book and/or educational toy, up to a value of £10 per volunteer.

We also plan to survey volunteers' personal experiences of research participation (including acceptability and tolerability of inoculation and maternal and infant sampling). This data (captured in volunteer CRFs and/or optional questionnaires) will help us identify areas for improvement in this study and future studies.

12.0 Defining study duration and volunteer participation

12.1 Definition of study start and end

The start of the study is defined as the date upon which recruitment activity for the study begins. The end of the clinical study is defined as the date upon which the final volunteer's final biological sample is processed. The end of the research study is defined as 5 years after the date of the last volunteer visit (to allow sufficient time for sample processing and data analysis).

The Sponsor will be informed of study completion. The clinical study may be terminated early at the discretion of the CI or Sponsor if there are safety concerns, concerns about compliance with International Conference on Harmonisation E6 Good Clinical Practice CPMP/ICH/135/95 (ICH GCP), or poor recruitment, or if new information becomes available which has an impact on the scientific validity or safety of the study.

An individual volunteer's participation will end when: they have completed their final visit; or when they are withdrawn from the study (whichever is earliest).

12.2 Volunteer withdrawal

A volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give their reasons for doing so.

The volunteer and their neonate may be withdrawn from the study at any time in the interests of their health and well-being, or for any of the following reasons:

- An administrative decision by the PI or CI.
- Ineligibility for inoculation (either arising after screening or retrospectively, having been overlooked at screening).
- A significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An adverse event (AE) that requires discontinuation of study involvement or results in inability to continue complying with study procedures.

The reason for withdrawal from the study will be recorded in the CRF. Withdrawn volunteers will be offered follow-up to manage AEs until resolution or stabilisation, or until a non-study-

related causation is assigned. Safety data collection will continue for any volunteers that have received the inoculum prior to withdrawal, unless the volunteer explicitly refuses this.

If a volunteer withdraws or is withdrawn from the study, any biological samples collected from the volunteer and their neonate prior to withdrawal will be maintained in storage and used in ongoing and future analyses, unless the volunteer explicitly refuses this. Source data or metadata relating to volunteers and infants withdrawn from the study will be included in results analysis. Any volunteer who withdraws consent or is withdrawn from the study may be replaced, at the PI's discretion.

12.3 Lost to follow-up

If contact with a volunteer is lost during the study, the study team will attempt to contact them by phone and/or email and/or post on a minimum of three separate occasions. If the volunteer has been inoculated with *N. lactamica*, the study team will then contact their GP and/or access their NHS patient notes to check if their contact details have changed. Volunteers will be considered lost to follow-up if there is no contact from the volunteer within two weeks of the final unsuccessful contact attempt by the study team.

Any biological samples collected from the volunteer and their neonate prior to being lost to follow-up will be maintained in storage and used in ongoing and future analyses. Source data or metadata relating to volunteers and neonates lost to follow-up will be included in results analysis. Any volunteer who is lost to follow-up may be replaced, at the PI's discretion.

13.0 Risks, safety and ethics

13.1 Definitions

13.1.1 Adverse event (AE)

An AE is any untoward medical occurrence in a volunteer or their neonate temporally related to the study, whether or not considered causally related to the study. AEs can include any unfavourable and unsolicited sign (including an abnormal laboratory finding), symptom, disease, or relevant procedural error (e.g. dosing error). Delivery of routine antenatal, postnatal or intra-partum care is not considered an AE.

13.1.2 Adverse reaction (AR)

An AR is any AE where a causal relationship between *N. lactamica* inoculation and the AE is at least a reasonable possibility and cannot be ruled out. Any case judged by the PI, CI or Sponsor as having a reasonable suspected causal relationship (i.e. possibly, probably or definitely related) to inoculation qualifies as an AR.

13.1.3 Unexpected adverse reaction (UAR)

A UAR is an AR for which the nature or severity is not consistent with the applicable information about the study intervention.

13.1.4 Serious adverse event (SAE)

An SAE is an AE that results in any of the following outcomes for the volunteer or their neonate, whether or not considered related to a study intervention:

- Death from any cause at any time.
- A life-threatening event; i.e. an AE that, in the view of the Investigator, presented an immediate risk of death. This does not include an AE that, if it had occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity, constituting substantial disruption of ability to carry out normal life functions.
- Hospitalisation (or prolongation of existing hospitalisation) for treatment or observation of a new condition, an unstable pre-existing condition, or a complication of

pregnancy or delivery. Hospitalisation (including inpatient and outpatient attendance) for routine antenatal, postnatal or intra-partum care, or for a stable pre-existing condition, does not constitute an SAE. Attendance to Accident and Emergency (A&E) or an Urgent Care Centre does not in itself constitute an SAE, although the reason for attendance may constitute an SAE.

- A serious adverse neonatal or pregnancy outcome, including (but not limited to) stillbirth, neonatal death, congenital anomaly or birth defect.
- Any other important medical event not resulting in hospitalisation, risk of death, or death, but that may (based on the Investigator's clinical judgement) require medical or surgical intervention to prevent the outcomes listed above. Examples of such events include severe allergic reactions, blood dyscrasias, or convulsions requiring emergency treatment in A&E or the NIHR-CRF.

13.1.5 Serious adverse reaction (SAR)

An AE (expected or unexpected) that is both serious and, in the opinion of the PI, CI or Sponsor, believed to be possibly, probably or definitely due to *N. lactamica* inoculation.

13.1.6 Suspected unexpected serious adverse reaction (SUSAR)

A SUSAR is an AE that is unexpected, serious and, in the opinion of the PI, CI or Sponsor, believed to be possibly, probably or definitely due to *N. lactamica* inoculation.

13.2 Potential adverse events and expected effects

Pharyngeal sampling and inoculation with *N. lactamica* suspension can cause minor irritation, resulting in stinging, coughing, sneezing, or crying. These minor expected effects are self-limiting within minutes, and do not constitute AEs. Similarly, localised discomfort and minor bruising resulting from venesection are expected effects that do not constitute AEs.

Mild, self-limiting respiratory tract infections are expected in healthy adult volunteers, so do not constitute AEs. However, any neonatal symptoms causing parental concern, or requiring medical attention or medication (over the counter or prescribed) will be treated as AEs, as will any neonatal fever.

Due to use of yeast-based and soya-based products in production of *N. lactamica* inoculum, there is a theoretical possibility of allergic reaction in volunteers with soya or yeast allergy. Volunteers reporting soya or yeast allergy at screening will not be enrolled onto the study. There is no evidence that soya or yeast antigens persist beyond inoculation, and we do not expect the neonate to have any exposure to these antigens. To date, there have been no SARs associated with *N. lactamica* inoculation. If a severe hypersensitivity reaction to the inoculum occurs, this will be reported as an SAR.

13.3 External Safety Committee (ESC)

An External Safety Committee (ESC) will be appointed to provide overall safety supervision and advice. The ESC will first convene and review this protocol prior to study initiation. The Investigator or the Sponsor may contact the ESC for advice and independent review in the following situations:

- Following any SAEs and SARs.
- For scheduled safety meetings (virtual or in person every 3-6 months).
- In any other situation where the Investigator or Sponsor feel that independent safety advice or review is important.

13.4 Adverse event (AE) management

All AEs will be detailed in the volunteer's CRF, and summarised in the AE log. The AE log will be stored in the Investigator Site File (ISF), and will be sent every 3 months to the Sponsor and the ESC, and included in the Annual Safety Report. AE severity, suspected causality, action, duration and outcome will be recorded for all AEs. Volunteers will be offered follow-up to manage AEs until resolution or stabilisation, or until a non-study-related causation is assigned.

13.4.1 AE causality assessment

For each AE, the Investigator will assess the relationship with the study intervention(s). The relationship will be categorised as unrelated, unlikely to be related, possibly related, probably related or definitely related. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, unrelated risk factors and the temporal relationship of the event to the study intervention(s), will be considered.

13.4.2 Serious adverse event (SAE) reporting procedures

SAEs will be documented accurately and reported to the PI as soon as anyone in the study team becomes aware of their occurrence. All SAEs will be reported to the Sponsor and ESC within 24 hours of the PI being alerted to their occurrence. SAEs will not normally be reported to the NHS Research Ethics Committee (REC), unless there is concern that the safety of other study volunteers may be affected, at the discretion of the CI.

13.4.3 Death and suspected unexpected serious adverse reaction (SUSAR) reporting procedures

All SUSARs and any deaths occurring during the study will be reported to the Sponsor and ESC within 24 hours of the PI being informed. The CI will report all SUSARs to the REC within required timelines, and will inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of volunteers. For all deaths, any autopsy reports and relevant medical reports will be made available to the relevant authorities.

13.5 Management of abnormal clinical findings

Abnormal clinical findings discovered through medical history, examination, or microbiological investigations will be assessed as to their clinical significance. If an investigation result is deemed clinically significant, it may be repeated or further validated, and the volunteer will be informed and advised to seek appropriate medical attention. The decision to withdraw, replace or exclude the volunteer from enrolment will be at the PI's discretion.

13.6 Ethics

This study will not begin until approval is granted by the REC and the Health Research Authority (HRA). A progress report will be submitted to the REC one year after the study is initiated, and annually until the study has ended. The Investigator will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki (updated 2013), and the ICH GCP.

14.0 Laboratory procedures

14.1 Managing biological samples

Study-specific and procedure-specific SOPs will be used to obtain and process biological samples from volunteers and their infants as per the study schedule. Investigators will follow established local and international laboratory standards.

Samples will be obtained in the NIHR-CRF, the volunteer's own home, or the clinical area where the volunteer is receiving care. Samples will be labelled immediately using a unique link-anonymised identifier (as per **SOP: Labelling specimens**). To minimise bias introduced by variation in sampling technique, one Investigator will aim to collect all pharyngeal and saliva samples in the study, with a different Investigator performing this duty only if the first Investigator is unavailable. The identity of the sampling Investigator will be documented in the relevant source form.

Labelled samples will be transported in a clean leak-proof container (as per **SOP: Transport of clinical samples**) to the CES department for initial processing and microbiological culture. The resulting sample aliquots and bacterial isolates will be stored in a secure, temperature-monitored -80^{o}C freezer in a lockable laboratory in the CES department. Sample receipt and storage will be recorded in laboratory source forms and the freezer log.

Although this study does not involve sequencing or other analysis of human genetic or cellular material, the pharyngeal and saliva samples taken will invariably contain human tissue. Thus, samples will be treated as relevant material under the Human Tissue Act 2004, and handled in facilities operating under the UoS Faculty of Medicine Human Tissue Authority (HTA) licence. Specific volunteer consent will be obtained at screening regarding storage of human tissue, with an explanation that no human genetic or cellular analysis will be undertaken. Although umbilical cord and infant venous blood will be collected, only serum (which is not considered relevant material under the Human Tissue Act 2004) will be stored and analysed.

Further research questions may become apparent during and after the study period, and it is desirable that the maximum amount of experimental data is obtained from these unique and valuable samples. At the end of the research study, any residual samples containing human tissue will be reviewed, and additional ethical approval will be sought if they are to be retained

for further use in this study or a related study. Alternatively, such samples may be transferred to a tissue bank (the HTA licenced Southampton Research Biorepository) at the end of the study, or disposed of in accordance with the HTA Code of Practice. Non-human material (including bacterial isolates derived from biological samples) may be retained and used after the end of the research study.

14.2 Initial processing of biological samples

Up to two nasopharyngeal swabs, two oropharyngeal swabs, two saliva swabs, and one breast milk sample will be collected from each volunteer and each infant at each study visit, where feasible and acceptable to the volunteer. Where two samples are obtained, one will be used to plate directly onto labelled selective agar during the study visit (as soon as possible after sampling), to maximise the possibility of identifying viable *N. lactamica* by microbiological culture (as per **SOP: Microbiological identification, quantification and storage of *N. lactamica***). The resulting inoculated agar plates will be transported along with all remaining samples to the CES department, and transit time will be recorded. At Visit 6, up to two saliva swabs and two oropharyngeal swabs may be obtained from each household contact under 5 years old. These will be processed as for maternal and neonatal oropharyngeal and saliva swabs.

All samples will be processed in a class 2 microbiological safety cabinet in the CES department. Initial processing of pharyngeal and saliva swabs will involve suspension of samples in appropriate storage medium and division into aliquots, as per the **SOP: Processing of biological samples**. Breast milk samples will also be divided into aliquots. One aliquot per sample will be used for microbiological identification, quantification and storage of *N. lactamica*. The remaining aliquots will be stored in a secure, temperature-monitored -80^{o}C freezer in a lockable laboratory in the CES department, and used in downstream investigations. All infant venous and umbilical cord blood samples will be processed to isolate serum, which will be frozen and stored in the CES department for future research.

14.3 Microbiological identification, quantification and storage of *N. lactamica*

Microbiological culture will be performed on a representative sample aliquot derived from every nasopharyngeal, oropharyngeal and saliva sample obtained in the study, as per the **SOP: Microbiological identification, quantification and storage of *N. lactamica***. Gonoccocal-selective GC agar supplemented with 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (X-gal) will be labelled, inoculated with a defined volume of sample, and incubated at 37^{o}C in 5% CO_2. Following 24-48 hours incubation, the agar plates will be visually inspected in a class 2 microbiological safety cabinet for colonies consistent in colour and morphology with *N. lactamica*. Oxidase reaction, Gram stain, microscopy, and API-NH (BioMérieux, France) or MALDI-TOF will be used to confirm *N. lactamica* identity.

Incubated agar plates will be photographed digitally, and the number of *N. lactamica* colonies will be counted and recorded in the laboratory source form. Positively identified *N. lactamica* will be sub-cultured onto fresh agar. Resulting isolates of *N. lactamica* will be stored at -80^{o}C, for use in downstream investigations.

14.4 Targeted PCR for *N. lactamica* Y92-1009

To confirm horizontal transfer of the inoculation strain from mother to neonate, any *N. lactamica* isolates obtained from infant biological samples will undergo targeted PCR for strain Y92-1009 (as per **SOP: *N. lactamica* Y92-1009 targeted PCR**). Our team have identified two chromosomal loci present in *N. lactamica* strain Y92-1009 that, when amplified in multiplex PCR and analysed by agarose gel electrophoresis, produce amplicons with a characteristic band pattern. Using the same multiplex PCR on isolates of naturally circulating strains of *N. lactamica*, this band pattern has not been reproduced. A third locus, present in all known strains of *N. lactamica*, is included to differentiate between PCR failure (no bands produced) and any non-Y92-1009 strain of *N. lactamica* (band produced, but Y92-1009-specific bands pattern not present).

14.5 Microbiome analysis

Once all biological samples have been collected, frozen aliquots obtained from initial processing of pharyngeal, saliva and breast milk samples will be transported to the University of Edinburgh (UoE) Centre for Inflammation Research (CIR). Samples will be transported by specialist courier in temperature-controlled conditions using leak-proof and shock-resistant transport containers, and will be received by a member of the study team to ensure safe handling and storage.

Thawed sample aliquots will be prepared for sequencing by DNA extraction, quantitative PCR of the 16S rRNA gene (indicating total bacterial load in the sample aliquot), and dilution of bacterial DNA to target concentrations. An amplicon library will be generated by sequencing one or more variable regions of the 16S rRNA gene. Resulting sequence reads will be processed by removing poor quality and chimeric reads, and clustering high quality reads for taxonomic classification and statistical analysis.

14.6 Sequencing of paired mother-infant *N. lactamica* isolates

N. lactamica isolates obtained from both the volunteer and their infant at a single time point (with neonatal isolates confirmed as strain Y92-1009 by targeted PCR) will be sequenced to assess for differences in *N. lactamica* microevolution in mother-infant pairs. Following completion of all study visits and collection of all biological samples, DNA from paired *N. lactamica* isolates will be extracted and subjected to whole genome sequencing. Resulting genomes will be mapped to a closed *N. lactamica* Y92-1009 reference genome for comparison.

14.7 Investigating for non-*N. lactamica* commensal transfer in mother-infant pairs

Microbiome community data obtained by 16S rRNA gene sequencing will be used to identify candidate commensal species, by choosing organisms that are (a) represented in both the volunteer and their neonate, and (b) readily amenable to culture using selective media. Choice of media, incubation conditions and microbiological identification methods will depend on the candidate organism chosen.

Thawed sample aliquots from mother-infant pairs will be plated onto selective media. Candidate organism isolates identified in both the volunteer and their neonate will be processed by DNA extraction and subjected to whole genome sequencing. Sequences derived from the volunteer's samples will be compared with those from their infant's samples to identify shared strains, suggesting horizontal transfer.

14.8 Investigating for *N. lactamica*-specific antibodies in mother-infant pairs

Presence of *N. lactamica*-specific immunoglobulin G (IgG) in serum derived from umbilical cord, infant venous and maternal venous blood will be investigated using enzyme-linked immunosorbent assay (ELISA), as described previously[27]. Plates coated with *N. lactamica* outer membrane vesicle (Nlac-dOMV) antigen will be blocked (e.g. using foetal calf serum) and then incubated with serially-diluted participant serum, followed by anti-IgG monoclonal antibodies and a chromogenic substrate. Negative controls (e.g. using bovine serum albumin in place of Nlac-dOMV) and positive controls (using single-donor positive control serum) will be performed. IgG titres will be calculated using a colorimetric plate reader and production of a serial dilution dose-response curve.

15.0 Analysis and statistical considerations

15.1 Sample size justification

This proof-of-concept study aims to establish whether horizontal *N. lactamica* colonisation can be induced in infants by maternal nasal inoculation in pregnancy. In the absence of any past CHI studies in pregnancy or early post-partum, it is not possible to accurately define the sample size needed to conclusively rule out such induced horizontal transfer. Rather, we have selected a pragmatic sample size of 20 inoculated volunteers, which is sufficient to address our primary study objective and aim.

Based on our previous work in non-pregnant adults (unpublished data), inoculation with 10^5 CFU *N. lactamica* results in colonisation in 90% of volunteers. If similar results are observed in pregnant volunteers, 18 out of 20 inoculated volunteers will become colonised. This sample would therefore fail to detect horizontal transfer only if it occurs in less than 1 in 18 infants (<6%); in this case, it would be impractical and even unethical to pursue horizontal *N. lactamica* CHI as a method of studying and manipulating infant commensalisation.

By one month of age, 2.3% of infants naturally carry *N. lactamica*[23]. Based on a plot of lower confidence limits against observed infant *N. lactamica* colonisation for a predicted sample of 18 experimentally-colonised volunteers, infant *N. lactamica* colonisation greater than 22% (95% CI [2.3%, 41.7%]) would demonstrate that CHI-induced carriage exceeds natural *N. lactamica* carriage (Figure 2). However, this is not an explicit end-point of this study. Indeed, we will use targeted PCR for *N. lactamica* strain Y92-1009 to confirm that horizontal transmission has occurred, and so do not require a comparison of *N. lactamica* carriage rates in inoculated versus uninoculated mother-infant pairs.

There is no evidence to suggest that colonisation efficiency will be lower in pregnant than non-pregnant adults. If 13 (65%) or fewer women become colonised, this sample is sufficient to detect significantly lower colonisation in pregnant than non-pregnant adults (n=16 needed for power=0.8, α=0.05). However, this is not an explicit end-point of this study.

In the absence of any published data on CHI in pregnancy and early infant life, it is not possible to accurately define the sample size needed to discern statistically significant microbiome changes associated with maternal *N. lactamica* inoculation in pregnancy. However, if some

infants become colonised and some do not, we will be able to compare microbiomes of colonised and uncolonised infants across study time points to estimate the effect, if any, of *N. lactamica* horizontal colonisation. Even if this comparison does not reach statistical significance, it will provide invaluable data for planning a future larger placebo-controlled study. This approach of using a pragmatic proof-of-concept sample to power a follow-up study has been employed in an interventional microbiome study involving healthy adults[28].

15.1.1 Lower confidence limit plot for CHI-induced N. lactamica *colonisation (Figure 2)*

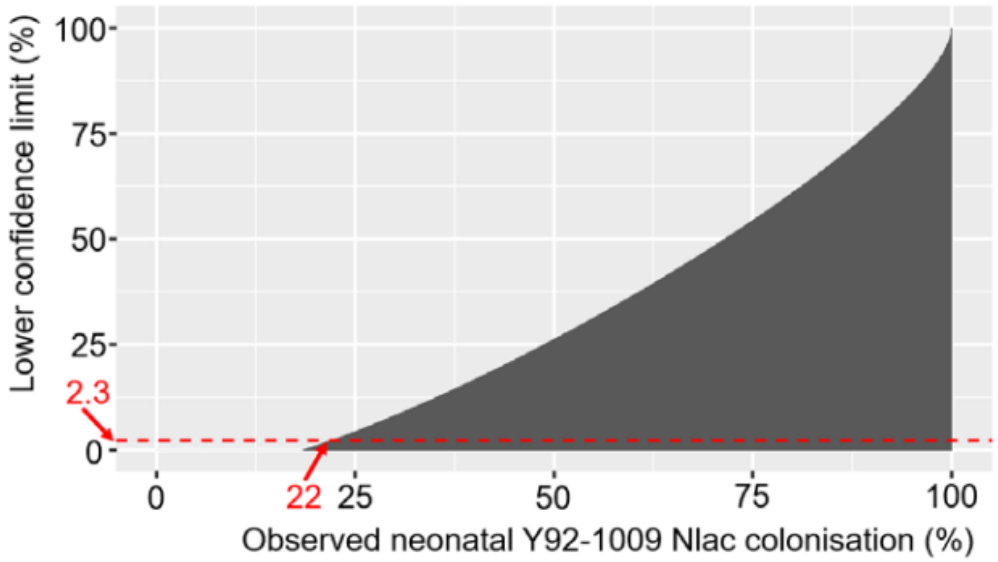

Figure 2: Lower confidence limit plot for inoculation-induced *N. lactamica* (Nlac) colonisation

15.1.2 Sample size contingency planning

It may be necessary to screen more than 20 volunteers in order to reach our target of 20 inoculations. Fewer than 10% of women aged 20-40 years naturally carry *N. lactamica*[24], and so we predict that 0-2 natural *N. lactamica* carriers will be identified at screening for every 20 *N. lactamica*-free women inoculated. At the PI's discretion, volunteers that have dropped out, been withdrawn or lost to follow-up can be replaced (estimated 10% based on our previous studies). Based on these considerations, we estimate that volunteer recruitment and associated biological sampling will take approximately nine months to complete, although (as with all studies involving recruitment in pregnancy) the recruitment rate is difficult to predict.

It is not clear if and how maternal and/or neonatal antibiotics may influence *N. lactamica* carriage and associated microbiome changes. Any women identified as needing antenatal /

perinatal antibiotics at screening (e.g. for planned elective caesarean section or group B streptococcus colonisation) will not be enrolled. Any volunteers (or their neonates) who receive antibiotics after *N. lactamica* inoculation will not be withdrawn from the study, but their antibiotic use will be documented. At the PI's discretion, an additional volunteer may be enrolled and inoculated for every volunteer (or neonate) who receives antibiotics after inoculation, up to a maximum of 10 additional volunteers.

15.2 Visit timing justification

From our past work, *N. lactamica* colonisation is well-established by 7 days post-inoculation, and maintained until at least 26 weeks, with no significant reduction in carriage prevalence over the first 8 weeks[20]. Inoculation at 36-38 weeks gestation thus allows enough time for colonisation before birth, whilst being late enough to minimise risk: beyond 36 weeks, new pregnancy complications are uncommon, and antenatal corticosteroids (which may have an immunosuppressive effect) are almost never needed.

Visit timings for infant microbiome sampling have been selected based on the literature to date regarding infant upper respiratory microbiome development. Within the first day of life, the infant demonstrates a highly diverse pioneer microbiome, with very little niche-specific differentiation[18]. By one week of life, site-specific differences have emerged, with reduction in species diversity and increased bacterial density at each site. Beyond one month, these differences become more pronounced, and microbiome samples can be reliably distinguished on the basis of anatomical niche[19].

15.3 Statistical analysis

Statistical and bioinformatic analysis will be performed by the study team.

The primary endpoint will be expressed as a proportion N/M, where:

- M = Number of mothers colonised with *N. lactamica;*
- N = Number of neonates (aged 0-31 days) colonised with the same strain of *N. lactamica* as their own mother;
- "Colonised" = evidence of *N. lactamica* by culture of upper respiratory swabs (using selective media and confirmation by API or MALDI-TOF) on at least one visit;
- "Same strain" = confirmed using Y92-specific *N. lactamica* PCR for inoculated volunteers, or whole genome sequencing for volunteers already naturally carrying *N. lactamica* at screening.

Subgroup analysis will be performed, to compare inoculated mother-infant pairs with naturally colonised mother-infant pairs, and to compare pairs where the mother and/or infant received antibiotics with pairs not receiving antibiotics.

Specific bioinformatic analysis strategies will depend on the quantity and quality of genomic data produced during the study, and on the rates of *N. lactamica* colonisation observed. Broadly speaking, we will use microbiome bioinformatic pipelines (e.g. QIIME2 and R packages) to perform taxonomic classification, calculation of diversity, and data visualisation.

16.0 Study quality and management procedures

16.1 Standard operating procedures (SOPs)

Approved study-specific SOPs will be used for all clinical and laboratory study activities. All team members will receive training to perform the procedures outlined in the SOPs relevant to their individual responsibilities within the team.

16.2 Monitoring, quality control, quality assurance and statutory inspection

The Sponsor will oversee monitoring that will be conducted by the study team and the UHS Research and Development (R&D) department Quality Assurance (QA) team, in line with NIHR-CRF protocols. The Monitor will verify that the study is conducted (and that data are generated, documented and reported) in compliance with the protocol, the approved SOPs, ICH GCP, and any other applicable regulatory requirements. The Monitor may also audit laboratory activities, and ICH GCP inspections may also be undertaken by the regulatory authority to ensure compliance with the protocol and national regulations.

16.3 Study amendments

No amendments to this protocol will be made without consulting and obtaining approval from the Sponsor and the REC. The Investigator is responsible for ensuring that changes to the study are not put into effect without approval, except to eliminate apparent immediate risk to the volunteers.

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the volunteers' safety, the objectives of the study or its progress. The Sponsor will be notified of any administrative changes, but these do not require REC or regulatory approval.

16.4 Protocol deviation

Any deviation from the protocol will be documented in a protocol deviation form filed in the volunteer's CRF, and summarised in the protocol deviation log. The protocol deviation log will be stored in the ISF and will be sent to the Sponsor and the ESC every 3 months.

16.5 Serious breaches

A serious breach is defined as a breach of ICH GCP or the study protocol that is likely to affect, to a significant degree, the safety or physical or mental integrity of the study subjects, or the scientific value of the study. In the event that a serious breach is suspected, the Sponsor and ESC will be informed within 24 hours of the PI being alerted to their occurrence, and the REC within required timelines.

16.6 Registration, exploitation and dissemination

The study will be registered on the ClinicalTrials.gov database. The Investigators will be involved in reviewing drafts of manuscripts, abstracts, press releases and any other publications arising from the study. Findings will be published in peer-reviewed journals as soon as is practicable, even where results prove negative. The authors will acknowledge that the study is funded by the Medical Research Council and supported by the NIHR-CRF. The results of the study will be disseminated at relevant international scientific meetings.

17.0 Data handling

17.1 Data management and policies

The CI will be the Data Processor, with overall responsibility for delegating the receiving, entering, analysing, maintaining, and archiving of all data that accrues from the study in the paper-based ISF held in the NIHR-CRF, and the electronic ISF stored on secure, password-protected UoS networks. The Sponsor will be the Data Controller (registration number Z6801020). The study will conform to UoS and UHS data and security policies, the Data Protection Act 2018 General Data Protection Regulation (GDPR), and ICH GCP. Adherence with data management policies will be regularly audited by the Monitor.

17.2 Source data and metadata

17.2.1 Clinical source data and case report forms (CRFs)

All relevant clinical source data (excluding NHS patient notes) will be filed in a study-specific and volunteer-specific CRF. Clinical source data are original documents and records, including but not limited to: volunteer demographic details, consent forms, results of microbiological investigations, correspondence between the study team and the volunteer or their GP / midwife, medical / medication history, physical examination / vital signs, records of study procedures / interventions, adverse event data, and any other records of study visits. In the majority of cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e. there is no other written record).

Only members of the clinical study team will have access to clinical records, and will be responsible for entering data into the paper CRF. Paper CRFs will be stored in a locked room in the NIHR-CRF, and The Director of the NIHR-CRF will ultimately be responsible for data security in relation to these. The Investigators will permit the Sponsor, Monitor, REC and relevant regulatory agencies to examine (and, when required by applicable law, to copy) records for the purposes of QA reviews, audits and evaluation of the study safety and progress.

17.2.2 Laboratory and genomic source data and metadata

Link-anonymised microbiological data derived from biological samples will be recorded in official laboratory workbooks and study-specific volunteer-specific paper laboratory source forms. These will be stored in a locked room in the CES department.

A database will be created into which linked-anonymised clinical and microbiological source data and metadata will be entered. The database will be compliant with GDPR, and will only be accessible within the NIHR-CRF through a secure, password-protected UHS NHS network. Any link-anonymised metadata resulting from database interrogation will be stored on secure, password-protected UoS networks accessible only by the Investigators.

Sequencing data (including read number, depth and coverage, quality metrics, and bioinformatic analyses) derived from biological samples processed at UoS, UoE, or another collaborating or third-party institution, will be stored on secure, password-protected UoS or UoE networks. Genome annotation will be performed in accordance with NCBI GenBank guidelines.

Data disseminated beyond the study team will be fully anonymised, including but not limited to: published scientific papers, conference presentations, public outreach events, and impact reports (e.g. ResearchFish, MRC Research Data Gateway). Following publication of our findings, fully anonymised sequencing data and metadata will be uploaded to an open-access curated online data repository (e.g. the European Nucleotide Archive).

17.3 Data storage, retention and archiving

Paper CRFs will be stored in the NIHR-CRF. After the study has ended, they will be retained for 15 years in an off-site secure warehouse (as per **SOP: Preparing paper and electronic documents for internal long term storage including archiving**). Sequencing data will be stored for 10 years on the UoS iSolutions FileStore (a high-security data storage facility with regular on- and off-site backup), after which the options for ongoing storage will be reviewed.

17.4 Volunteer confidentiality

All volunteers will be assigned a unique linked-anonymised alphanumeric identifier, which will be used in lieu of identifiable data in all documentation except the volunteer's CRF (which will allow the identifier to be matched to the volunteer's identifiable data). Identifiable data will be stored in the paper-based ISF in a locked room in the NIHR-CRF, and will only be available to the clinical study team, the Sponsor, the REC and the Monitor. Identifiable data will not be stored in the electronic ISF. Non-clinical researchers (e.g. individuals involved in processing or analysing biological samples only) will not be able to access identifiable volunteer data.

18.0 Financing and insurance

18.1 Financing

The study will be funded primarily by the MRC via the MRC Clinical Research Training Fellowship awarded to Dr Anastasia A Theodosiou (grant reference MR/V002015/1). In addition, central funding will be available from the Southampton NIHR-CRF to assist with overheads, administration and support staff.

18.2 Insurance

UoS has a specialist insurance policy in place, which would operate in the event of any volunteer suffering harm as a result of their involvement in the research.

19.0 References

1. Roser M et al. Child and Infant Mortality. OurWorldInData.org Nov 2019. Retrieved Dec 2020 from: https://ourworldindata.org/child-mortality.
2. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 2018;18(11):1191-1210.
3. GBD 2016 Meningitis Collaborators. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2018;17(12):1061-1082.
4. Gostin L et al. The public health crisis of underimmunisation: a global plan of action. Lancet Infect Dis 2020;20(1):e11-16.
5. Laxminarayan R et al. Access to effective antimicrobials: a worldwide challenge. Lancet 2016;387(10014):168-75.
6. Teo S et al. Dynamics of airway microbiota identify a critical window for interplay of pathogenic bacteria and allergic sensitization in childhood respiratory disease. Cell Host Microbe 2018;24(3):341–352.
7. Luna P et al. The association between anterior nares and nasopharyngeal microbiota in infants hospitalized for bronchiolitis. Microbiome 2018; 6(1):2.
8. Bosch A et al. Maturation of the infant respiratory microbiota, environmental drivers, and health consequences.Am J Respir Crit Care Med 2017;196(12):1582–1590.
9. Thorsen J et al. Infant airway microbiota and topical immune perturbations in the origins of childhood asthma. Nat Commun 2019;10(1):5001.
10. Man W et al. Loss of microbial topography between oral and nasopharyngeal microbiota and development of respiratory infections early in life. Am J Respir Crit Care Med 2019;200(6):760–770.
11. de Steenhuijsen Piters W et al. The role of the local microbial ecosystem in respiratory health and disease. Philos Trans R Soc B Biol Sci 2015;370(1675):20140294.
12. Shinefield H et al. Bacterial interference: its effect on nursery-acquired infection with Staphylococcus aureus. V. An analysis and interpretation. Am J Dis Child 1963;105:683–8.
13. Sprunt K et al. Abnormal colonization of neonates in an ICU: conversion to normal colonization by pharyngeal implantation of alpha haemolytic Streptococcus strain 215. Pediatr Res 1980;14(4):308–313.
14. Hao Q et al. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev 2015;(2):CD006895.
15. Elias J et al. Are probiotics safe for use during pregnancy & lactation? Can Fam Physician 2011;57(3):299-301.
16. Hjelmsø M et al. Prenatal dietary supplements influence the infant airway microbiota in a randomized factorial clinical trial. Nat Commun 2020;11(1):426.
17. Ferretti P et al. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. Cell Host Microbe 2018;24(1):133–145.
18. Man W. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 2017;15(5):259–270.
19. Chu D et al. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med 2017;23(3):314–326.
20. Deasy A et al. Nasal Inoculation of the Commensal Neisseria lactamica Inhibits Carriage of Neisseria meningitidis by Young Adults. Clin Infect Dis 2015;60(10):1512-20.
21. Pandey A et al. Microevolution of Neisseria lactamica during nasopharyngeal colonisation induced by controlled human infection. Nat Commun 2018;9(1):4753.

22. Dorey R et al. The nonpathogenic commensal Neisseria: friends and foes in infectious disease. Curr Opin Infect Dis. 2019;32(5):490-496.
23. Bennett J et al. Genetic diversity and carriage dynamics of Neisseria lactamica in infants. Infect Immun 2005;73(4):2424.
24. Olsen S et al. Pharyngeal carriage of N meningitidis and N lactamica in households with infants within areas with high and low incidences of meningococcal disease. Epidemiol Infect 1991;106(3):445-57.
25. Gold R et al. Carriage of N meningitidis and N lactamica in infants and children. J Infect Dis 1978;137(2):112.
26. Li Y et al. Immunization with live Neisseria lactamica protects mice against meningococcal challenge and can elicit serum bactericidal antibodies. Infect Immun 2006;74(11):6348-55.
27. Laver JR et al. A recombinant commensal bacteria elicits heterologous antigen-specific immune responses during pharyngeal carriage. Sci Transl Med 2021;13(601):eabe8573.
28. de Steenhuijsen Piters W et al. Interaction between the nasal microbiota and S. pneumoniae in the context of live-attenuated influenza vaccine. Nat Commun 2019;10(1):2981.