Nasopharyngeal resistome evolution under selective pressure and association with adverse health outcomes in a paediatric population in Blantyre, Malawi

Protocol V7.0

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Glossary of abbreviations

Abbreviation	Term
AMR	Antimicrobial resistance
ARG	Antibiotic resistance gene
CEI	Community engagement and involvement
COMREC	College of Medicine Research and Ethics Committee
CRF	Case report form
DNA	Deoxyribonucleic acid
EDC	Electronic data capture
GPCC	Gateway Primary Care Centre
HGT	Horizontal gene transfer
HIV	Human Immunodeficiency Virus
ICF	Informed consent form
LSTM	Liverpool School of Tropical Medicine
MGE	Mobile genetic elements
MOP	Manual of procedures
NPM	Nasopharyngeal microbiome
NPR	Nasopharyngeal resistome
NPS	Nasopharyngeal swab
QA	Quality assurance
QC	Quality control
QECH	Queen Elizabeth Central Hospital
REC	Research Ethics Committee
RSV	Respiratory Syncytial Virus
SOP	Standard operating procedure
SQL	Structured Query Language
VacAMR	Vaccines to control respiratory pathogens and Antimicrobial Resistance
WHO	World Health Organisation

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Executive Summary

Study type: This is a multi-site, extended observational case-control study with a preliminary cohort component.

Problem: Based on modelling, Sub-Saharan Africa is the region most affected by death due to antimicrobial resistance (AMR), but there is little AMR data from the region. Pneumonia remains a leading cause of death in children under 5 years old worldwide. To change this, we must understand how antibiotic selective pressure and healthcare exposure impacts AMR development and how AMR influences health outcomes in resource limited settings. The nasopharynx is a reservoir of organisms that cause pneumonia and their associated AMR. Metagenomic deep-sequencing enables identification of the nasopharyngeal resistome (NPR), all the genetic determinants of AMR within the nasopharyngeal bacterial niche. No study has examined whether human resistome composition is associated with health outcomes.

Objectives: Our study aims to answer the following research question; does hospital admission and antibiotic treatment for pneumonia generate a persistent hospital-acquired nasopharyngeal resistome in Malawian children, and is this associated with adverse health outcomes in a resource limited setting? The primary objectives of this study are:

1. To measure how the diversity and relative abundance of the nasopharyngeal resistome of children hospitalised with pneumonia differs from the nasopharyngeal resistome of healthy children in the community.
2. To measure how the diversity and relative abundance of the nasopharyngeal resistome of children hospitalised with pneumonia differs from the nasopharyngeal resistome of children with pneumonia treated with antibiotics in the community.
3. To investigate whether there is measurable persistence of a hospital-acquired nasopharyngeal resistome post-discharge, and whether this is associated with adverse health outcomes, including recurrent hospitalisation.

Methodology: This study will recruit 350 children aged 12-36 months in four groups: healthy children in the community, children with pneumonia in the community, children hospitalised with pneumonia and children re-hospitalised with pneumonia. The study will be conducted at Ndirande community healthcare centre, Gateway primary care centre and Queen Elizabeth Central Hospital. Nasopharyngeal swabs (NPS) and urine samples will be taken at recruitment and follow up study visits; NPS will undergo metagenomic sequencing to identify the nasopharyngeal microbiome (NPM) and NPR, urine samples will be used for an

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antibiotic assay. Demographics, co-morbidities, health outcomes and antibiotic exposure will be recorded at recruitment for all participants, and subsequent study visits where applicable.

Expected results and dissemination: The study is expected to describe NPR evolution following antibiotic selective pressure and healthcare exposure for pneumonia in Malawian children. It will provide contemporary data on AMR carriage in the community, hospital admission and re-admission rates with pneumonia, and antibiotic use patterns. A report of the study will be submitted to the College of Medicine Research Ethics Committee (COMREC), Blantyre district health office and Liverpool School of Tropical Medicine (LSTM). The findings will be presented at international scientific conferences and published in peer reviewed medical journals.

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1.0 Background

Antimicrobial resistance (AMR) is a threat to global public health, predicted to cause 10 million deaths annually by 2050 [1]. Based on modelling, Sub-Saharan Africa is the region most affected by AMR-attributable death, but there is little AMR data from the region [2]. To change this, we must understand how antibiotic selective pressure and healthcare exposure impacts AMR development and how AMR influences health outcomes. We need comprehensive microbiological and epidemiological data from resource limited settings, where data are sparse [2,3], to design appropriate public health interventions [4].

Pneumonia is a leading cause of death in children under 5 years old worldwide [5] and one of three syndromes that dominate the global burden of AMR [2]; it remains a diagnostic challenge [6]. The nasopharynx is a reservoir for pneumonia pathogens, including *Streptococcus pneumoniae* and *Staphylococcus aureus*, which are common causes of AMR-attributable death [2].

Metagenomic deep-sequencing enables identification of all the antibiotic resistance genes (ARGs; genetic code that produces antibiotic resistance in a bacteria) and mobile genetic elements (MGEs; genetic code that carries ARGs between different bacterial species), that form the resistome in a microbial niche. There is limited data on NPR evolution [5]; studies are characterised by small samples sizes and single sampling time points. Comparison between studies is limited by differing populations, deoxyribonucleic acid (DNA) extraction methods and sequencing techniques [5].

Many of these studies do not examine the NPM; they use targeted sequencing techniques to focus on specific resistance determinants [7–9], or culture enrichment to focus on Streptococcal species, particularly *S. pneumoniae* [7,9–12]. This biased analysis creates the misleading impression that the NPM and NPR are dominated by *S. pneumoniae* [13]; other pathogens in the nasopharynx do exhibit AMR, including *H. influenzae* and *S. aureus* [14]. Importantly, a more diverse microbiome may facilitate exchange of ARG within a resistome [13]. *S. pneumoniae* readily acquires ARG by horizontal gene transfer (HGT; movement of genetic code from one organism to another) on MGE [15], particularly in the context of multiple serotype carriage [11], and from environmental DNA by transformation [15].

Existing NPR studies do highlight some interesting themes: whilst many ARG are associated with streptococcal species, β lactamase genes are predominantly found on non-

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streptococcal species, which may provide a source for HGT between species [12]. The neonatal nasopharynx is colonised by ARG from environmental sources [8,16]; this is particularly relevant in hospitalised neonates, who may acquire nosocomial bacteria with ARG on MGE that have potential for HGT to pneumonia pathogens within the NPM.

1.1 Rationale

There is limited data on NPR evolution under antibiotic selective pressure and following exposure to a hospital environment. No study has examined whether human resistome composition (ARG abundance and diversity) is associated with health outcomes.

Malawi has limited healthcare resources and a high incidence of pneumonia in children aged 12-36 months [17]. Our study will describe the impact of antibiotic selective pressure and exposure to the hospital environment on the NPR of Malawian children with pneumonia. The study will investigate the hypothesis that among these children a hospital-acquired NPR is associated with adverse health outcomes following discharge, by examining whether NPR composition is associated with hospital readmission.

We have chosen to focus on hospital re-admission as an adverse outcome related to AMR in pneumonia, because severe pneumonia requiring hospitalisation is more commonly bacterial in aetiology [6]; thus, re-hospitalisation is more likely to reflect the impact of AMR in bacterial pneumonia. Alongside the World Health Organisation (WHO) markers of severity in pneumonia, a range of adverse health outcomes of AMR in bacterial pneumonia will be recorded, including additional episodes of pneumonia, additional courses of antibiotics and their duration, and mortality outcomes.

Antibiotic use prior to seeking healthcare input is common in Malawi; 70% of patients report self-administration of antibiotics in this setting [18], which correlates with recent unpublished evidence of antibiotic activity detected in urine assays in hospitalised children.

Disentangling the impact of antibiotics and hospital admission on the resistome could inform public health interventions, including prescribing practices and hospital admission policies.

1.2 Hypothesis

Our hypothesis is that antibiotic use will select for a less diverse group of ARGs, dominated by ARGs that produce phenotypic resistance to the class of antibiotics to which an individual has been exposed; that ARGs will be more abundant in the NPR at discharge from

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hospital, due to microbiome dominance by the pneumonia pathogen; and that exposure to the hospital environment will drive acquisition of ARGs on MGEs, which could lead to more HGT. Children who are re-admitted to hospital with pneumonia within 3 months of a previous hospitalisation will have a more 'high-risk' NPR than those who remain in the community after hospital discharge, with a greater abundance of ARGs, less diverse NPR and greater prevalence of MGEs.

1.3 Study Objectives

1.3.1 Broad Objective

To determine whether hospital admission and antibiotic treatment for pneumonia generate a persistent hospital-acquired nasopharyngeal resistome in Malawian children, and whether this is associated with adverse health outcomes in a resource limited setting?

1.3.2 Primary Objectives

1. To measure how the diversity and relative abundance of the nasopharyngeal resistome of children hospitalised with pneumonia differs from the nasopharyngeal resistome of healthy children in the community.
2. To measure how the diversity and relative abundance of the nasopharyngeal resistome of children hospitalised with pneumonia differs from the nasopharyngeal resistome of children with pneumonia treated with antibiotics in the community.
3. Investigate whether there is measurable persistence of a hospital-acquired nasopharyngeal resistome post-discharge, and whether this is associated with adverse health outcomes, including recurrent hospitalisation.

1.3.3 Specific Secondary Objectives

1. To measure the rate of hospital re-admission with pneumonia in children aged 12-36 months over a 4-week period in a single tertiary referral hospital in Malawi.
2. To measure antibiotic use in the community in children aged 12-36 months in Malawi.

2.0 Methodology

2.1 Study design

This is a multi-site, extended case-control study with a preliminary cohort component to assess the evolution of the NPR under selective pressure of antibiotic use and hospital admission for pneumonia in children aged 12-36 months in Malawi. The study will be led by the PhD student, Lucy O'Connor, as a PhD fellowship.

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In the preliminary cohort component, healthy children in the community will have a NPS collected at the first study visit, to determine the baseline community resistome through metagenomic sequencing (see Figure 1).

To assess the impact of oral antibiotics on the NPR, children presenting with pneumonia to community healthcare clinics, Ndirande and Gateway Primary Care Centre (GPCC), will have a NPS collected at the first study visit and 3-months post-recruitment, to determine the antibiotic-exposed resistome through metagenomic sequencing (see Figure 1).

To understand the impact of broad-spectrum intravenous antibiotics and exposure to a hospital environment on the NPR, children hospitalised with pneumonia (without any hospital admission 6 months prior) to Queen Elizabeth Central Hospital (QECH) will have a NPS collected at the first study visit, discharge and 3-months post-recruitment to determine a hospital-acquired resistome through metagenomic sequencing (see Figure 1).

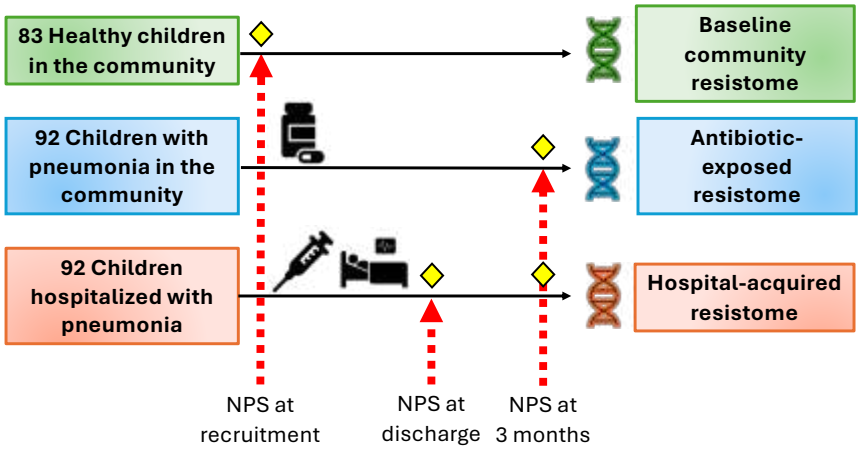

Figure 1. Preliminary cohort component of the study. The yellow diamonds represent the key samples groups for NPR comparison.

The case-control component of the study will examine whether a hospital acquired NPR is associated with adverse health outcomes, specifically re-admission to hospital with pneumonia. Children hospitalised to QECH with pneumonia (without admission 6 months prior), who are not re-admitted within 3 months will form the control group (see Figure 2); these participants will have a NPS will be collected at the first study visit (within 72 hours of admission to hospital), discharge from hospital and 3-months post-recruitment to assess for a hospital-acquired resistome. Those hospitalised within the preceding 6 months will be

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excluded from this control group, to prevent confounding. Children admitted to QECH with pneumonia within 3 months of a previous hospital admission with pneumonia will form the case group; these participants will have a NPS collected at the first study visit (within 72 hours of admission) only, to assess for a hospital-acquired resistome. Health passport screening and carer-reporting will be used to establish prior hospitalisation.

A 4-week audit of hospital re-admission rates of children aged 12-24 months with pneumonia will be conducted at QECH before starting study recruitment, to further inform sample size calculations.

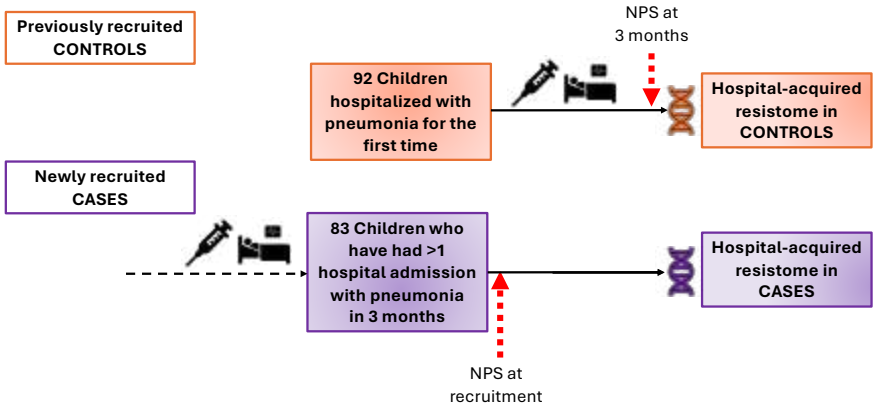

Figure 2. Case-control study design to examine whether a hospital-acquired NPR is associated with re-admission to hospital with pneumonia.

Two flocked NPS will be taken at the first study visit from all study participants with pneumonia; one NPS will be stored for viral PCR analysis (outside this fellowship), the other NPS will undergo metagenomic sequencing. Viral PCR analysis of the NPS will seek to identify RNA pathogens most associated with severe acute respiratory infections requiring hospitalisation in this population: Respiratory Syncytial Virus (RSV) and Influenza A [19]. Only one NPS for metagenomic sequencing will be taken from study participants at subsequent study visits e.g. discharge from hospital and 3-month follow-up. All NPS will be stored at -80°Celsius, following an established protocol [20], prior to DNA extraction using mechanical and chemical lysis to optimise the yield of DNA from low-biomass samples dominated by gram-positive organisms. Extracted DNA will undergo metagenomic sequencing. Metagenomic data analysis will be performed using an open-source bioinformatic pipeline that I will develop to determine NPM and NPR composition. A urine

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sample will also be collected from each participant at the first study visit, and stored for antibiotic assays (outside this fellowship), to assess the impact of antibiotic use prior to seeking healthcare input on NPR composition.

Age, gender, co-morbidities, mid-upper arm circumference, pneumococcal vaccination status, antibiotics received 7 days prior to recruitment, haemoglobin level and malaria screening results will be recorded for all participants at recruitment; exposure to tobacco smoke from a household contact and whether the participant was breastfed in their first 3 months of life will also be recorded, as these are independently associated with pneumonia requiring hospitalisation in high-income settings [21]. Mode of childbirth (vaginal versus caesarean section) will also be recorded, as this influences early NPM development, and may be associated with childhood respiratory health [22]. To assess for acquisition of MGEs from the hospital environment, we will record data on duration of hospitalization and incidence of shared bed-spaces for hospitalised study participants, including number of occupants and number of days in a shared bed-space. Shared hospital bed-spaces will be sampled using a swab for metagenomic sequencing. Groups undergoing repeat NPS at 3 months will have health outcomes (including mortality), healthcare interactions (including healthcare facility and hospital attendance/admission) and antibiotic exposure recorded.

Groups being admitted to hospital will have WHO markers of severity in pneumonia recorded, including presence of the following at the time of admission: central cyanosis, oxygen saturations less than 90%, inability to feed, vomiting, convulsions, lethargy and impaired consciousness. Anonymised blood culture results and chest radiographs that are taken as part of routine clinical care for hospitalised participants will also be recorded. To assess for potential influence of healthcare seeking behaviour on hospital re-admission, groups presenting to healthcare clinics and/or hospitals with pneumonia will have the educational level of the parents recorded, and the distance travelled to the healthcare clinic.

2.2 Key characteristics of the organisation and site

Healthy community participants will be recruited in Ndirande wards, which are under the Blantyre District Health Office. These wards are likely to be representative of the baseline community resistome for children admitted to QECH, due to the geographical proximity of this ward to the hospital. Community participants with pneumonia will be recruited in Ndirande health centre and GPCC, a primary care clinic near to QECH. Hospitalised participants will be recruited from QECH, a tertiary referral hospital in central Blantyre.

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The Malawi Liverpool Wellcome Programme (MLW) was founded in 1995 and is a partnership between Kamuzu University of Health Sciences (KUHeS), LSTM and the University of Liverpool; core funding is provided by the Wellcome trust. MLW has modern ISO15189 accredited diagnostic laboratories, and dedicated laboratories for microbiology and molecular biology. MLW also has a dedicated clinical research pharmacy and runs several clinical sites around the country. MLW has also invested in a strategy for integrated data collection platforms, with robust management systems and transparent, safe and GCP-compliant data sharing, allowing clinical, researcher and collaborator data access.

2.3 Study population

The study population will be healthy children aged 12-36 months in Ndirande, children aged 12-36 months with pneumonia presenting to GPCC or Ndirande Health Centre and children aged 12-36 months hospitalised with pneumonia at QECH. Children with severe anaemia (haemoglobin level < 70 grams per Litre), on long-term antibiotic treatment or prophylaxis, children on immunosuppressive medications or with an immunosuppressive illness, including HIV infection, will be excluded from the study.

2.4 Study period

This study will be conducted over a three-year period as a PhD fellowship, with participant recruitment over an 18-month period; see Figure 3. Due to the budget, our study team will not have the capacity to recruit in the community and hospital concurrently; community participants will be recruited prior to hospital participants.

We have chosen a 3-month follow up timepoint, to allow the resistome to return to a post-antibiotic baseline; a previous study demonstrated microbiome recovery within 3 months of infective-asthma exacerbation [23].

Importantly, there is evidence of seasonal variation in composition of the NPM and NPR [24]. Unfortunately, our study team size is restricted by budgetary constraints, therefore we are unable to recruit in the community and hospital simultaneously. We plan to conduct most of the community patient recruitment intensively within a 6-month period, before starting hospital recruitment. To restrict potential confounding due to seasonal effect, we plan to return to the community on an infrequent basis (approximately 3-4 weekly) for healthy community sampling over the following 6 months to ensure a baseline healthy resistome has been sampled across all seasons.

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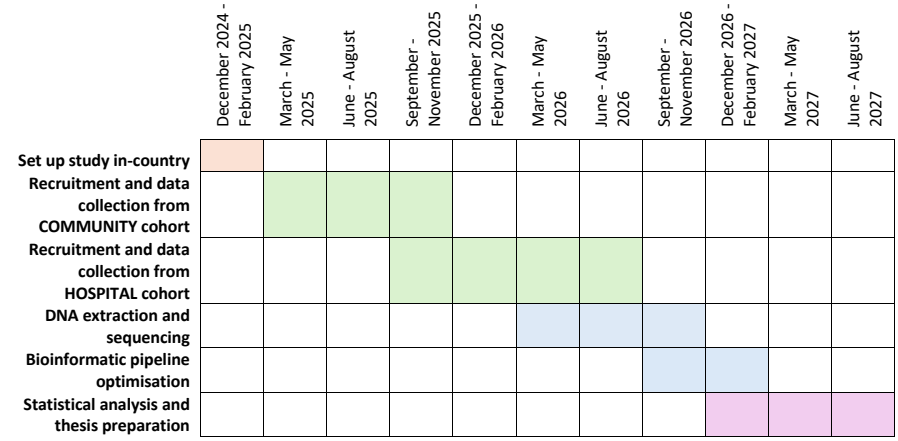

Figure 3. Study timeline

Samples collected from study participants will be stored for 5 years after the conclusion of the study at Malawi Liverpool Wellcome program. The study will be completed within 18 months of the start of recruitment, or when the recruitment targets have been achieved and all samples collected (if this occurs in a time frame shorter than 18 months). Upon study completion, the Sponsor (LSTM), RECs and MLW will be notified; a report of the study findings will be submitted to COMREC, Blantyre district health office and LSTM.

2.5 Sample size and calculations

There is no established method for sample size calculation in resistome studies; we use an approach for microbiome studies [25]. Differences in gut plasmidome composition have been detected with 41 participants per group [26], but NPR has a lower biomass than gut resistome, therefore the resistome is likely to be less diverse, with a lower relative abundance of ARGs. NPR analysis 3.5 years post-antibiotic exposure in a cluster-randomised trial of mass-azithromycin administration shows a trend toward decreased NPR diversity (p = 0.07) in azithromycin clusters when compared to placebo clusters, with just 25 samples per group (unpublished data).

We have used the measured difference in relative abundance of a specific macrolide ARG (ermX) 3.5 years after mass-azithromycin administration (unpublished data) as a medium effect size that maybe clinically relevant (Δ= 0.505). Using the approach for microbiome studies, accepting 90% power and 5% type I error (95% confidence interval),

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we've calculated that we will require a sample size of 83 participants in each group to detect this effect size, using the formula $n=2(Z_{1-\alpha/2}+ Z_{1-\beta})^2/\Delta^2$ [25].

In total we aim to recruit 350 study participants: 83 healthy community participants, 92 participants with pneumonia in the community, 92 participants admitted to hospital with pneumonia and 83 participants re-admitted to hospital with pneumonia. Study groups with a sample size of 92 participants permit a 10% loss to follow up.

2.6 Community Engagement

Our study population of children in a resource limited setting are vulnerable, and not represented by a patient advocacy group. This study will be embedded within a larger programme of vaccine evaluation and AMR research at MLW, which has been underpinned by longstanding community engagement and involvement (CEI); the NIHR global health research group for vaccines to control respiratory pathogens and AMR (VacAMR) in Malawi. Working with the VacAMR CEI team, we will conduct community engagement activities prior to, during and after study recruitment to engage all key stakeholders, including community leaders, community health workers and hospital clinical staff. This will ensure a clearer understanding of the study within the communities, which will help recruitment and enable meaningful dissemination of study results. Initial community engagement meetings will involve a presentation of the background, purpose and procedures in the proposed study, and an introduction to the study team; the anticipated impact of the study will be highlighted. After study completion, key findings will be shared with participants, communities and local government entities, including key stakeholders through applicable platforms.

2.7 Participant recruitment, enrolment and consent

Healthy children will be recruited from Ndirande community using established random recruitment protocols for pneumococcal carriage surveys [20]. Children with pneumonia in the community will be recruited at time of presentation to Ndirande community healthcare centre and GPCC. Children hospitalised with pneumonia (without admission 6 months prior) will be recruited within 72 hours of admission to QECH. Children re-hospitalised with pneumonia within 3 months will be recruited within 72 hours of admission to QECH; health passport screening and carer-reporting will be used to establish prior hospitalisation.

A trained member of the study field team will approach and explain the study to the parent/legally acceptable representative of potential study participants that meet the eligibility

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criteria in the community setting. Nursing staff at Ndirande community healthcare centre, GPCC and QECH will identify and approach patients eligible for potential study enrolment, for which they will receive a stipend.

The parent/legally acceptable representative of each eligible participant will be asked to sign an Informed Consent Form (ICF). Consent will be sought for sample storage, indicating whether the parent/legally acceptable representative authorises storage of NPS and urine samples for 5 years after study completion. In situations where the parent/legally acceptable representative is illiterate, an impartial witness will be present to verify the participant's understanding and will also sign on the ICF; the participant's parent/legally acceptable representative will acknowledge their agreement with a thumbprint. Each participant's parent/legally acceptable representative will be given a copy of the signed ICF, which includes contact information for the principal investigator and the participant identifier.

2.7.1 Withdrawal from study

During the consent process, it will be clearly communicated to the participant's parent/legally acceptable representative that they are free to withdraw from the study at any time. If any participant withdraws before completing the study, we will seek consent from the participant's parent/legally acceptable representative to use already collected data. Participants will not have to provide a reason for withdrawing from the study. All participant's parents/legally acceptable representatives will be informed prior to consent and upon withdrawal that there will be no consequences for their withdrawal from the study at any time.

2.7.2 Participant compensation

Each participant's parents/legally acceptable representatives will receive the equivalent of $10 (United States Dollars) as travel reimbursement and compensation for time spent on study visits. At the current exchange rate of $1.00 for MK 1,700, each participant's parents/legally acceptable representatives will be given MWK17,000 for time spent on study activities at each visit in accordance with the guidelines set by COMREC. We will review the currency exchange rates regularly and update the compensation as necessary.

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2.7.3 Eligibility criteria

Pneumonia will be defined clinically using WHO criteria as the presence of fever, cough, dyspnoea and tachypnoea requiring antibiotic treatment. Current diagnostic guidelines do not recommend chest radiographs for uncomplicated pneumonia.

Patients with HIV infection or severe anaemia (<70g/L) will be excluded; these variables are independently associated with adverse health outcomes. Severe anaemia is the leading cause of recurrent hospitalisation in African children [27]. HIV is associated with post-discharge mortality in childhood pneumonia [28].

2.7.3.1 Inclusion criteria for healthy children in the community

- Child aged between12-36 months.

2.7.3.2 Exclusion criteria for healthy children in the community

- Presence of any of the following symptoms: fever, cough, difficulty in breathing or fast breathing.
- Currently taking long-term antibiotic prophylaxis, TB treatment or immunosuppressive medications.
- Diagnosis of an immunosuppressive illness, including HIV infection.
- Hospital admission within the past six months.

2.7.3.3 Inclusion criteria for children with pneumonia in the community

- Child aged between12-36 months.
- Presence of all of the following symptoms: fever, cough, difficulty in breathing and fast breathing.
- Participant has been prescribed antibiotics for treatment of a lower respiratory tract infection on this presentation.

2.7.3.4 Exclusion criteria for children with pneumonia in the community

- Severe anaemia, with a recorded haemoglobin level < 70 grams per Litre.
- Currently taking long-term antibiotic prophylaxis, TB treatment or immunosuppressive medications.
- Diagnosis of an immunosuppressive illness, including HIV infection.
- Hospital admission within the past six months.

2.7.3.5 Inclusion criteria for children hospitalised with pneumonia

- Child aged between 12-36 months.

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- Presence of all of the following symptoms: fever, cough, difficulty in breathing and fast breathing.
- Participant has been prescribed antibiotics for treatment of a lower respiratory tract infection on this presentation.

2.7.3.6 Exclusion criteria for children hospitalised with pneumonia

- Severe anaemia, with a recorded haemoglobin level < 70 grams per Litre.
- Currently taking long-term antibiotic prophylaxis, TB treatment or immunosuppressive medications.
- Diagnosis of an immunosuppressive illness, including HIV infection.
- Hospital admission within the past six months.
- Resident outside of Blantyre district.

2.7.3.7 Inclusion criteria for children re-hospitalised with pneumonia

- Child aged between12-36 months.
- Presence of all of the following symptoms: fever, cough, difficulty in breathing and fast breathing.
- Participant has been prescribed antibiotics for treatment of a lower respiratory tract infection on this presentation.
- Hospital admission to ANY hospital with a lower respiratory tract infection within the past 3 months.

2.7.3.8 Exclusion criteria for children re-hospitalised with pneumonia

- Severe anaemia, with a recorded haemoglobin level < 70 grams per Litre.
- Currently taking long-term antibiotic prophylaxis, TB treatment or immunosuppressive medications.
- Diagnosis of an immunosuppressive illness, including HIV infection.

2.8 Safety

Participants' safety will be prioritised throughout the study. All potential, foreseeable safety risks will be fully outlined and disclosed verbally during the consent process, and in writing on the participant information sheet and ICF. The process of NPS collection can cause slight discomfort and irritation for children; in rare instances (with certain underlying conditions) the child may experience light bleeding after sample collection. Staff collecting NPS will be trained to minimise discomfort, and provide participant and parent/legally acceptable representative reassurance during the sample collection process.

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2.9 Statistical analysis

The statistical analysis plan is briefly summarised below. A detailed statistical analysis plan will be developed prior to starting recruitment.

2.9.1 Primary analysis

Participants will be gender-matched across groups. To establish differences in NPR composition the mean NPR diversity in a sample (measured using the Shannon index), mean relative abundance of all ARGs in a sample and mean relative abundance of specific ARGs in a sample will be compared between study groups using unadjusted t-tests, as summarised in Figure 4. Statistical analysis will be performed in R. Results will be presented in tables and illustrative heatmaps.

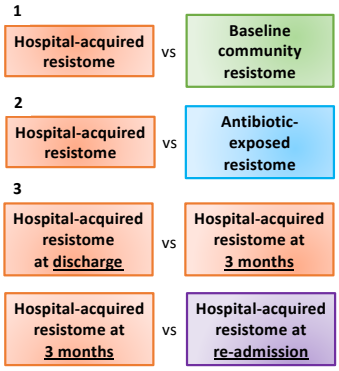

Figure 4. Plan for statistical analysis of NPR composition.

2.9.2 Secondary analyses

Principal component analyses and non-metric multidimensional scaling will be used to determine whether there is a distinct hospital-acquired NPR in hospitalised participants. Conditional logistic regression will be used to test for association between NPR composition and re-hospitalisation with pneumonia within a 3-month period, controlling for potential confounders including co-morbidities, nutritional status, duration of hospital admission and severity of pneumonia.

2.10 Data management

Human subject demographic and clinical metadata will contain confidential and personal information. Case report forms (CRFs) will be entered digitally on password encrypted tablets using the electronic data capture (EDC) system ODK, then transferred daily to the SQL server, a password encrypted database, using a secure connection at MLW in

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compliance with Malawian data transfer legislation. Only trained study field team members will have access to this encrypted database; access will be controlled by unique user ID and password setup to maintain confidentiality. Data entry, modification, and download will be restricted to specific roles in the study team. All password encrypted tablets will be locked in a secure location when not in use on field visits. Data cleaning and database locking will be conducted at MLW. The database will be locked shortly after study completion; anonymised data will then be transferred via the MLW data portal to a password encrypted folder on the LSTM shared (S:) network drive and a password encrypted external hard drive.

Paper documents will be used for recording the process of data collection in the laboratory; a member of study staff will deliver paper documents to a data centre with locked cabinets. Study documentation, including consent forms, will be archived in-country for 10 years, as per LSTM policy.

NPS and urine samples will be labelled with a barcode linked to a unique identifier, which will be recorded on the CRFs during data entry. Metagenomic sequence data will be labelled with this unique identifier. Raw metagenomic sequence data will contain confidential human genome sequence data; this will be removed in the bioinformatic pipeline and not stored long-term.

2.11 Data sharing and access

Access to the study database and metagenomic sequence data will be restricted to the clinical study team, or approval granted by the principal investigator (PI), to ensure participant confidentiality.

No human genome sequence data will be shared with researchers. Anonymised human genome, microbiome and resistome sequence data will be stored in .fast5, .fastq and .csv format on a password encrypted folder on the LSTM shared (S:) drive space, a password encrypted external hard drive and in the Amazon Elastic Compute Cloud, a secure web platform for data storage.

As a population health study, anonymised digital patient and metagenomic data will be stored on password-encrypted external hard drives for a minimum of 10 years after study completion; these will be retained by the PI and clinical fellow at LSTM. Open access publication and sharing of curated metagenomic data on the github repository will ensure long-term data preservation. All electronic databases will be stored behind institutional firewalls, backed-up on-site and off-site according to standard operating procedures (SOPs).

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This will be overseen by the investigators. The processes and specific details of data sharing and access will be described in the Data Management and Access Plan.

2.12 Quality assurance and quality control

To ensure the quality of processes, data and documentation, a manual of procedures (MOP) will be prepared. This will encompass both quality control (QC) and quality assurance (QA) activities, with SOPs to ensure confidentiality and data integrity and to prevent human and processing errors. Key elements covered by the SOPs will include:

- Data storage: Utilization of unique participant identification numbers for data storage.
- Data Evaluation: Ensuring protocol compliance and source document accuracy.
- Document Review: Establishing responsibilities and frequency for document review, including specimen tracking logs and questionnaires.
- QA and QC Issue Resolution: Outlining responsibilities for addressing QA issues (correcting procedures not compliant with the protocol) and QC issues (correcting errors in data entry).

During study conduct, the MLW Clinical Research Support Unit may look closely at field data quality, specimen quality and laboratory examination processes. In the event of identifying a protocol deviation, documentation must include a detailed description, root cause analysis, plan for correction and prevention of future deviations. Any deviations to the protocol will be submitted to LSTM as sponsor; any proposed amendments to the protocol will be submitted to LSTM as sponsor and the LSTM research ethics committee (REC) for approval.

2.13 Dissemination of the results

The study results will be shared with COMREC and the Ministry of Health after study completion and data analysis. All research outputs from this study will be published on open-source platforms or in open-source scientific journals. I will work with the VacAMR CEI team to share our findings with participating communities and local stakeholders at QECH, to ensure research outputs benefit the local population. I will also communicate our findings to patients and the public in Malawi and globally, to generate a wider dialogue around AMR, through collaborations with non-governmental organisations such as One Health Trust, The Foundation to Prevent Antibiotic Resistance, Antibiotic Research UK and the British Society for Antimicrobial Chemotherapy. I will create public information bulletins that summarise our findings, to improve accessibility to key information.

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2.14 Ethical considerations

Written study information and consent material will be provided in Chichewa. Consent for participation in this observational study will be sought from the legal guardians of participants, as our study population aged 12-36 months will not be able to consent for themselves. The consent process will detail the collection of NPS for metagenomic sequencing and viral PCR, urine samples for antibiotic assays and recording of demographic data, co-morbidities and health outcomes for analysis. The consent process will explain that anonymised microbiome and resistome data will be shared with other researchers. No human genome sequence data will be shared.

Study participants will be renumerated for study visits in keeping with current guidance [29]. Study visits and nasopharyngeal sampling from children will be minimised to ensure participant retention.

2.14.1 Ethical compliance

The study protocol will be reviewed and approved by COMREC and LSTM REC. Any amendment will require COMREC and LSTM REC approval before implementation; any amended protocol would then supersede the initial protocol. Prior to study enrolment, parents/legally acceptable representatives of study participants will be provided with all the necessary information about the study and voluntary study participation. Written informed consent will be sought and documented from at least one parent/legally acceptable representative of all study participants. The study will be conducted in compliance with the protocol and applicable global and local ethical requirements.

2.15 Project risks and constraints

Potential challenges to study completion and risks of participant enrolment are considered below.

2.15.1 Failure to recruit sufficient participant numbers

A major potential challenge to study completion is identifying enough participants being re-hospitalised within 3 months to meet our recruitment targets. There is no data on hospital re-admission rates due to pneumonia in Sub-Saharan Africa. The leading cause of recurrent hospitalisation among children aged under 5 years in Sub-Saharan Africa is severe anaemia, with a readmission rate of 18% over 6 months [27]. A study conducted at QECH between April 2013 and August 2016 recruited 1600 children presenting with pneumonia that met the WHO's Integrated Management of Childhood Illness (IMCI) definition: cough or

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difficult/fast breathing [30]; this is broader than our clinical definition, but based on this we could expect to recruit approximately 500 children hospitalised with pneumonia in a 12-month period. If the hospital re-admission rate were equal to severe anaemia at 18% over 6 months, this would equate to just 90 cases in a 12-month period.

A 4-week audit of hospital re-admission rates of children aged 12-24 months with pneumonia will be conducted at QECH before starting study recruitment, to further inform sample size calculations. We will use parent/carer reporting and patients' health passports as screening tools to identify those being re-admitted within 3 months of a previous hospitalisation with pneumonia. Our budget includes a stipend for hospital nursing staff to identify potentially eligible patients for screening during the admission process.

If the study experiences low participant enrolment numbers within the first 4 months, we will reassess and renew our CEI activities. Potential adjustments to the study design could include a wider age-range of study participants, although this would likely result in greater variability of baseline NPM and NPR; modifying the case to control ratio to 0.75 to 2; or adjusting the case definition to include hospital re-admissions with any infection requiring antibiotics within 3 months of hospitalisation with pneumonia, which may enable us to capture the extra-pulmonary adverse health effects of AMR.

2.15.2 Recruitment of critically unwell patients

Where appropriate, we will seek to enrol critically unwell patients in the study, as data from these participants is key to understanding the impact of AMR on health outcomes. However, there may be times when it is not appropriate to approach a parent/legally acceptable representative to discuss participant enrolment, for example during emotional distress. Importantly, participant enrolment will never interrupt or disrupt provision of medical care to an acutely unwell patient.

2.15.3 Perception bias

To ensure voluntary study participation and avoid participant enrolment driven by the perception that patients will receive better care if enrolled in the study, the ICF will clearly explain to parents/legally acceptable representative s of participants that participation and/or withdrawal from the study will not affect the standard of care they will receive.

2.15.4 Challenges to identifying the legally acceptable representative for consent process

It may be difficult to identify the appropriate legally acceptable representative to consent for a child's participation in the study. The adult accompanying a potential participant

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to a study enrolment site (i.e. Ndirande healthcare centre, GPCC or QECH) will be asked whether they are the child's parent; if not, they will be asked to identify the child's parent to obtain consent for study participation. If the child does not have a parent able to consent on their behalf, the accompanying adult will be asked to identify the child's legally acceptable representative to obtain consent for study participation. If no legally acceptable representative can be identified for the consent process, the child will not be enrolled in the study.

2.15.5 Failure of long read metagenomic sequencing

Preliminary laboratory work for this study was performed on small numbers of NPS in a UK laboratory setting, with varying yields of long-read DNA following metagenomic sequencing; this was attributed to use of sequencing equipment (MinION flow cells) beyond their recommended storage period. There is a chance that DNA extraction and long-read sequencing will not yield the expected results in a different laboratory setting. Prior to study recruitment, trials of DNA extraction and long-read sequencing will be performed on NPS and a laboratory reference standard, the ZymoBIOMICS microbial community standard, to ensure reliability and reproducibility of sequencing results.

2.15.6 Reliable antibiotic consumption data

A major challenge to the reliability of this study will be the ability to collect data on antibiotic consumption by study participants from their parent/legally acceptable representative. Previous studies of antibiotic adherence for fast-breathing pneumonia in children in Malawi have shown that up to 20% are non-adherent with the prescribed antibiotic regimen [31], due to challenges in preparing and administering medication, community pressures to share drugs and occasionally the complexity of regimens [32]. Another study of antibiotic use in adults in rural Malawi, found that 70% of the population self-medicated with antibiotics [18]. In these studies, attempts to improve reliability of recorded antibiotic consumption include pill counting and use of a "drug bag" visual aid depicting the oral formulations of common antibiotics for pneumonia in children, to assist parents/legally acceptable representative s in recalling their antibiotic use [18].

We will use an antibiotic record card, attached to patients' health passports to assist accurate recall of the duration of oral antibiotics consumed in the community, and the "drug bag" visual aid when completing the CRFs with participants' parents/legally acceptable representatives. We will also collect urine samples at the recruitment study visit for an antibiotic assay on a separate grant.

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2.15.7 Global pandemic/Local epidemic

As this study will be conducted in a hospital setting, and involves nasopharyngeal sampling, there is the potential impact of a global pandemic equivalent to COVID-19, or epidemics equivalent to Monkeypox or viral haemorrhagic fevers, such as Marburg/Ebola. All sample collection in the study will be conducted using appropriate personal protective equipment, as outlined in SOPs, to protect study participants and staff. In the event of a global pandemic or local epidemic, the study will follow the appropriate MLW contingencies.

2.16 Future use of stored samples

Upon completion of the study, with participant approval, MLW will store the remaining samples (NPS and urine) for up to 5 years, maintaining confidentiality. During the consent process, participants will be provided with the choice to opt in or out of consenting for storage of specimens for future research. When necessary, the investigators will seek approval from the relevant RECs to consider the stored samples for further study.

2.17 Budget and finance

	Year 1		Year 2		Year 3		TOTAL	
	GBP	MWK	GBP	MWK	GBP	MWK	GBP	MWK
Project staff salaries	37,730.00	83,006,000	20,751.50	45,653,300	0	0	58,481.50	128,659,300
Research materials and consumables	9,770.00	21,494,000	13,013.00	28,628,600	350.00	770,000	23,133.00	50,892,600
Equipment: 2x Samsung Galaxy tablets	400.00	880,000	0	0	0	0	400.00	880,000
Miscellaneous	12,520.65	27,545,430	7,500.65	16,501,430	2,995.65	6,590,430	23,016.95	50,637,290
Participant compensation	2,484.00	5,464,800	2,484.00	5,464,800	0	0	4,968.00	10,929,600
Total	62,904.65	138,390,230	43,749.15	96,248,130	3,345.65	7,360,430	109,999.45	241,998,790

Table 1. Estimated study budget (1 GBP = 2200 MWK)

2.17.1 Budget justification

This study, the principal investigator and study field personnel are being funded by the Liverpool Clinical PhD Wellcome fellowship. No contribution is being requested from MLW or KUHeS.

2.17.1.1 Project staff and salaries

This project will require the following personnel:

- 1 research nurse
- 1 field research assistant
- 1 laboratory technician

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The study team will be recruited through MLW, and be eligible for generic MLW/KUHeS training for both academic and operations teams as determined by the Training Committee; £100 of the study budget will be contributed as a training fee.

2.17.1.2 Research materials and consumables

This includes field materials, laboratory materials and consumables, alongside international shipping charges for NPS transport.

2.17.1.3 Equipment

Data collection processes require equipment for sample collection and storage, and data storage; this includes computer tablets, power banks, external hard drives, transport to field sites, laboratory equipment and reagents.

2.17.1.4 Miscellaneous costs

This includes general project costs including IT support and telecommunications, translation costs and research support costs including data entry and management costs, statistical support, stipends for support staff, participant compensation, fees for COMREC/QECH applications and 10% KUHeS finance and administration fee.

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References

1. O'Neill, J. (2016) Tackling drug-resistant infections globally: Final report and recommendations. *Arch. Pharm. Pract.* 7, 110
2. Murray, C.J. *et al.* (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399, 629–655
3. Lester, R. *et al.* (2022) Effect of resistance to third-generation cephalosporins on morbidity and mortality from bloodstream infections in Blantyre, Malawi: a prospective cohort study. *The Lancet. Microbe* 3, e922–e930
4. Walsh, T.R. *et al.* (2022) Global burden of antimicrobial resistance: essential pieces of a global puzzle.*Lancet (London, England)*, 3992347–2348
5. O'Connor, L. and Heyderman, R. (2023) The challenges of defining the human nasopharyngeal resistome. *Trends Microbiol.* DOI: 10.1016/j.tim.2023.02.008
6. O'Brien, K.L. *et al.* (2019) Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet* 394, 757–779
7. Bojang, A. *et al.* (2020) Impact of Intrapartum Oral Azithromycin on the Acquired Macrolide Resistome of Infants' Nasopharynx: A Randomized Controlled Trial. *Clin. Infect. Dis.* 71, 3222–3225
8. Cason, C. *et al.* (2021) Microbial Contamination in Hospital Environment Has the Potential to Colonize Preterm Newborns' Nasal Cavities. *Pathog. (Basel, Switzerland)* 10, 615
9. Keenan, J.D. *et al.* (2018) The Effect of Antibiotic Selection Pressure on the Nasopharyngeal Macrolide Resistome: A Cluster-randomized Trial. *Clin. Infect. Dis. an Off. Publ. Infect. Dis. Soc. Am.* 67, 1736–1742
10. Allemann, A. *et al.* (2019) Nasal Resistome Development in Infants With Cystic Fibrosis in the First Year of Life. *Front. Microbiol.* 10, 212
11. Manenzhe, R.I. *et al.* (2020) Characterization of Pneumococcal Colonization Dynamics and Antimicrobial Resistance Using Shotgun Metagenomic Sequencing in Intensively Sampled South African Infants. *Front. public Heal.* 8, 543898
12. Manenzhe, R.I. *et al.* (2020) Longitudinal changes in the nasopharyngeal resistome of South African infants using shotgun metagenomic sequencing. *PLoS One* 15, e0231887–e0231887

Approved by
College of Medicine
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(COMREC)
Research and Ethics Committee

13. García-Rodríguez, J.A. and Fresnadillo Martínez, M.J. (2002) Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J. Antimicrob. Chemother.* 50 Suppl S, 59–73
14. Unger, H.W. *et al.* (2015) Impact of intermittent preventive treatment in pregnancy with azithromycin-containing regimens on maternal nasopharyngeal carriage and antibiotic sensitivity of Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus: a cross-sectiona. *J. Clin. Microbiol.* 53, 1317–1323
15. Aminov, R.I. (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.* 1, 134
16. Hendriksen, R.S. *et al.* (2019) Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* 10, 1124
17. Tizifa, T.A. *et al.* (2021) Incidence of clinical malaria, acute respiratory illness, and diarrhoea in children in southern Malawi: a prospective cohort study. *Malar. J.* 20, 473
18. Limwado, G.D. *et al.* (2024) Prevalence of antibiotic self-medication and knowledge of antimicrobial resistance among community members in Neno District rural Malawi: A cross-sectional study. *IJID Reg.* 13, 100444
19. Peterson, I. *et al.* (2016) Respiratory Virus-Associated Severe Acute Respiratory Illness and Viral Clustering in Malawian Children in a Setting With a High Prevalence of HIV Infection, Malaria, and Malnutrition. *J. Infect. Dis.* 214, 1700–1711
20. Swarthout, T.D. *et al.* (2020) High residual carriage of vaccine-serotype Streptococcus pneumoniae after introduction of pneumococcal conjugate vaccine in Malawi. *Nat. Commun.* 11, 1–12
21. Man, W.H. *et al.* (2019) Bacterial and viral respiratory tract microbiota and host characteristics in children with lower respiratory tract infections: a matched case-control study. *Lancet Respir. Med.* 7, 417–426
22. Bosch, A.A.T.M. *et al.* (2016) Development of Upper Respiratory Tract Microbiota in Infancy is Affected by Mode of Delivery. *EBioMedicine* 9, 336–345
23. Hou, J. *et al.* (2022) Temporal Dynamics of the Nasopharyngeal Microbiome and its Relationship with Childhood Asthma Exacerbation. *Microbiol. Spectr.* 10, e0012922
24. Flynn, M. and Dooley, J. (2021) The microbiome of the nasopharynx. *J. Med. Microbiol.* 70, 001368
25. Ferdous, T. *et al.* (2022) The rise to power of the microbiome: power and sample size

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College of Medicine
15/04/2026
(COMREC)
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calculation for microbiome studies. *Mucosal Immunol.* 15, 1060–1070

26. Willmann, M. *et al.* (2019) Distinct impact of antibiotics on the gut microbiome and resistome: A longitudinal multicenter cohort study. *BMC Biol.* 17, 1–18
27. Connon, R. *et al.* (2021) Incidence and predictors of hospital readmission in children presenting with severe anaemia in Uganda and Malawi: a secondary analysis of TRACT trial data. *BMC Public Health* 21, 1480
28. Ngari, M.M. *et al.* (2020) Mortality during and following hospital admission among school-aged children: a cohort study. *Wellcome open Res.* 5, 234
29. Gordon, S.B. *et al.* (2018) A Malawi guideline for research study participant remuneration [version 2; peer review: 2 approved]. *Wellcome Open Res.* 3
30. Iroh Tam, P.-Y. *et al.* (2022) Clinical pneumonia in the hospitalised child in Malawi in the post-pneumococcal conjugate vaccine era: a prospective hospital-based observational study. *BMJ Open* 12, e050188
31. Nightingale, R. *et al.* (2016) Non-adherence to community oral-antibiotic treatment in children with fast-breathing pneumonia in Malawi– secondary analysis of a prospective cohort study. *Pneumonia* 8, 21
32. King, C. *et al.* (2018) Non-adherence to oral antibiotics for community paediatric pneumonia treatment in Malawi – A qualitative investigation. *PLoS One* 13, e0206404

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