Justification for Changes in Protocol Version

These justifications were submitted to, and approved by:

1. The local ethics committee Kamuzu University of Health Sciences Research Ethics Committee (formerly College of Medicine Research Ethics Committee) in Malawi
2. The ethics committee of the sponsor institution, Liverpool School of Tropical Medicine in the United Kingdom.

Protocol V6.0 (Submitted on 01/10/2025, approved on 14/10/2025, implemented on 03/12/2025)

Since the study started recruiting on 19/08/2025, lower numbers of community pneumonia participants have been enrolled than anticipated. If enrolment of community pneumonia participants continues at the current rate, it will take 9 months to achieve the recruitment target of 92 community pneumonia participants, producing a 3-month delay in the study timeline.

The study screening logs indicate that most (74%) of patients with cough that have been screened in the paediatric outpatient clinic at Ndirande Health Centre are not eligible for enrolment because they fall outside of the target age-range of 12-24 months. Of those who were ineligible for enrolment at screening, 40% are aged between 25-36 months. Thus, extending the inclusion criteria to include children aged 12-36 months of age at recruitment will allow the study to achieve the recruitment target within the original timeframe.

Protocol V7.0 (Submitted on 10/03/2026, approved on 15/04/2026, implemented on 13/05/2026)

The following protocol amendments were requested to further improve the quality of the findings from the study:

1. To provide further information about the participants' presenting illness, we would like to record the results of blood cultures and chest radiographs taken as part of routine clinical care for NP Resistome study participants at Queen Elizabeth Central Hospital (QECH). If a potential pneumonia pathogen is identified in the blood culture of a study participant, it would help to address objective 3 of the study: examining the association between the carriage of antimicrobial resistance, and antimicrobial resistance causing adverse health outcomes in pneumonia. This would be achieved by comparison of the blood culture isolate and antibiotic resistance profile, with those identified in the nasopharyngeal microbiome and resistome (NPR).

   Chest radiographs will also help to address objective 3 of the study. Current World Health Organisation and local Malawian diagnostic guidelines only recommend chest radiographs for paediatric patients with severe pneumonia, to identify potential pneumonia complications including pleural effusions, empyema, pneumothoraces and pneumatoceles. This data will help the study to identify potential confounding in the causal pathway between a persistent 'hospital-acquired NPR', by differentiating cases where hospitalisation is likely due to anatomical complications of pneumonia, from cases where hospitalisation may be attributed to adverse pneumonia outcomes due to antibiotic resistance.

2. We would also like to focus the recruitment of participants admitted to QECH with pneumonia (the first pneumonia admission cohort) to residents of Blantyre district; this is to make sure that we are able to achieve the 3-month follow-up study visit to the participant's home. We do not have the capacity to conduct 3-month follow up study visits outside of Blantyre district. The follow up of our participants at the 3-month study visit is crucial to achieving the primary study outcome: determining whether there is a persistent 'hospital-acquired' resistome.

3. We are currently only recruiting participants with pneumonia at QECH during weekday working hours. However, following study commencement, we have found that a significant number of patients are referred from district health facilities to this tertiary centre on a Friday, then admitted on a Saturday, and therefore are currently excluded from the study, as they are outside the first 24 hours of their hospitalisation. This will impact our ability to achieve our recruitment target within the study timeframe. Due to limited study resources, it is not possible to expand our recruitment activities to weekend hours. We therefore request an extension of this recruitment time-window to the first 72 hours of hospitalisation. This change will not impact on the validity of study. We will record the date and time of the admission, so that the variability can be accounted for in statistical analysis of the data.

Protocol V8.0 (Submitted on 22/05/2026, approved on 22/05/2026, implemented on 13/08/2026)

Since the study started recruiting hospitalised participants at Queen Elizabeth Central Hospital (QECH) on 24/02/2026, we have enrolled lower numbers of hospital readmission participants than anticipated. Unfortunately, if enrolment of hospital readmission participants continues at the current rate (approximately 1 participant per week), we will not be able to reach our recruitment target of 83 hospital readmission participants within the study timeline.

Consequently, we are requesting an amendment to extend the inclusion criteria for hospital readmission participants to include children being admitted to QECH for treatment of pneumonia, following hospitalisation for pneumonia, meningitis or any infection requiring treatment with an intravenous beta-lactam antibiotic (penicillin/cephalosporin) and/or an intravenous aminoglycoside (gentamicin/amikacin) within the past 3-months. Whilst this amendment would broaden the inclusion criteria from just readmissions following previous hospitalisation with pneumonia, specifying that these participants must have documentation in their health passport of a meningitis diagnosis or treatment with an intravenous beta-lactam antibiotic and/or aminoglycoside in the preceding admission, will ensure that all hospital readmission participants have been exposed to a similar systemic antibiotic selective pressure.

Our data thus far show that most hospitalised pneumonia patients receive intravenous penicillin X and gentamicin for antibiotic therapy, or a third-generation cephalosporin. World Health Organisation and local guidelines at QECH for meningitis advise treatment with at least 7 days of intravenous ceftriaxone or cefotaxime for children aged 12-36 months. Therefore, this amendment would permit analysis of the impact of beta lactam, aminoglycoside and third-generation cephalosporin antibiotics on nasopharyngeal resistome composition, and the association with hospital readmission.