

Streptococcus pneumoniae carriage and antimicrobial resistance among Kenyan children
discharged from hospital

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Abstract

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Streptococcus pneumoniae is a leading cause of morbidity and mortality among children under five in sub-Saharan Africa, particularly in the vulnerable period following hospital discharge. This dissertation investigates the dynamics of pneumococcal carriage and antimicrobial resistance (AMR) in this high-risk population through a series of analyses nested within the Toto Bora trial, a randomized, placebo-controlled study of azithromycin in 1,398 Kenyan children discharged from hospital and followed for six months. This collection of nested studies aims to inform strategies for preventing AMR and improving child health by evaluating the impact of azithromycin treatment, identifying risk factors for carriage and resistance, and assessing the clinical consequences of pneumococcal carriage.

The first study evaluates the effect of a 5-day course of azithromycin versus placebo administered at hospital discharge on *S. pneumoniae* carriage and AMR. No significant differences were observed in carriage prevalence or azithromycin resistance at 3- or 6-months post-discharge

between treatment arms. This may be due in part to high inpatient antibiotic use in this population, reducing any further impact of azithromycin. Notably, resistance to multiple antimicrobial classes was already prevalent at discharge, with nearly 35% of isolates classified as multidrug-resistant. This study underscores the importance of considering inpatient antibiotic use when evaluating the downstream effects of post-discharge antimicrobial interventions.

The second study explores clinical and environmental risk factors for pneumococcal carriage and AMR using longitudinal data from nasopharyngeal swabs collected at discharge and follow-up. Pneumococcal carriage at hospital discharge was inversely associated with inpatient antibiotic use and longer hospital stays whereas carriage at 3 and 6 months was linked to household-level exposures such as unimproved water sources, untreated drinking water, and shared sanitation facilities. Resistance to doxycycline was more common among children with multiple young siblings, while receipt of the recommended doses of pneumococcal conjugate vaccine (PCV) was associated with reduced resistance to doxycycline and clindamycin. These findings highlight the role of water, sanitation, and hygiene (WASH) interventions in shaping post-discharge colonization and support the role of PCVs in AMR mitigation strategies.

The third study investigates whether *S. pneumoniae* carriage at discharge is associated with increased risk of rehospitalization or death. Notably, carriage was not linked to adverse outcomes and was associated with a significantly lower risk of all-cause mortality, even after adjusting for key confounders. This inverse association may reflect protective mucosal immunity conferred by colonization with less virulent strains. Given the persistently high post-discharge mortality observed among children without *S. pneumoniae* carriage, this group may warrant particular attention in future interventions targeting vulnerable populations. Azithromycin-resistant pneumococcal carriage was associated with poorer clinical outcomes among children treated with azithromycin, underscoring the importance of appropriate antimicrobial therapy.

Together, this dissertation provides a comprehensive view of pneumococcal dynamics in the period following discharge from hospital. The findings suggest that short-course azithromycin does not meaningfully alter carriage or antimicrobial resistance patterns in settings with high inpatient antibiotic use, and that household environmental conditions play a critical role in shaping post-discharge colonization. Importantly, pneumococcal carriage at discharge was not associated with increased morbidity or mortality. These insights have implications for antimicrobial policy, vaccine strategy, and post-discharge care in low-resource settings, emphasizing the need for integrated approaches that address both clinical and environmental drivers of AMR and child health.

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Finally, I dedicate this dissertation to my daughter, whose laughter reminds me why this work matters. May this research help build a world where every child has the chance to thrive.

Chapter 1: Introduction

Streptococcus pneumoniae (*S. pneumoniae* or pneumococcus) is a leading cause of morbidity and mortality among children under five, particularly in low- and middle-income countries (LMICs). In sub-Saharan Africa alone, pneumococcal disease is responsible for an estimated 973,000 cases of severe disease and 166,000 deaths annually in this age group, with a mortality rate over 11 times higher than that of the Americas or Europe.¹ The most common clinical manifestations of pneumococcal disease in children are pneumonia, meningitis, bacteremia or sepsis, and otitis media.²

Transmission pathways

S. pneumoniae is an opportunistic pathogen that colonizes the mucosal surfaces of the human nasopharynx. Colonization, or carriage, is a required precursor for disease and is the source of spread from person to person.³ Carriers of *S. pneumoniae* shed the virus in nasal secretions and can spread the bacteria through coughing, sneezing, or other close contact.² Transmission primarily occurs via inhalation of airborne respiratory droplets or via person-to-person contact (e.g., hand-to-nose).^{4,5} *S. pneumoniae* can also be transmitted through fomites, or inanimate objects, when surfaces become contaminated by the secretions of carriers.^{4,6} Pneumococci have been shown to survive in the environment for a few hours to several days or months (140 days in soil)^{7,8} and are tolerant to long periods of desiccation (up to 4 weeks)⁶ owing in part to their protective polysaccharide capsule.⁹ Therefore, adequate hygiene is essential to prevent spread, especially in healthcare facilities and daycare centers where it has been isolated from surfaces such as soft toys and crib linen.¹⁰

The household environment also plays an important role in the transmission of *S. pneumoniae*. Household crowding, living with siblings under the age of five, poor hygiene, and inadequate ventilation increase the risk of transmission.^{2,4,11} Over-crowded conditions facilitate transmission through close contact and increased environmental contamination. The number of young children in a household is predictive of *S. pneumoniae* colonization as children in the household are the primary source of transmission to other household members, though both parents and siblings can transmit disease to pneumococcus-naïve infants.^{12,13}

Antimicrobial resistance

Development of antimicrobial resistance (AMR) in *S. pneumoniae* is of growing concern as reduced susceptibility can lead to treatment failure.^{14–17} Although *S. pneumoniae* AMR data from

sub-Saharan Africa are limited, existing data suggests the proportion of isolates resistant to beta-lactams (first-line therapy) is as high as 45%.^{15,18} *S. pneumoniae* can acquire resistance-conferring genes in the environment from other streptococci and related bacteria and develop resistance through successive genetic mutations due to selective pressure, such as from antibiotics.¹⁵ Selection of resistant clones occurs commonly among children because they often carry pneumococcus for longer durations, and are more frequently exposed to antibiotics, compared to adults.¹⁵ Risk factors for acquisition of antibiotic resistance in *S. pneumoniae* include hospitalization in the last 3 months, antibiotic use in the last 6 months, day-care attendance, and HIV infection.¹⁵

The hospital environment provides ample opportunity for bacteria of diverse genera to intermingle¹⁹ and be exposed to antibiotics. Hospitals contain a high-density of individuals at high risk of infection, such as patients with wounds or IV catheters, those with recent surgery, and those undergoing hemodialysis. Immunocompromised patients who disproportionately experience hospitalizations, often take prophylactic antibiotics for prolonged duration also increasing the likelihood of selection for resistance.²⁰ In the absence of adequate infection control, pathogens can be spread patient-to-patient via staff and equipment.

While environmental factors, such as inadequate sanitation and hygiene and contact with animals, have been associated with AMR among gram-negative bacteria of the gastrointestinal tract, fewer studies have examined these factors in respiratory pathogens, including *S. pneumoniae*.^{21,22} These factors, along with household crowding, can promote the spread of pathogens, thus increasing the demand for antibiotics and keeping selective pressure for resistance high.²⁰ A better understanding of the dynamics of pneumococcal carriage and AMR is therefore critical for informing strategies to reduce disease burden and improve child survival.

Pneumococcal conjugate vaccines

Efficacious pneumococcal conjugate vaccines (PCVs) have been rolled out in 40 of 48 countries in sub-Saharan Africa.²³ In 2011, Kenya introduced PCV10-GSK, a 10 valent vaccine by GlaxoSmithKline²³ using a three-dose schedule administered to infants at 6, 10, and 14 weeks.²⁴ Catch-up vaccination for children up to 4 years of age was conducted in selected sites. As of 2021, national coverage is estimated at 92%.²³ PCVs target only a subset of *S. pneumoniae* serotypes, with higher valency vaccines (e.g., PCV13, PCV 20) incorporating additional high priority serotypes based on virulence and disease burden. In 2022, the Kenya National Vaccines and Immunization Program replaced PCV10-GSK with Pneumosil™ (PCV10-SII, Serum Institute

of India), a 10-valent PCV featuring a modified serotype composition—incorporating serotypes 6A and 19A, while excluding 4 and 18C—designed to be a more cost-effective option optimized for the serotype distribution in high-burden regions.²⁵

The introduction of pneumococcal conjugate vaccines (PCVs) has significantly reduced the incidence of invasive pneumococcal disease (IPD) and vaccine-type carriage in many settings, including Kenya.²⁶ However, serotype replacement—where non-vaccine serotypes fill the ecological niche left by vaccine-targeted strains—has led to a resurgence in carriage and, in some cases, disease.¹⁸ Moreover, while PCVs have been associated with reductions in antimicrobial resistance (AMR) among vaccine-type strains,²⁷ the overall impact on resistance is complex and context-dependent. In high-burden settings, where antibiotic use is widespread and often unregulated, the selective pressure exerted by antibiotics can drive the emergence and persistence of resistant pneumococcal lineages, even in the presence of high vaccine coverage.²⁸

Post-discharge mortality

Children recently discharged from hospital represent a particularly vulnerable population. Post-discharge mortality rates in LMICs are alarmingly high—often exceeding in-hospital mortality—and are driven by a combination of factors including incomplete resolution of the initial illness, nosocomial infections, and disruptions to the microbiota from broad-spectrum antibiotic use.^{29,30} In this context, the role of pneumococcal carriage and AMR in shaping post-discharge outcomes is poorly understood.

While mass drug administration (MDA) of azithromycin has shown promise in reducing child mortality in some settings,³¹ concerns about promotion of AMR have limited its widespread adoption.²⁸ Azithromycin MDA has been associated with increased macrolide resistance in *S. pneumoniae* and other commensal organisms,³² and may also select for resistance to unrelated antibiotic classes through co-selection mechanisms.³³ As an alternate to MDA, more targeted use of azithromycin to individuals at highest risk of mortality – such as those recently discharged from hospital – may reduce mortality while minimizing population-wide development of resistance.

This dissertation addresses these critical knowledge gaps through three interrelated studies nested within the Toto Bora trial,³⁴ a randomized, placebo-controlled study of 1,398 Kenyan children discharged from hospital and followed for six months. **Chapter 2** evaluates the effect of a 5-day course of azithromycin on the prevalence of *S. pneumoniae* carriage and resistance to multiple antimicrobial classes. **Chapter 3** investigates the clinical and environmental risk factors associated with pneumococcal carriage and AMR over time, with a focus on modifiable

household-level exposures such as water, sanitation, and hygiene (WASH) conditions. **Chapter 4** explores the clinical implications of pneumococcal carriage and resistance by assessing their associations with risk of rehospitalization and death during the post-discharge period.

Together, these studies provide a comprehensive examination of pneumococcal epidemiology in a high-risk pediatric population. By integrating microbiological, environmental, and clinical data, this work offers new insights into the drivers and consequences of pneumococcal carriage and resistance in the months following hospital discharge. The findings have important implications for antimicrobial stewardship, vaccine policy, and the design of interventions to reduce post-discharge mortality and curb the spread of AMR in low-resource settings.

Chapter 2: The effect of a 5-day course of azithromycin on *Streptococcus pneumoniae* carriage and antimicrobial resistance among Kenyan children discharged from hospital

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Abstract

Background: Mass azithromycin distribution reduces child mortality in some settings, potentially through reductions in nasopharyngeal carriage of *Streptococcus pneumoniae*, but has been associated with increased antimicrobial resistance. Data are lacking on the impact of azithromycin on *S. pneumoniae* carriage and antimicrobial resistance over time.

Methods: We analyzed data from a double-blind, randomized placebo-controlled trial (ClinicalTrials.gov; NCT02414399) which followed 1,398 hospitalized Kenyan children to evaluate the impact of a 5-day course of oral azithromycin at discharge from hospital on carriage of *S. pneumoniae* and antimicrobial resistance at 3- and 6-months post-discharge. Randomization to azithromycin or placebo (1:1) was stratified by enrollment county (Kisii or Homa Bay). We calculated the prevalence of *S. pneumoniae* carriage and the proportion of isolates resistant to azithromycin among a random sample of 844 children. For each outcome, we calculated prevalence ratios (PRs) and 95% confidence intervals for the intervention using generalized estimating equations, adjusting for enrollment site.

Findings: Overall, 1,253/1398 (89.6%) enrolled children received antibiotics during their hospitalization. Baseline carriage of *S. pneumoniae* was similar among children randomized to the azithromycin group (158/702 [22.5%]) compared to the placebo group (171/696 [24.6%]; $p=0.4$) and did not differ at month 3 (65.6% vs 67.0%; adjusted PR 0.98 [95% CI 0.90, 1.06]) or month 6 (66.7% vs 66.5%; PR:1.00 0.92, 1.08]. At baseline, 15.7% of isolates were resistant to azithromycin and there was no difference between azithromycin-treated and placebo groups at month 3 (35/266 [13.2%] vs 32/256 [12.5%]; PR:1.06[0.86, 1.66]) or month 6 (41/245 [16.7%] vs 43/243 [17.6%]; PR: 1.01[0.69,1.49]).

Conclusions: We did not observe an effect of azithromycin treatment on the prevalence of *S. pneumoniae* carriage and antimicrobial resistance 3- or 6-months post-treatment. This may be due in part to high inpatient antibiotic use in this population, reducing any further impact of azithromycin.

Introduction

More than 2.8 million deaths occur each year among children under five years of age in sub-Saharan Africa,³⁵ primarily due to preventable and treatable infectious conditions, most commonly pneumonia.³⁶ Children who survive hospitalization continue to experience an elevated risk of death, with mortality rates averaging 3-5% in the 6 months following hospitalization.^{29,37} This elevated risk may arise from inadequate treatment of the acute presenting condition, nosocomial infections, untreated underlying conditions, and/or alterations to the bacterial flora of the gut and nasopharynx.

Mass drug administration (MDA) of azithromycin to all young children in a community is being considered as a mortality-preventing intervention in some high-mortality settings following promising results seen from trials conducted in Ethiopia and Niger.^{31,38,39} However, guidance from the World Health Organization for the mass use of azithromycin for mortality prevention is limited due to concerns about promotion of antimicrobial resistance (AMR).²⁸ Azithromycin MDA has been associated with an increase in azithromycin resistance in commensal bacteria, including *Streptococcus pneumoniae*, the leading bacterial cause of pneumonia.^{33,40} Limited evidence suggests repeated MDA rounds may increase resistance to non-macrolide antibiotics, such as beta-lactams, which are first-line therapy for pneumococcal pneumonia.³² Existing data on the effect of MDA programs on *S. pneumoniae* are limited to cross-sectional population-level surveys. There is a lack of individual-level longitudinal data on the trajectory of resistance in *S. pneumoniae* among children treated with azithromycin.

As an alternative to MDA, targeted use of azithromycin among children at highest risk of mortality, such as those recently discharged from the hospital, may reduce mortality while minimizing population-wide development of resistance. We previously reported results from a randomized, placebo-controlled trial³⁴ evaluating the effect of a 5-day course of azithromycin on risk of rehospitalization and death among Kenyan children recently discharged from the hospital. The intervention was not found to reduce re-hospitalization and mortality in this population.⁴¹ Here, we investigate whether nasopharyngeal carriage of *S. pneumoniae* and resistance to azithromycin and other relevant antimicrobial classes varied by azithromycin treatment arm at 3- and 6-months post-discharge. We hypothesized that five days of azithromycin may lower the prevalence of *S. pneumoniae* carriage in the short term and select for azithromycin-resistant strains of *S. pneumoniae*, resulting in a higher proportion of carried isolates that are resistant. From a public health perspective, we sought to determine whether any difference in resistant carriage was

sustained for 3 to 6 months. A better understanding of AMR in azithromycin-treated children could inform the rollout of MDA programs.

Methods

Study design and population

We conducted a longitudinal analysis using data collected from children enrolled in a double-blind, placebo-controlled, randomized clinical trial called Toto Bora (registered at ClinicalTrials.gov; NCT02414399).³⁴ The trial received ethical approval from the Institutional Review Boards of the Kenya Medical Research Institute (KEMRI SERU #3086), the Kenyan Pharmacy and Poisons Board (Ref. No. PPB/ECCT/15/10/04), and the University of Washington (IRB #49120). Written informed consent was obtained from all participating caregivers in their preferred language (English, Kiswahili, Kisii, or Luo). For individuals who were not literate, the consent form was read aloud in their preferred language, and consent was documented using a thumbprint in the presence of a witness.

The trial enrolled children 1-59 months of age, admitted to the hospital for non-traumatic conditions from June 2016–November 2019 and subsequently discharged, with weight >2 kilograms and plans to remain in the study area for at least 6 months. Details of the trial protocol and statistical analysis plan have been published.^{34,41} Recruitment was completed in the inpatient wards of Kisii Regional and Homa Bay District Hospitals in [formerly] Nyanza Province in western Kenya, a low-resource setting where the under-five mortality rate was estimated to be 55.7 per 1,000 live births in 2022.⁴² Randomization to the azithromycin or placebo arm (1:1) in blocks of 10 was stratified by enrollment site (Kisii or Homa Bay).

AMR sub-study: A random sample of 844 children from the parent trial was selected for antimicrobial susceptibility testing (AST). To maintain randomization, the sample was drawn irrespective of whether *S. pneumoniae* was isolated at a given time point. The final sample size of the AMR sub-study was selected to achieve a target of at least 1,200 *S. pneumoniae* isolates for AST testing across three time points.

Data collection

Caregivers of enrolled children were interviewed to collect medical history and sociodemographic information, including household characteristics and environmental exposures. Hospital records were abstracted to ascertain clinical history, diagnoses, antibiotic use, and laboratory data. At 3- and 6-month follow-up visits, caregivers were interviewed about any illnesses, re-hospitalizations,

or treatments given to the child, and about study drug adherence at the 3-month visit. Child anthropometric measurements were collected at enrollment and 3- and 6-month follow-up visits. Vaccination history was determined by immunization record when available and supplemented by caregiver self-report. If a participant failed to return for a scheduled follow-up visit, study staff attempted to make contact via cellphone with primary caregivers, and then followed up via a home visit. Participants were considered lost to follow-up if they did not attend either follow-up visit despite one month of active tracing.

Nasopharyngeal swabs (Copan Diagnostics, USA), the gold standard method for measuring pneumococcal carriage in children,⁴³ were collected from all children at enrollment (post-discharge, before administration of study medication) and from all surviving children at 3- and 6-month follow-up visits. Swabs were immediately placed in skim milk, tryptone, glucose, and glycerin (STGG) media, vortexed to make a back-up sample, and frozen within 1 hour of collection, in onsite -80°C freezers with generator and solar-power back-up, and then shipped, on dry ice, monthly to the Kenya Medical Research Institute (KEMRI) in Nairobi for long-term -80°C storage.

Nasopharyngeal culture was done on all available swab samples (baseline, 3-months, and 6-months) on blood agar with gentamicin, and all suspected *S. pneumoniae* colonies were confirmed by optochin test at the KEMRI-Wellcome Trust Research Programme laboratory in Kilifi, Kenya following established protocols.⁴³ For children randomly selected for the AMR sub-study, one *S. pneumoniae* isolate per child from all available timepoints underwent AST with the disc diffusion method for azithromycin, oxacillin (for penicillin susceptibility), doxycycline, levofloxacin, co-trimoxazole, chloramphenicol, clindamycin, and linezolid and by E-TEST for ceftriaxone. Isolates were classified as susceptible, intermediate, or resistant according to 2024 Clinical and Laboratory Standards Institute (CLSI) zone diameter breakpoints or minimum inhibitory concentration (MIC) breakpoints for ceftriaxone.⁴⁴ Intermediate and resistant isolates were considered non-susceptible.

Intervention

After hospital discharge and baseline sample collection, enrolled children received the first daily dose of either oral suspension azithromycin (10mg/kg) or a similar appearing placebo under observation by study staff. The subsequent four doses (5mg/kg azithromycin or placebo) were administered daily by caregivers at home.

Outcomes

The primary outcomes were a) the prevalence of *S. pneumoniae* carriage, defined as the proportion of children with microbiological isolation of *S. pneumoniae* from a nasopharyngeal swab, and b) the proportion of carried pneumococci that were resistant to azithromycin at baseline (discharge) and 3- and 6-month follow-up visits. Secondary outcomes included the proportion of carried pneumococci that were resistant to other classes of antimicrobials and that were multidrug-resistant (MDR) defined as non-susceptible to three or more antimicrobial classes tested.

Statistical analysis

Given the sample size from the parent trial (N=1400) and assuming alpha 0.05 and 67% carriage in placebo-treated children, we were powered to detect an 11% reduction in carriage (prevalence ratio of 0.89) associated with the azithromycin treatment. Among the AMR sub-study (N=844), we expected approximately 280 *S. pneumoniae* isolates in each arm at each follow-up visit. Assuming an alpha level of 0.05 and 15% of isolates resistant to azithromycin in the placebo group, we had 80% power to detect a 57% increase in the proportion of isolates that were resistant, which is comparable to prevalence ratios ranging from 1.6 to 2.3 in MDA trials for mortality prevention.⁴⁵

For each outcome, we compared the prevalence of *S. pneumoniae* carriage or the proportion of isolates that were antimicrobial resistant in the intervention vs. control groups. First, we conducted an unadjusted analysis comparing the prevalence in the two groups using a z-test. Next, we performed an adjusted individual-level analysis using generalized estimating equations (GEE) with a Poisson link function, an exchangeable correlation structure to account for correlation within individuals over time, and stratification by enrollment site (via adjustment). Our primary analysis was intention-to-treat (ITT), adjusting only for enrollment site. In per-protocol analyses, we included only participants who self-reported full adherence to the 5-day course of azithromycin. We calculated prevalence ratios and 95% confidence intervals (CI) for *S. pneumoniae* carriage associated with the intervention. To assess changes over time, we included an interaction term between follow-up visit and the intervention. In sensitivity analyses, we adjusted for factors known to be strongly predictive of *S. pneumoniae* carriage, including age, nutritional status, exclusive breastfeeding for the first six months of life, HIV status, living in a crowded household (>3 persons/room), and *S. pneumoniae* status at baseline.

We assessed effect modification on the multiplicative scale by baseline *S. pneumoniae* status (carriage/non-carriage) and by antibiotic use during the initial hospitalization (any/none). We conducted a pre-specified subgroup analysis stratified by enrollment site. We conducted two-tailed tests with alpha 0.05. All analyses were completed with R version 4.0.3.

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The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From June 2016–November 2019, 1,400 children were enrolled in the Toto Bora trial; two were deemed ineligible after randomization. Baseline characteristics of the 1,398 children included in the ITT analysis are summarized in table 1. The median age was 18 months (IQR 9-32), median duration of hospitalization was 3 days (IQR 2-5), and 1,253/1398 (89.6%) children received antibiotics during their hospitalization, most commonly penicillin (60%) and gentamicin (54%). Over 93% of children were up to date on pneumococcal conjugate vaccine (PCV) vaccine doses for their age: 561/604 (92.9%) among those with vaccination cards and 735/788 (93.3%) among those without (based on caregiver-report). Overall, 1324 (95.1%) received at least one dose, 1304 (93.7%) two doses, and 1246 (89.5%) three doses. Participant characteristics were generally balanced between the azithromycin and placebo arms, except for slightly more children in the azithromycin group being partly (rather than exclusively) breastfed and slightly more children in the azithromycin group living in crowded households.

The first dose of azithromycin or placebo was given under observation for all children and 658/691 (95.2%) in the azithromycin group and 653/682 (95.7%) in the placebo group received all five doses of the study drug as reported by caregivers. Non-study use of azithromycin during follow-up was reported for 3/1398 (0.2%) participants; two in the placebo arm and one in the azithromycin treatment arm. During the 6-month follow-up, 132 (9.4%) children experienced the combined outcome of death or rehospitalization; 34 (2.4%) children died and 115 (8.2%) were re-hospitalized at least once.

S. pneumoniae carriage

Nasopharyngeal swabs were available for testing for 1395/1398 (99.8%) children at baseline, 1255 (89.8%) at month 3, and 1201 (85.9%) at month 6. Baseline carriage of *S. pneumoniae* was

similar among children randomized to the azithromycin group (158/701 [22.5%]) compared to the placebo group (171/694 [24.6%]) (p=0.4).

In ITT analysis, adjusting for enrollment site, azithromycin treatment compared to placebo was not associated with carriage of *S. pneumoniae* at 3 months (420/640 [65.6%] vs 412/615 [67.0%]; PR 0.98 [95% CI 0.90, 1.06]) or 6 months (405/607 [66.7%] vs 395/594 [66.5%]; PR 1.00 [0.92, 1.08]) (figure 1). It was not uncommon for a child to clear or acquire *S. pneumoniae* carriage between follow-up visits, as shown in the figure of individual trajectories (appendix, figure S1). There was no evidence for a change in the effect of the intervention over time (p=0.51). While baseline *S. pneumoniae*, age, breastfeeding in the first 6 months of life, and household crowding were predictors of later colonization, including them in the model did not change the point estimate or standard error for the effect of the intervention (appendix table S1).

The baseline prevalence of carriage was higher among children who did not receive any inpatient antibiotics (41/74 [55.4%] azithromycin-treated children and 33/70 [47.1%] placebo-treated children). However, neither baseline carriage (interaction p=0.52) nor inpatient antibiotic use (p=0.16) modified the treatment effect (appendix, figure S2). We did not observe a difference between enrollment sites in baseline carriage of *S. pneumoniae* or in the effect of the intervention on carriage (appendix, figure S3).

Antimicrobial resistance

The 844 children (429 azithromycin-treated and 415 placebo-treated) randomly selected for the AMR sub-study were representative of the overall trial participants in terms of baseline characteristics and *S. pneumoniae* prevalence (appendix, table S2). *S. pneumoniae* was isolated and AST testing completed for 1220 nasopharyngeal swabs collected from these children across the three timepoints (210 isolates at baseline, 522 at month 3, and 488 at month 6). At hospital discharge (prior to randomization), resistance was observed to co-trimoxazole (205/210 [97.6%]), oxacillin (198/210 [94.3%]), doxycycline (68/210 [32.4%]), azithromycin (33/210 [15.7%]), clindamycin (22/210 [10.5%]), chloramphenicol (1/210 [0.5%]) and linezolid (1/210 [0.5%]). 34.8% (73/210) of baseline isolates were resistant to 3 or more antimicrobial classes.

Comparing azithromycin and placebo arms, the percent of isolates resistant to azithromycin did not differ at month 3 (35/266 [13.2%] vs 32/256 [12.5%]; PR 1.06 [0.86, 1.66]) or month 6 (41/245 [16.7%] vs 43/243 [17.6%]; PR 1.01 [0.69, 1.49]) (figure 2). Results were similar when adjusting for baseline *S. pneumoniae*, breastfeeding, and household crowding. In per-protocol analyses,

results did not meaningfully differ when children who did not complete the full 5-day course were excluded (appendix, table S3).

Baseline carriage did not modify the effect of the intervention on azithromycin-resistance among *S. pneumoniae* isolates ($p=0.38$). There was some evidence that the effect of the intervention on azithromycin resistance varied by antibiotic use during the enrollment hospitalization ($p=0.053$; appendix, figure S4). However, among the subset of children that did not receive any inpatient antibiotics, we observed imbalance in the proportion of isolates that were resistant to azithromycin in the two treatment arms at baseline (prior to randomization), likely due to the small sample size (appendix, table S4). Therefore, we performed a post-hoc analysis among this subset of children, adjusting for baseline resistance to azithromycin, and found the intervention to be associated with a higher proportion of azithromycin resistance at 6 months (PR 5.4 [1.53, 19.1]) and not significantly so at 3 months (PR 1.33 [0.31, 5.66]) (appendix, table S4). While the proportion of azithromycin-resistant pneumococci was higher in Homa Bay compared to Kisii at baseline (PR 1.63 [1.20, 2.20]; $p=0.002$), we did not observe an effect of county on the relationship between the intervention and azithromycin resistance ($p=0.85$; appendix, figure S5).

We did not observe a difference between treatment arms in the proportion of carried pneumococci resistant to penicillin (via oxacillin), doxycycline, or clindamycin, or in the proportion of isolates that were MDR (figure 3). We did not have sufficient resistant isolates to examine treatment effects for resistance to clindamycin, chloramphenicol, or linezolid.

Discussion

In this randomized, double-blind clinical trial, we did not find evidence of an effect of a 5-day course of azithromycin prescribed at hospital discharge on the carriage prevalence of *S. pneumoniae* or proportion of carried *S. pneumoniae* that were antimicrobial resistant at 3- or 6-months post-discharge. Both treatment arms had similar carriage prevalence at baseline, therefore, adjusting for baseline carriage of *S. pneumoniae* did not affect the prevalence ratios at outcome nor did baseline carriage prevalence modify the effect of azithromycin on carriage prevalence. The lack of an effect on *S. pneumoniae* carriage is consistent with findings from MDA trials of azithromycin for mortality prevention, which found no difference in the prevalence of carriage among children in azithromycin communities compared to control.^{40,45,46} However, our AMR findings differ from the MORDOR (Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance) I trial, which found children living in azithromycin MDA communities in Niger and Malawi had 9.4% and 11.1% higher proportion, respectively, of azithromycin resistance

among carried *S. pneumoniae* isolates 6 months after the fourth treatment round.^{40,45,47} Of note, this trial was conducted in community settings, which differ from the population of hospitalized children included here.

The lack of an observed effect of the azithromycin intervention on resistance in *S. pneumoniae* to azithromycin was unexpected but is encouraging. In contrast to the single dose of azithromycin used in most MDA programs, the five-day course of azithromycin may have effectively killed all present *S. pneumoniae* rather than selecting for resistant clones. Our sub-study was powered to detect an 8% absolute difference in the proportion of carried isolates that were resistant, so we cannot rule out the possibility of a smaller intervention effect, though the observed 1% differences are unlikely to be meaningful. There are other possible explanations for these findings. First, a transient increase in azithromycin resistance may have occurred prior to the collection of nasopharyngeal swabs at the 3-month follow-up visit. This interval was selected from a public health perspective but may have been too long to assess an impact of azithromycin treatment due to high turnover of carried *S. pneumoniae* strains. While azithromycin has an extended half-life (2-4 days),⁴⁸ selective pressure would begin to wane several days after the last dose. Given an estimated mean carriage duration of 31 days and an acquisition rate of 6% per day,⁴⁹ most children would have reacquired *S. pneumoniae* strains during follow-up that were representative of resistance levels in their community. Lastly, the high rate of inpatient antibiotic use and exposure to resistant bacteria during hospitalization may have minimized the remaining potential effect of the intervention in this population.¹⁵ Indeed, when we examined the effect among children without inpatient antibiotic exposure (who had a higher prevalence of *S. pneumoniae* at baseline), there was a moderate signal for increasing azithromycin resistance in the azithromycin-treated group compared to placebo.

Overall, the prevalence of carriage of *S. pneumoniae* nearly tripled from hospital discharge to 6-month follow-up. The relatively low prevalence of carriage post-discharge is consistent with prior research showing reductions following antibiotic use,⁵⁰ as 90% of participants had at least one antibiotic during their hospitalization. The prevalence of carriage in this study 6-months post-discharge is similar to that observed in cross-sectional surveys of Kenyan children under 5 years: 59% in rural Asembo, 76% in Kilifi County, and 83% in urban Kibera.^{18,26} The lack of an effect of the azithromycin intervention on *S. pneumoniae* carriage is consistent with previous studies which found no reduction in carriage or a transient reduction for one month.⁵¹

At hospital discharge, nearly all isolates tested were resistant to at least one antimicrobial, and nearly 35% were resistant to three or more antimicrobial classes. We observed high levels of non-

susceptibility to oxacillin, which is likely a reflection of the level of inpatient penicillin exposure in this population. Recent hospitalization is a risk factor for Beta-lactam and macrolide resistance in *S. pneumoniae*.¹⁵ The relatively high proportion of resistance at discharge to antimicrobials which are not commonly used in the study population (e.g., doxycycline and azithromycin) may be attributed to co-selection pressure mediated by other antibiotics such as cephalosporins.⁵² The proportion of isolates resistant to azithromycin was similar to that seen in cross-sectional surveys in Asembo (12%) and lower than that observed in Kibera (24%).¹⁸ Of note, azithromycin resistance was lower in *S. pneumoniae* compared to commensal *E. coli* isolated from our study participants at baseline (37.7% resistant; data previously published).⁴¹ This may be related to the high bacterial diversity, selective pressure within the gut, mobile genetic element exchange between Gram negative species, and longer exposure time to oral antimicrobials.

Strengths of this study include longitudinal follow-up, the large sample of nasopharyngeal swabs tested, AST of nine antimicrobial classes, high adherence to the intervention (including 100% adherence to the first dose), high participant retention (>99%), and a well-characterized study population. This work is also subject to several limitations. We were underpowered for the observed prevalence ratios; however, they were notably small, suggesting that if a true difference exists, it is unlikely to be impactful. Given the high rate of *S. pneumoniae* acquisition in this population, a shorter interval (e.g., 2-4 weeks) to the first swab collection may have increased the likelihood of observing an intervention effect. Adherence to the trial medication was assessed by caregiver report, which may have overestimated the true adherence. There was potential for non-study azithromycin use, however, we expect this was rare based on data collected at follow-up visits and data from the MAL-ED cohort from Tanzania and South Africa, suggesting macrolide use in the region is rare.⁵³ Serotyping data for *S. pneumoniae* were not available, which limits our ability to distinguish between new and continued colonization. While less than 3% of participants died during follow-up, survival bias may be a concern if *S. pneumoniae* carriage and AMR profiles differed for children who survived until follow-up visits. Finally, because our study population is limited to recently hospitalized children, these results are not generalizable to the community.

In summary, among Kenyan children recently discharged from the hospital, a 5-day course of azithromycin did not affect carriage of *S. pneumoniae* or resistance to azithromycin and other antimicrobials as measured 3- or 6-months post-discharge. While a 5-day course of azithromycin might have a short-term impact on the resistance profile of carried *S. pneumoniae*, this study suggests it is unlikely to last up to three months and is therefore unlikely to be impactful at the public health level.

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We would like to thank the children and their caregivers who participated in the Toto Bora study and the clinical and laboratory staff at each study site. We are thankful to Paul Ndungu, Gerald Okeyo and Hannah Atlas for their support in study coordination, and Mame Mareme Diakhate for their data management expertise. The azithromycin and placebo were donated by Pfizer.

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Tables and Figures

Table 1. Baseline characteristics of children enrolled in Toto Bora by randomization arm (n=1398)

Characteristic	Overall, N = 1,398 ¹	Azithromycin, N = 702 ¹	Placebo, N = 696 ¹
County of recruiting hospital			
Homa Bay	576 (41%)	290 (41%)	286 (41%)
Kisii	822 (59%)	412 (59%)	410 (59%)
Age (months)	18 (9, 32)	18 (9, 31)	18 (9, 33)
Age category (months)			
0-5	188 (13%)	94 (13%)	94 (14%)
6-11	277 (20%)	141 (20%)	136 (20%)
12-23	406 (29%)	202 (29%)	204 (29%)
24-59	527 (38%)	265 (38%)	262 (38%)
Sex			
Male	828 (59%)	426 (61%)	402 (58%)
Female	570 (41%)	276 (39%)	294 (42%)
Caregiver reported income			
≥5,000 KSh	454 (32%)	229 (33%)	225 (32%)
<5000 KSh	874 (63%)	433 (62%)	441 (63%)
Refused/unknown	70 (5.0%)	40 (5.7%)	30 (4.3%)
Highest level of education completed by primary caregiver			
Secondary school or greater	752 (54%)	368 (52%)	384 (55%)
Primary school or less	644 (46%)	333 (48%)	311 (45%)
Household crowding			
<3 persons per room	1,127 (81%)	559 (80%)	568 (82%)
≥3 persons per room	271 (19%)	143 (20%)	128 (18%)
Water source			
Improved	1,154 (83%)	577 (82%)	577 (83%)
Unimproved	243 (17%)	125 (18%)	118 (17%)
Type of toilet used by the household			
Flush	135 (9.7%)	74 (11%)	61 (8.8%)
Pit latrine	1,209 (87%)	599 (85%)	610 (88%)
Other (e.g., bush)	53 (3.8%)	28 (4.0%)	25 (3.6%)
Breastfeeding for first 6 months of life (or until enrollment if child <6 m)			
Never breastfed	24 (1.7%)	14 (2.0%)	10 (1.4%)
Exclusively breastfed	663 (47%)	322 (46%)	341 (49%)
Partially breastfed	632 (45%)	334 (48%)	298 (43%)
Unknown	79 (5.7%)	32 (4.6%)	47 (6.8%)
Acute malnutrition			
None	1,266 (91%)	638 (91%)	628 (90%)
Moderate	75 (5.4%)	36 (5.1%)	39 (5.6%)
Severe	57 (4.1%)	28 (4.0%)	29 (4.2%)
Child HIV status			
HIV unexposed	1,192 (85%)	597 (85%)	595 (85%)
HIV exposed, uninfected	140 (10%)	70 (10%)	70 (10%)

Characteristic	Overall, N = 1,398 ¹	Azithromycin, N = 702 ¹	Placebo, N = 696 ¹
HIV infected	18 (1.3%)	9 (1.3%)	9 (1.3%)
HIV uninfected, exposure status unknown	41 (2.9%)	23 (3.3%)	18 (2.6%)
HIV exposed, infection status unknown	7 (0.5%)	3 (0.4%)	4 (0.6%)
Has recommended number of PCV doses for this age (with grace of 4 weeks)	1,296 (93%)	651 (93%)	645 (93%)
Any antibiotic used during admission	1,253 (90%)	628 (89%)	625 (90%)
Penicillin	839 (60%)	417 (59%)	422 (61%)
Gentamicin	757 (54%)	375 (53%)	382 (55%)
Ceftriaxone	538 (39%)	270 (38%)	268 (39%)
Macrolides	52 (3.7%)	28 (4.0%)	26 (3.7%)
Any antibiotic prescribed at discharge	867 (62%)	447 (64%)	420 (60%)
<i>S. pneumoniae</i> detected at baseline	329 (24%)	158 (23%)	171 (25%)

KSh, Kenyan Shilling; PCV, pneumococcal conjugate vaccine

¹n (%), median (IQR)

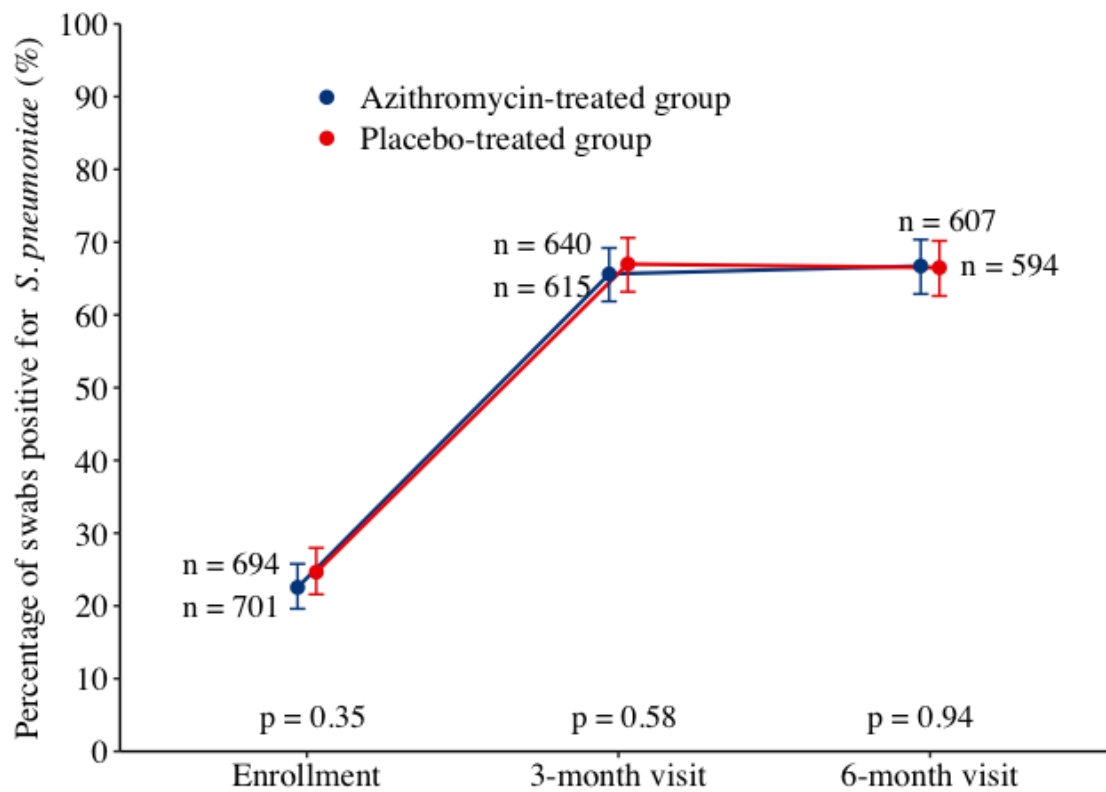


Figure 1. Percentage of swabs positive for *S. pneumoniae* by treatment arm. Error bars are 95% confidence intervals. The p-values are from a generalized estimating equation model with time by intervention interaction, adjusting for enrollment site. There was no evidence that the difference between treatment arms changes over time ($p=0.51$). The prevalence of carriage significantly increased from enrollment to month 3 ($p<0.001$).

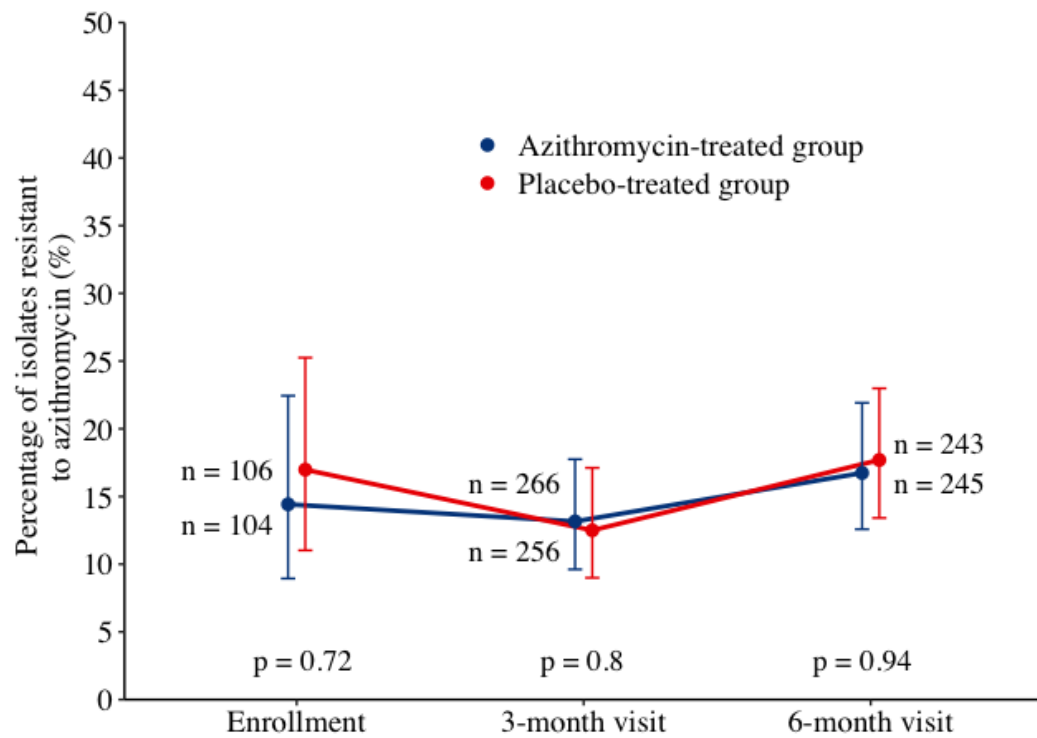


Figure 2. Percentage of *S. pneumoniae* isolates resistance to azithromycin by treatment arm. Error bars are 95% confidence intervals. The p-values are from a generalized estimating equation model with time by intervention interaction, adjusting for enrollment site.

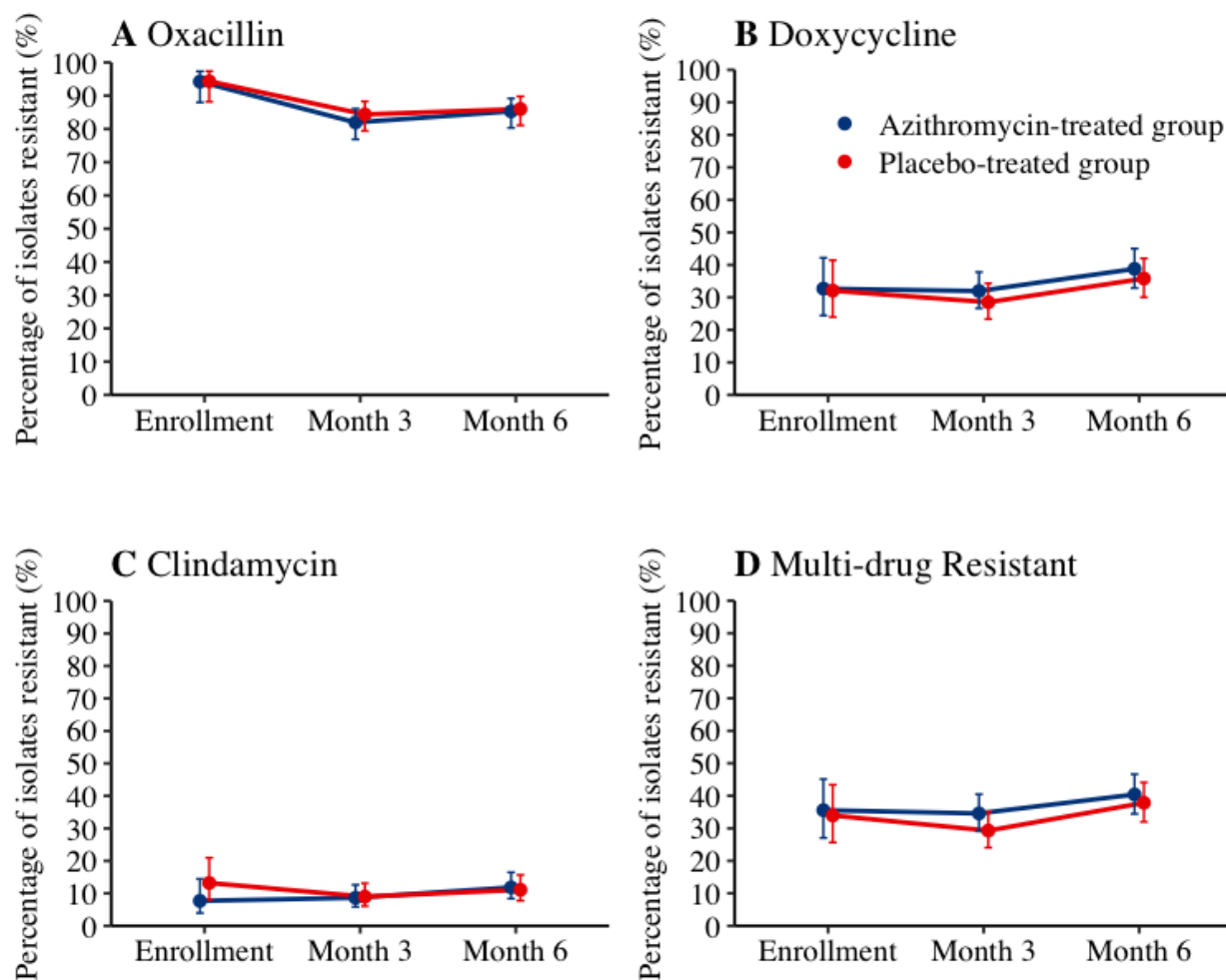


Figure 3. Percentage of isolates resistant to other antimicrobials by treatment arm
Error bars are 95% confidence intervals.

Supplementary Appendix

The effect of a 5-day course of azithromycin on *Streptococcus pneumoniae* carriage and antimicrobial resistance among Kenyan children discharged from the hospital

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Figure S1. Trajectory of *S. pneumoniae* carriage and azithromycin resistance over the 6 months post-discharge by treatment arm (n=844 children)

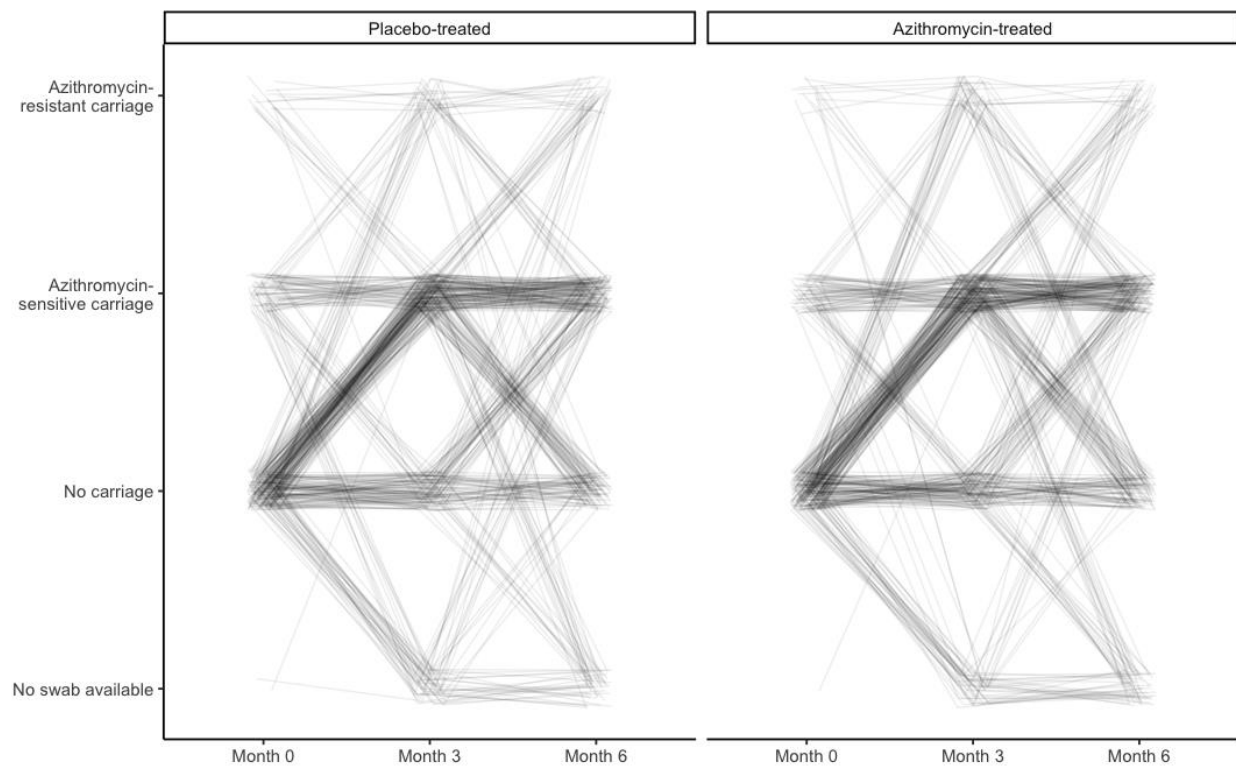


Table S1. Effect of azithromycin intervention on *S. pneumoniae* carriage at 3- and 6-month follow-up using generalized estimating equations, adjusting for county of enrollment and predictors of *S. pneumoniae* carriage

Characteristic	Model 1			Model 2		
	PR	95% CI	p-value	PR	95% CI	p-value
Azithromycin (vs. placebo)	1.00	0.94, 1.06	0.88	0.99	0.93, 1.05	0.632
County of enrollment						
Homa Bay	REF	—	—	REF	—	—
Kisii	0.97	0.91, 1.03	0.34	0.96	0.89, 1.02	0.177
<i>S. pneumoniae</i> at baseline	1.30	1.22, 1.38	<0.001	1.26	1.19, 1.34	<0.001
Age at enrollment (months)						
0-5				1.19	1.08, 1.31	<0.001
6-11				1.19	1.09, 1.30	<0.001
12-23				1.13	1.05, 1.23	0.002
24-59				REF		
Breastfeeding in the first 6 months of life						
Exclusively breastfed				REF	—	—
Never breastfed				1.01	0.82, 1.24	0.923
Partially breastfed				1.09	1.02, 1.17	0.010
Unknown				0.84	0.71, 1.00	0.053
Household crowding (≥ 3 persons per room) at enrollment				1.08	1.01, 1.16	0.035

PR = Prevalence Ratio, CI = Confidence Interval

Model 1 is adjusted for county of enrollment and *S. pneumoniae* at baseline

Model 2 is adjusted for county of enrollment and *S. pneumoniae* at baseline, age category, breastfeeding status, and household crowding.

Figure S2. The effect of azithromycin on the percentage of swabs positive for *S. pneumoniae*, stratified by whether the child received any antibiotics during their hospitalization

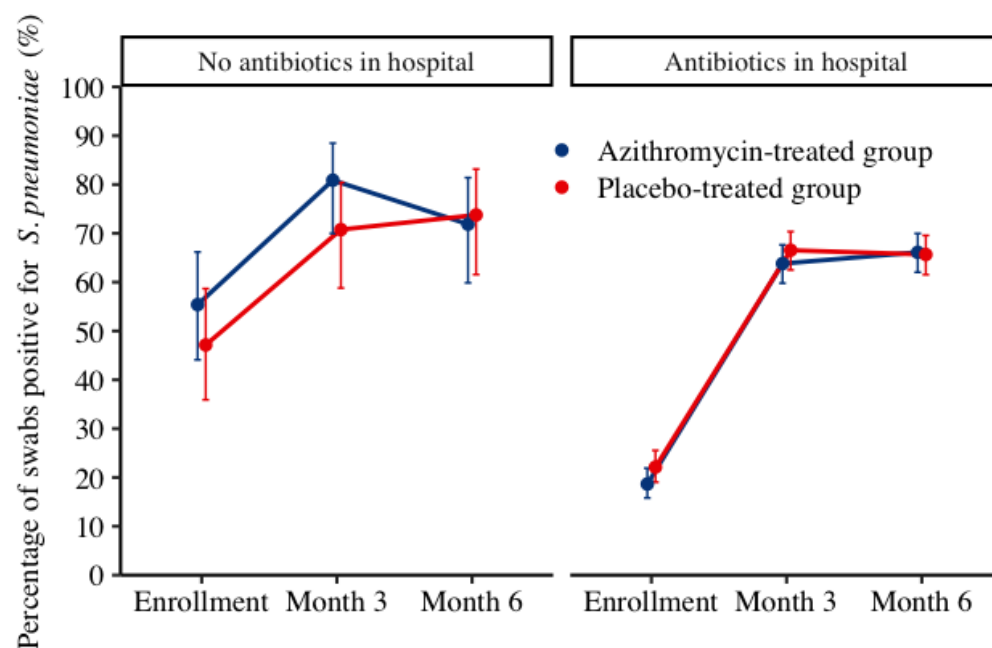


Figure S3. The effect of azithromycin on percentage of swabs positive for *S. pneumoniae*, stratified by enrollment county

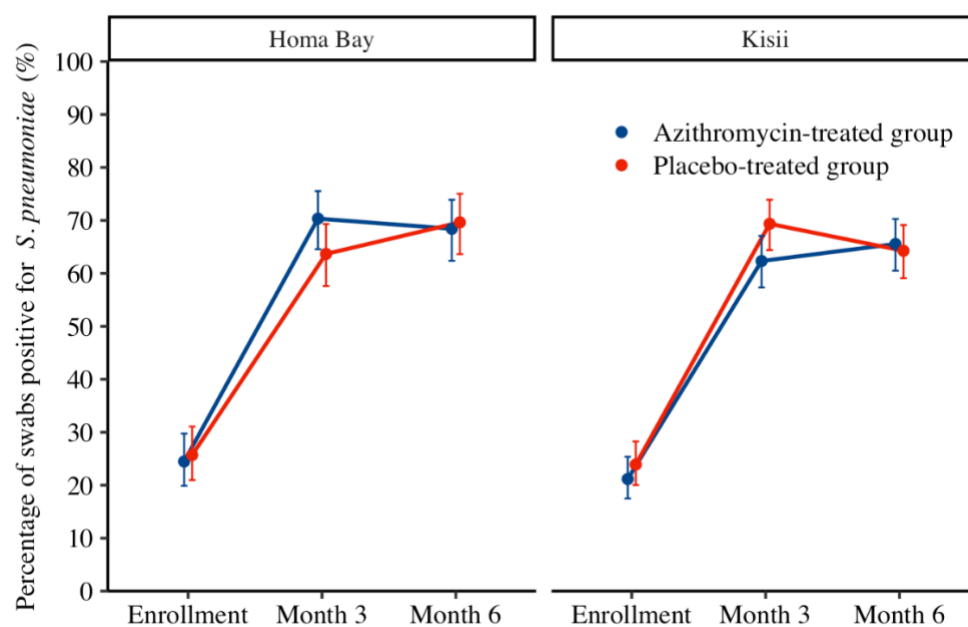


Table S2. Characteristics of children randomly selected for antimicrobial susceptibility testing (AST)

Characteristic	Overall, N = 1,398 ¹	AST Sample, N = 844 ¹	Not Sampled, N = 554 ¹
Randomization arm			
Placebo	696 (50%)	415 (49%)	281 (51%)
AZM	702 (50%)	429 (51%)	273 (49%)
County of recruiting hospital			
Homa Bay	576 (41%)	337 (40%)	239 (43%)
Kisii	822 (59%)	507 (60%)	315 (57%)
Age at enrollment (months)	18 (9, 32)	18 (9, 32)	18 (9, 32)
Child age category (months)			
0-5 mo	188 (13%)	109 (13%)	79 (14%)
6-11 mo	277 (20%)	168 (20%)	109 (20%)
12-23 mo	406 (29%)	250 (30%)	156 (28%)
24-59 mo	527 (38%)	317 (38%)	210 (38%)
Sex			
Male	828 (59%)	495 (59%)	333 (60%)
Female	570 (41%)	349 (41%)	221 (40%)
Caregiver reported income			
Income $\geq$ 5000 ksh	454 (32%)	267 (32%)	187 (34%)
Income < 5000 ksh	874 (63%)	535 (63%)	339 (61%)
Unknown or refuse to answer	70 (5.0%)	42 (5.0%)	28 (5.1%)
Highest level of education completed by primary caregiver			
Secondary or greater	752 (54%)	450 (53%)	302 (55%)
Primary school or less	644 (46%)	394 (47%)	250 (45%)
Household crowding			
No crowding (< 3 persons per room)	1,127 (81%)	684 (81%)	443 (80%)
Crowding ($\geq$ 3 persons per room)	271 (19%)	160 (19%)	111 (20%)
Water source			
Improved	1,153 (83%)	713 (85%)	440 (79%)
Unimproved	244 (17%)	130 (15%)	114 (21%)
Type of toilet used by the household			
Flush toilet	135 (9.7%)	88 (10%)	47 (8.5%)
Pit latrine	1,209 (87%)	728 (86%)	481 (87%)

Characteristic	Overall, N = 1,398 ¹	AST Sample, N = 844 ¹	Not Sampled, N = 554 ¹
Other(bush/cat)	53 (3.8%)	28 (3.3%)	25 (4.5%)
Exclusive breastfeeding for first 6 months of life (or until enrollment if child <6 m)			
Never breastfed	24 (1.7%)	16 (1.9%)	8 (1.4%)
Exclusively breastfed	663 (47%)	397 (47%)	266 (48%)
Partially breastfed	632 (45%)	381 (45%)	251 (45%)
Unknown	79 (5.7%)	50 (5.9%)	29 (5.2%)
Acute malnutrition based on WLZ, MUAC (in 6+ month olds), and/or presence of edema			
None	1,266 (91%)	757 (90%)	509 (92%)
MAM	75 (5.4%)	44 (5.2%)	31 (5.6%)
SAM	57 (4.1%)	43 (5.1%)	14 (2.5%)
Child HIV status			
HIV unexposed	1,192 (85%)	722 (86%)	470 (85%)
HIV exposed, uninfected/infection status unknown	147 (11%)	85 (10%)	62 (11%)
HIV infected	18 (1.3%)	13 (1.5%)	5 (0.9%)
Uninfected/exposure status unknown	41 (2.9%)	24 (2.8%)	17 (3.1%)
Has recommended number of vaccines for this age (with grace of 4 weeks)			
No	96 (6.9%)	58 (6.9%)	38 (6.9%)
Yes	1,296 (93%)	783 (93%)	513 (93%)
Any antibiotic used during enrollment admission	1,253 (90%)	757 (90%)	496 (90%)
Any antibiotic prescribed at discharge	867 (62%)	530 (63%)	337 (61%)
Antibiotic use reported during the follow-up	312 (22%)	194 (23%)	118 (21%)
Child died during follow-up	34 (2.4%)	20 (2.4%)	14 (2.5%)
Child was rehospitalized at least once during follow-up	115 (8.2%)	71 (8.4%)	44 (7.9%)

¹n (%); Median (IQR)

Table S3. Per-protocol analysis results: Effect of azithromycin on the proportion of *S. pneumoniae* isolates resistant to azithromycin at 3- and 6-months post-discharge among those who reported taking all five doses

	Timepoint	Azithromycin-treated		Placebo-treated		Adjusted Prevalence ratio (95% CI)
Analysis		n/N	%	n/N	%	
Per Protocol I*						
	Enrollment	14/97	14.4	17/102	16.7	0.92 (0.49, 1.75)
	Month 3	32/254	12.6	31/248	12.5	1.01 (0.63, 1.62)
	Month 6	38/237	16.0	38/233	16.3	1.06 (0.71, 1.59)
Per Protocol II**						
	Enrollment	8/49	16.3	8/40	20.0	0.82 (0.35, 1.96)
	Month 3	13/114	11.4	14/108	13.0	0.90 (0.44, 1.84)
	Month 6	20/101	19.8	15/106	14.2	1.43 (0.77, 2.65)

Prevalence ratio adjusted for county of enrollment

*includes only those who reported taking all 5 doses of study drug via adherence questionnaire

**includes only those who reported taking all 5 doses of study drug via bottle tick-mark

Figure S4. The effect of azithromycin on the proportion of *S. pneumoniae* isolates resistant to azithromycin, stratified by whether the child received any antibiotics during their hospitalization

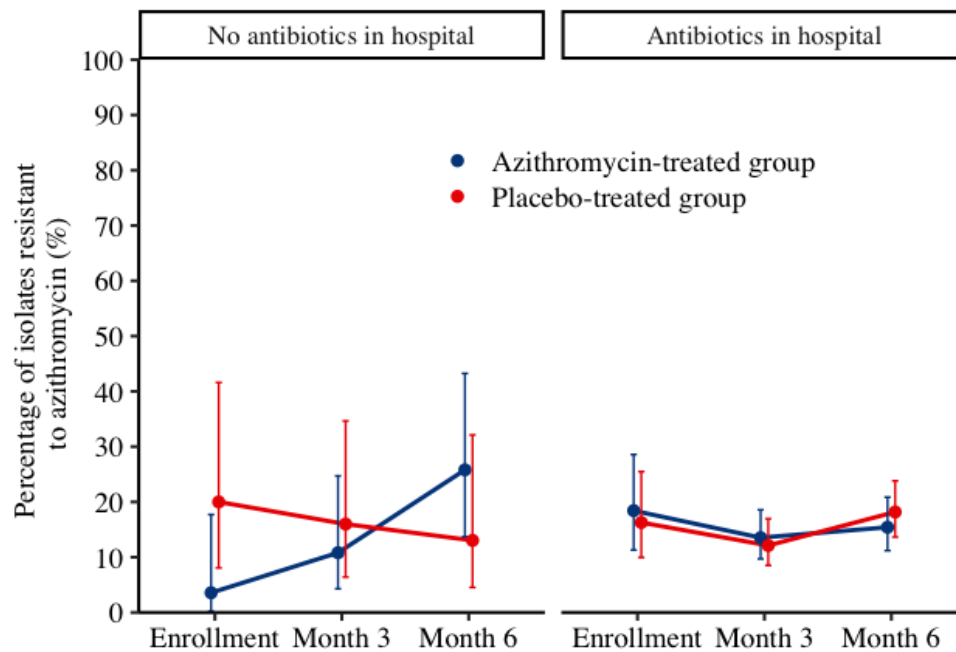


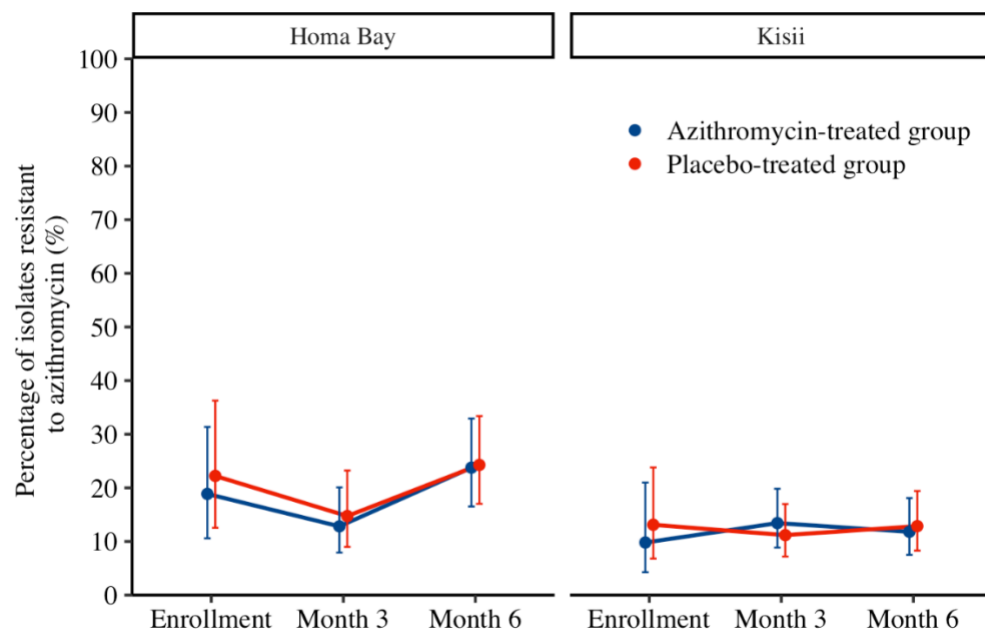
Table S4. The effect of azithromycin on the proportion of *S. pneumoniae* isolates resistant to azithromycin, stratified by whether the child received any antibiotics during their hospitalization

	Timepoint	Azithromycin-treated		Placebo-treated		Prevalence Ratio (95% CI), adjusted for county ¹	Prevalence Ratio (95% CI) adjusted for county and baseline resistance ²
		n/N	%	n/N	%		
No antibiotics during hospitalization							
	Enrollment	1/28	3.6	4/20	20.0	0.22 (0.03, 1.61)	0.37 (0.09, 1.51)
	Month 3	4/37	10.8	4/25	16.0	0.71 (0.19, 2.67)	1.33 (0.31, 5.66)
	Month 6	8/31	25.8	3/23	13.0	2.18 (0.67, 7.09)	5.4 (1.53, 19.1)
Antibiotics during hospitalization							
	Enrollment	14/76	18.4	14/86	16.3	1.17 (0.61, 2.27)	1.06 (0.74, 1.51)
	Month 3	31/229	13.5	28/231	12.1	1.12 (0.69, 1.80)	1.13 (0.71, 1.81)
	Month 6	33/214	15.4	40/220	18.2	0.91 (0.60, 1.38)	0.91 (0.60, 1.39)

¹GEE model, with Poisson link and exchangeable correlation structure, adjusted for enrollment county

²GEE model, with Poisson link and exchangeable correlation structure, adjusted for enrollment county and resistance to azithromycin at enrollment

Figure S5. The effect of azithromycin on the proportion of *S. pneumoniae* isolates resistant to azithromycin, stratified by enrollment county



Chapter 3: Pneumococcal carriage and antimicrobial resistance among Kenyan children during the 6 months following hospital discharge: temporal trends in clinical and household risk factors

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Abstract

Background: Children discharged from hospital in low-resource settings are at high risk for morbidity and mortality related to *Streptococcus pneumoniae*. A better understanding of the modifiable factors that predict *S. pneumoniae* carriage and antimicrobial resistance (AMR) could inform interventions for this high-risk group.

Methods: Using nasopharyngeal specimens collected at hospital discharge and 3- and 6-months after among 1,398 Kenyan children under five enrolled in a randomized trial, we assessed risk factors for *S. pneumoniae* carriage and AMR. Using generalized estimating equations, we calculated prevalence ratios (PR) and 95% CIs, overall and for each timepoint, adjusting for age, sex, county, caregiver education, and randomization arm (azithromycin or placebo).

Findings: At discharge, carriage was inversely associated with inpatient antibiotic use (PR=0.38, 95% CI 0.31–0.46) and duration of hospitalization (PR=0.69, 95% CI 0.57–0.84). Across all timepoints, carriage was associated with use of an unimproved water source, untreated drinking water, and a non-flushing toilet. Having three or more siblings under five years was associated with higher doxycycline resistance (PR=1.33, 95% CI 1.01–1.75) and having the recommended doses of pneumococcal conjugate vaccine (PCV) was associated with decreased clindamycin (PR=0.54, 95% CI 0.29–1.00) and doxycycline resistance (PR=0.74, 95% CI 0.59–0.94).

Conclusions: Our findings highlight the importance of household-level interventions and PCV to prevent *S. pneumoniae* carriage and AMR, respectively, among recently hospitalized children.

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is a major cause of morbidity and mortality globally, particularly in low and middle-income settings. *S. pneumoniae* causes an estimated 973,000 cases of severe disease and 166,000 deaths among African children under five every year, with a mortality rate over 11 times higher than that of the Americas or Europe.¹ Common manifestations of the disease in children include pneumonia, meningitis, sepsis, and otitis media.² Nasopharyngeal colonization of *S. pneumoniae* is a required precursor for disease and is the source of spread from person to person. The prevalence of carriage is as high as 80% among children under five years of age in sub-Saharan Africa and is associated with household crowding and sharing the home with siblings under five.^{11,18,26,50}

Development of antimicrobial resistance (AMR) in *S. pneumoniae* is of growing concern as reduced susceptibility can lead to treatment failure.^{14–17} Although *S. pneumoniae* AMR data from sub-Saharan Africa are limited, the prevalence of resistance to beta-lactams (first-line therapy) is as high as 45%.^{15,18} *S. pneumoniae* can acquire resistance-conferring DNA in the environment from other streptococci and related bacteria and develop resistance through successive genetic mutations due to selective pressure, such as from antibiotics.¹⁵ Selection of resistant clones occurs commonly among children because they often carry pneumococcus for longer durations, and are more frequently exposed to antibiotics, compared to adults.¹⁵ Risk factors for acquisition of antibiotic resistance in *S. pneumoniae* include hospitalization in the last 3 months, antibiotic use in the last 6 months, day-care attendance, and HIV infection.¹⁵

While environmental factors, such as inadequate sanitation and hygiene and contact with animals, have been associated with AMR among gram-negative bacteria of the gastrointestinal tract,^{54,55} few studies have examined these factors in *S. pneumoniae*.^{21,22} These factors, along with household crowding, can promote the spread of pathogens, thus increasing the demand for antibiotics and selective pressure for resistance.^{19,20} In addition to the nasopharynx, *S. pneumoniae* is found in the gastrointestinal tract of humans and animals, including ruminants, where mixing of bacterial genes encoding AMR is common. A recent pilot study in Bangladesh tested soil and cow dung from 10 rural households and found 15 streptococcus species, including *S. pneumoniae* and *S. equinus* in cow dung from all 10 households and in soil from three and five households, respectively.⁵⁶ Additionally, aerosol transport of pathogens and AMR genes from untreated wastewater has been documented.⁵⁷ Given children in rural settings have a high risk of contact with soil, cow dung, and untreated water, these findings suggest AMR in *S. pneumoniae* could be impacted by these environmental factors.

Children discharged from hospital in low-resourced settings are an ideal population in which to study *S. pneumoniae* carriage and AMR because they are a population at high risk for pneumococcus-related morbidity and mortality^{29,37}, are frequently exposed to broad-spectrum antibiotics that exert selective pressure on *S. pneumoniae*, and are returning to a home environment with possible household crowding, lack of clean water and sanitation, and close proximity to animals. A better understanding of the modifiable factors that predict carriage and AMR in *S. pneumoniae* during this transition from the hospital to home environment could help target interventions for this high-risk group. We leveraged nasopharyngeal specimens from a recently completed randomized clinical trial conducted among 1,398 children in western Kenya to assess risk factors for *S. pneumoniae* carriage and resistance to nine antibiotic classes over a 6-month period following hospital discharge. We hypothesized that inpatient antibiotic use and duration of hospitalization would be associated with carriage and resistance at hospital discharge, and that household and environmental factors would predict carriage and resistance during the 6-month post-discharge period.

Methods

Study population and procedures

We conducted a secondary, longitudinal analysis using data from Toto Bora, a double-blind, placebo-controlled, randomized clinical trial (registered at ClinicalTrials.gov; NCT02414399) of azithromycin for the prevention of death and rehospitalization, which enrolled children 1-59 months of age, admitted to the hospital for non-traumatic conditions from June 2016 – November 2019 and subsequently discharged.³⁴ Details regarding participant enrollment and follow-up procedures have been previously published.^{34,41} We interviewed caregivers of enrolled children to collect medical history and sociodemographic information, including household characteristics and environmental exposures. We abstracted hospital records to ascertain clinical history, diagnoses, and antibiotic use. Vaccination history was determined by immunization record when available and supplemented by caregiver self-report. In the primary trial, we did not observe an effect of a 5-day course of azithromycin on the combined outcome of death or rehospitalization,⁴¹ or on the secondary outcome of *S. pneumoniae* carriage and AMR.

The present study included all 1,398 children enrolled in Toto Bora. A random sample of 844 children were selected for the AMR sub-study, irrespective of pneumococcal carriage.

Ethics statement

The Toto Bora trial received approval from the Institutional Review Boards of the Kenya Medical Research Institute (KEMRI SERU #3086), the Kenyan Pharmacy and Poisons Board (Ref. No. PPB/ECCT/15/10/04), and the University of Washington (IRB #49120). Written informed consent was obtained from all participating caregivers in their preferred language (English, Kiswahili, Kisii, or Luo). For individuals who were not literate, the consent form was read aloud in their preferred language, and consent was documented using a thumbprint in the presence of a witness.

Laboratory methods

Nasopharyngeal swabs (Copan Diagnostics, USA), recognized as the gold standard for detecting pneumococcal carriage in pediatric populations,⁴³ were obtained from all participants at enrollment (following hospital discharge) and again at 3- and 6-month follow-up visits for those who survived. Each swab was promptly placed into skim milk–tryptone–glucose–glycerol (STGG) transport medium, vortexed to create a duplicate sample, and stored at –80°C within one hour of collection in on-site freezers equipped with both generator and solar power backup systems. Samples were shipped monthly on dry ice to the Kenya Medical Research Institute (KEMRI) in Nairobi for long-term storage at –80°C.

All collected swabs (baseline, 3-month, and 6-month) were cultured on gentamicin-supplemented blood agar to isolate *S. pneumoniae*. Suspected colonies were confirmed using optochin susceptibility testing at the KEMRI-Wellcome Trust Research Programme laboratory in Kilifi, Kenya, following standardized procedures.⁴³ For children randomly selected for the AMR sub-study, one pneumococcal isolate per child per timepoint (if available) underwent antimicrobial susceptibility testing (AST). The disc diffusion method was used to assess susceptibility to azithromycin, oxacillin (as a proxy for penicillin), doxycycline, levofloxacin, co-trimoxazole, chloramphenicol, clindamycin, and linezolid, while ceftriaxone susceptibility was determined using E-TEST. Classification of isolates as susceptible, intermediate, or resistant was based on the 2024 Clinical and Laboratory Standards Institute (CLSI) breakpoints for zone diameters or minimum inhibitory concentrations (MICs).⁴⁴ Intermediate and resistant isolates were considered non-susceptible.

Outcomes

The primary outcomes were a) the prevalence of *S. pneumoniae* carriage, defined as the proportion of children with microbiological isolation of *S. pneumoniae* from a nasopharyngeal

swab, and b) the proportion of carried pneumococci that were resistant to penicillin, azithromycin, doxycycline, clindamycin, or that were multidrug-resistant (MDR) defined as non-susceptible to three or more antimicrobial classes tested.

Secondary outcomes included the proportion of carried pneumococci that were resistant to ceftriaxone, levofloxacin, co-trimoxazole, chloramphenicol, and linezolid and the proportion of children that carried *S. pneumoniae* that were resistant to each antimicrobial tested (using the 844 children in the AMR sub-study as the denominator).

Statistical methods

Among all children enrolled in Toto Bora (n=1389), we calculated the prevalence of *S. pneumoniae* carriage at discharge and 3- and 6-months post-discharge. Among all children randomly selected for the AMR sub-study (n=844), we calculated the proportion of *S. pneumoniae* isolates that were resistant and the proportion of children that carried *S. pneumoniae* that were resistant to each antimicrobial at each timepoint. Among children with *S. pneumoniae* isolated at more than one timepoint, we calculated the frequency (number of timepoints) of resistance to each antimicrobial and the percentage of children who carried a resistant strain that had resistance to the same antimicrobial at one or more follow-up visits.

We assessed risk factors for each primary outcome and changes over time using generalized estimating equations (GEE) with a Poisson link and exchangeable correlation structure to account for intra-subject correlation of repeated measurements. Our primary risk factors of interest were household crowding (≥ 3 persons per room), number of children < 5 years of age sleeping in the home, number of other children < 5 years who are cared for by the primary caregiver, use of improved water source, treatment of drinking water (using filters, boiling, or chlorinating), type of toilet (flush, pit latrine with or without a slab, or open defecation), use of a toilet shared by more than one household, and livestock ownership (cows, goats, sheep, pigs and/or chickens). Having an improved water source was determined by the reporting of water piped to the home or community, a bore hole or tube well, spring water through a pipeline, a lined well with a pump or bucket or bottled water as a primary source of water. We also examined duration of hospitalization (< 4 or ≥ 4 days), receipt of the recommended number of pneumococcal conjugate vaccine (PCV) doses for the child's age (allowing a 4-week window), and inpatient use of antibiotics (macrolides, cephalosporins, penicillin, or other antibiotics). Finally, we categorized inpatient antibiotic use into four mutually exclusive categories: none, any penicillin (but no cephalosporin), any cephalosporin, and other (non-Beta-lactam) antibiotics.

Multivariate models adjusted for randomization arm and study site, as well as age and sex as *priori* confounders. In sensitivity analyses we additionally adjusted for caregiver education as a measure of socioeconomic position and any additional covariates found to be associated with the outcome in univariate analyses. To assess changes over time, we included an interaction term between follow-up visit and the predictor of interest. Associations were considered statistically significant at an alpha of <0.05. Analyses were performed in R version 4.0.3.

Results

Characteristics of children included in the *S. pneumoniae* carriage analyses (n=1398) and AMR sub-study (n=844) are shown in Table 1. Children randomly selected for the AMR sub-study were representative of the overall study population. Over 93% of children were up to date on PCV vaccine doses for their age: 561/604 (92.9%) among those with vaccination cards and 735/788 (93.3%) among those without (based on caregiver-report). Overall, 1324 (95.1%) received at least one dose, 1304 (93.7%) two doses, and 1246 (89.5%) three doses.

Nasopharyngeal swabs were available for testing for 1395/1398 (99.8%) children at baseline, 1255 (89.8%) at month 3, and 1201 (85.9%) at month 6. Thirty-four (2.4%) children died during the 6-months post-discharge. Other reasons for missing swabs are summarized in the supplemental Table S1. *S. pneumoniae* was isolated from 329/1395 (23.6%) children at enrollment, 832/1255 (66.3%) at month 3, and 800/1201 (66.6%) at month 6, representing a significant increase over time ($p<0.001$).

S. pneumoniae carriage at hospital discharge was associated with age younger than 24 months, caregiver education level of primary school or less, use of untreated drinking water, and a discharge diagnosis of diarrhea (table 1). At discharge, *S. pneumoniae* carriage was inversely associated with hospital stay greater than 3 days (PR 0.69, 95% CI 0.57–0.84) and use of any antibiotic during hospital admission (PR 0.38, 95% CI 0.31–0.46), particularly cephalosporins (PR 0.10, 95% CI 0.07–0.15); these associations with carriage were attenuated at 3- and 6-month follow-up visits (time interaction p-values <0.01; figure 1). Having other children under five sleeping in the home or cared for by the primary caregiver were associated with carriage at 3- and 6-month follow-up visits, but not at enrollment. In the GEE model including all timepoints, and adjusting for age, sex, county, and randomization arm, we found carriage to be associated with use of an unimproved water source, untreated drinking water, use of a slab latrine, pit latrine, or open defecation versus flush toilet, and use of a shared versus private toilet (figure 2). When

additionally adjusting for caregiver education, use of an untreated drinking water, pit latrine and open defecation were no longer associated with carriage (supplemental figure S1).

Among the 844 children selected for the AMR sub-study, *S. pneumoniae* was isolated and AST completed for all nasopharyngeal swabs collected from these children across the three timepoints (210 isolates at baseline, 522 at month 3, and 488 at month 6). At hospital discharge, resistance was observed to co-trimoxazole (205 out of 210 isolates; 97.6%), penicillin (198/210; 94.3%), doxycycline (68/210; 32.4%), azithromycin (33/210; 15.7%), clindamycin (22/210; 10.5%), and was rare for both chloramphenicol and linezolid (1/210 each; 0.5%). MDR was identified in 73/210 (34.8%) isolates. Among children with carriage, there was no change over time in the proportion of isolates that were resistant, except for an observed decrease in the proportion resistant to penicillin at months 3 and 6 ($p < 0.001$) (figure 3). The proportion of all *children* in the AMR sub-study with carriage of *S. pneumoniae* resistant to penicillin, doxycycline, azithromycin, and clindamycin increased from enrollment to month 3, in accordance with the increase in carriage (supplemental figure S2). Among the 426/844 (50.5%) children who had *S. pneumoniae* isolated at more than one timepoint, resistance to the same antimicrobial at two or more timepoints was common for penicillin 341/402 (84.8%), and less so for children with resistance to doxycycline (82/238; 34.5%), azithromycin (32/113; 28.3%), clindamycin (20/79; 25.3%), or MDR (90/249; 36.1%). The distribution of the number of timepoints (0, 1, 2, or 3) each child had *S. pneumoniae* resistant to each antimicrobial is summarized in supplemental figure S3.

We did not observe trends over time in risk factors associated with the proportion of isolates resistant to azithromycin (figure 4), doxycycline, oxacillin, or MDR (supplemental figures S4, S5, S6). Household crowding was associated with resistance to doxycycline (PR 1.29, 95 CI 1.01–1.66) at month 6. When assessing overall associations (considering all timepoints), adjusting for county, randomization arm, age, sex, and caregiver education, we found having 3 or more siblings under 5 years of age was associated with higher doxycycline resistance (PR 1.33, 95% CI 1.01–1.75); having the recommended doses of PCV vaccine was associated with lower doxycycline (PR 0.74, 95% CI 0.59–0.94) and clindamycin resistance (PR 0.54, 95% CI 0.29–1.00); use of untreated drinking water was associated with lower azithromycin resistance (PR 0.61, 95% CI 0.42–0.87); and open-defecation compared to a flush toilet was associated with lower doxycycline (PR 0.30, 95% CI 0.14–0.64] and azithromycin resistance (PR 0.29, 95% CI 0.09–0.93) (figure 5). In sensitivity analyses, results did not meaningfully differ when models were adjusted for household income and HIV status (supplemental figures S7 and S8).

Discussion

Among children recently discharged from the hospital in western Kenya, nasopharyngeal carriage of *S. pneumoniae* was inversely associated with duration of hospitalization and inpatient antibiotic use, especially 3rd generation cephalosporins. These associations were attenuated at 3- and 6-months post-discharge, at which point carriage was associated with household factors such as sharing a home with other children under five, having an unimproved water source, using untreated drinking water, sharing toilet facilities with other households, and using a pit latrine or open defecation. We observed a relatively high burden of antimicrobial resistance in *S. pneumoniae*. Among children with carriage, the proportion of isolates that were resistant to each antimicrobial class was largely stable over the 6 months following discharge, though the proportion of all children with antibiotic resistant carriage increased due to the notable recolonization of *S. pneumoniae* during the post-discharge period. Having the recommended doses of PCV was associated with a lower proportion of doxycycline and clindamycin resistance among children with *S. pneumoniae* carriage which is notable, and consistent with growing appreciation of the role vaccines play in preventing AMR.^{27,58}

As expected, the prevalence of carriage in this recently hospitalized population was lower than that observed in population-based surveys of Kenyan children^{18,26} most likely due to inpatient antibiotic exposure. We did not observe an effect of PCV vaccine doses on carriage of *S. pneumoniae*, consistent with literature describing serotype replacement following the rollout of PCV-10 in Kenya in 2011.^{26,59} The strongest predictors of carriage at discharge were age younger than 24 months, no inpatient antibiotics prescribed, discharge diagnosis of diarrhea, caregiver education less than primary school, and use of untreated drinking water. Once children transitioned from the hospital environment to the home environment, we observed a stronger association with household factors (siblings or other children under five in the home, unimproved water source, pit latrine, open defecation, shared toilet), but no longer observed an association with hospital duration or with inpatient antibiotic exposure other than cephalosporins. These findings are consistent with research suggesting a high rate of clearance and acquisition of *S. pneumoniae* among Kenyan children⁴⁹ and highlight the relevance of interventions to prevent household transmission of pneumococcus.

We observed resistance patterns similar to recently published data from 2017 in Kenya¹⁸ and from 2018-2019 in Mozambique⁶⁰, including nearly universal resistance to co-trimoxazole, high resistance to oral penicillin, rare resistance to ceftriaxone, and universal susceptibility to levofloxacin. While prior access to healthcare and treatment is a strong predictor of AMR in *S.*

pneumoniae, we did not observe an association between individual-level use of inpatient antibiotics or duration of hospitalization and AMR in our study. However, because all children in our study were recently hospitalized, our ability to detect these associations may be diluted. Community-wide use of antibiotics and the resistance profiles of the strains circulating in the child's community may be stronger predictors of AMR than individual-level antimicrobial use.¹⁵ Further, given the transmissibility of antibiotic resistance genes, children returning home from hospitals with *S. pneumoniae* carriage may be an unappreciated source of household AMR and could particularly benefit from water, sanitation, and hygiene (WASH) or other interventions.⁶¹

Among children with pneumococcal carriage, we expected the proportion of *S. pneumoniae* isolates that were resistant to be higher at discharge, when susceptible strains would presumably be cleared by antibiotic exposure, compared to the proportion resistant at 3- and 6-month follow-up visits. However, except for penicillin, the proportion of *S. pneumoniae* resistant to other antimicrobial classes was largely stable over the 6-month follow-up. This may indicate that community levels of AMR are similar to what was observed in this hospitalized population, though we are unable to confirm this with our data. Sustained resistance to penicillin was commonly observed among individuals with *S. pneumoniae* isolated at more than one timepoint. However, azithromycin, clindamycin, and doxycycline resistance were more transiently observed. This could be related to less common exposure to these antibiotics post-discharge in the community. Macrolide, tetracycline, and clindamycin use was rarely reported by caregivers during follow-up visits, though caregivers may have been reticent to report non-prescribed antibiotic use and may not have been able to correctly identify the drug name.

We did not observe our hypothesized associations between WASH factors and AMR in *S. pneumoniae*, although our analyses may have been underpowered due to lower-than-expected resistance to azithromycin and clindamycin and higher than expected resistance to penicillin. While open defecation was inversely associated with AMR, confidence intervals were wide due to small numbers (very few households reported open defecation and none from Kisii county were selected for the AMR sub-study). The observed inverse association between treated drinking water and azithromycin resistance may reflect reporting bias or residual confounding by socioeconomic status, as adjustment for caregiver-reported education and household income may have been imperfect. WASH factors were highly predictive of *S. pneumoniae* carriage, which is in turn a requirement for isolate-based susceptibility testing, so our ability to detect an additional effect on AMR was reduced.

To our knowledge, this is the first study to evaluate the trajectory of *S. pneumoniae* carriage and resistance in the months following hospital discharge, a time of a presumed high risk of developing disease from pathogen carriage and when the high burden of resistant pathogens may pose a high transmission risk to other household members. Existing data on *S. pneumoniae* carriage and AMR in sub-Saharan Africa were primarily collected prior to or during the roll-out of PCV vaccines⁶² and from urban centers. Our findings provide data from a rural setting in western Kenya with high PCV-10 coverage, and from a period more than 5 years after vaccine rollout. Additional strengths of this study include the large sample size of nasopharyngeal swabs tested, high retention over the follow-up period, and use of both medical records and caregiver interview to gather complete sociodemographic and clinical history.

The study is subject to several limitations. First, isolation of *S. pneumoniae* by bacterial culture has less than 100% sensitivity and is known to vary by serotype and prior antibiotic use, which could have led to an overestimation of the effect of antibiotic use on carriage. Misclassification of antimicrobial resistance was possible, since only a single isolate was selected for AST for each child at each timepoint. Clindamycin resistance could be underestimated because we were unable to conduct follow-up testing for erythromycin-inducible clindamycin resistance as is recommended for isolates that are susceptible to clindamycin but resistant to erythromycin by disc diffusion.⁴⁴ There was potential for recall and social desirability bias when assessing household-level characteristics (e.g., treatment of drinking water, household income) that were self-reported by caregivers at enrollment and for vaccination history for children without immunization cards available. We expect misclassification of these exposures was non-differential with respect to the outcomes, thus attenuating the effect estimates. We are unable to account for variation in antibiotic use (adherence, duration, changes) during admission or during the follow-up period, which may impact pathogen selection and resistance patterns. Our study population is limited to individuals who survived hospitalization, which may select for individuals who are less likely to be colonized with resistant *S. pneumoniae* at baseline. While less than 3% of participants died during follow-up, survival bias may be a concern if *S. pneumoniae* carriage or AMR profiles differed from children who survived until 3- and 6-month follow-up visits. Some children were missing nasopharyngeal swabs at one or more follow-up visits. Although we assume that missingness was unrelated to the outcomes(s), estimates from the GEE model—which incorporates all available observations—may be biased if the data were not missing completely at random.

We found a high burden of AMR among *S. pneumoniae* isolated from Kenyan children at hospital discharge and 6 months later, highlighting the importance of reducing AMR transmission in

healthcare and community settings. Our findings suggest PCVs and minimizing household crowding could reduce risk of AMR and that access to improved water, sanitation, and hygiene are associated with lower carriage of *S. pneumoniae*. The World Health Organization recommends WASH and wastewater management be incorporated into National Action Plans for AMR.⁶¹ Such interventions, as well as education targeted to caregivers of recently discharged children, could reduce acquisition of *S. pneumoniae* during a period of high risk for pneumococcus-related morbidity and mortality.

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Tables and figures

Table 1. Baseline characteristics of children enrolled in Toto Bora, overall (n=1398) and among the random sample selected for the antimicrobial resistance (AMR) sub-study (n=844)

Characteristic	Overall, N = 1,398 ¹	AMR sub- study, N = 844 ¹	Not sampled, N = 554 ¹
Randomization arm			
Placebo	696 (50%)	415 (49%)	281 (51%)
Azithromycin	702 (50%)	429 (51%)	273 (49%)
County of recruiting hospital			
Homa Bay	576 (41%)	337 (40%)	239 (43%)
Kisii	822 (59%)	507 (60%)	315 (57%)
Age at enrollment (months)	18 (9, 32)	18 (9, 32)	18 (9, 32)
Age category (months)			
0-5	188 (13%)	109 (13%)	79 (14%)
6-11	277 (20%)	168 (20%)	109 (20%)
12-23	406 (29%)	250 (30%)	156 (28%)
24-59	527 (38%)	317 (38%)	210 (38%)
Sex			
Male	828 (59%)	495 (59%)	333 (60%)
Female	570 (41%)	349 (41%)	221 (40%)
Caregiver reported income			
Income >= 5000 ksh	454 (32%)	267 (32%)	187 (34%)
Income < 5000 ksh	874 (63%)	535 (63%)	339 (61%)
Unknown or refuse to answer	70 (5.0%)	42 (5.0%)	28 (5.1%)
Highest level of education completed by primary caregiver			
Secondary or greater	752 (54%)	450 (53%)	302 (55%)
Primary school or less	644 (46%)	394 (47%)	250 (45%)
Household crowding			

Characteristic	Overall, N = 1,398¹	AMR sub- study, N = 844¹	Not sampled, N = 554¹
No crowding (< 3 persons per room)	1,127 (81%)	684 (81%)	443 (80%)
Crowding (>=3 persons per room)	271 (19%)	160 (19%)	111 (20%)
Water source			
Improved	1,153 (83%)	713 (85%)	440 (79%)
Unimproved	244 (17%)	130 (15%)	114 (21%)
Type of toilet used by the household			
Flush toilet	135 (9.7%)	88 (10%)	47 (8.5%)
Pit latrine	1,209 (87%)	728 (86%)	481 (87%)
Open defecation	53 (3.8%)	28 (3.3%)	25 (4.5%)
Exclusive breastfeeding for first 6 months of life (or until enrollment if child <6 m)			
Never breastfed	24 (1.7%)	16 (1.9%)	8 (1.4%)
Exclusively breastfed	663 (47%)	397 (47%)	266 (48%)
Partially breastfed	632 (45%)	381 (45%)	251 (45%)
Unknown	79 (5.7%)	50 (5.9%)	29 (5.2%)
Acute malnutrition based on WLZ, MUAC (in 6+ month olds), and/or presence of edema			
None	1,266 (91%)	757 (90%)	509 (92%)
MAM	75 (5.4%)	44 (5.2%)	31 (5.6%)
SAM	57 (4.1%)	43 (5.1%)	14 (2.5%)
Child HIV status			
HIV unexposed	1,192 (85%)	722 (86%)	470 (85%)
HIV exposed, uninfected/infection status unknown	147 (11%)	85 (10%)	62 (11%)
HIV infected	18 (1.3%)	13 (1.5%)	5 (0.9%)
Uninfected/exposure status unknown	41 (2.9%)	24 (2.8%)	17 (3.1%)

Characteristic	Overall, N = 1,398¹	AMR sub- study, N = 844¹	Not sampled, N = 554¹
Has recommended number of vaccines for this age (with grace of 4 weeks)			
No	96 (6.9%)	58 (6.9%)	38 (6.9%)
Yes	1,296 (93%)	783 (93%)	513 (93%)
Any antibiotic used during enrollment admission	1,253 (90%)	757 (90%)	496 (90%)
Any antibiotic prescribed at discharge	867 (62%)	530 (63%)	337 (61%)
Antibiotic use reported during the follow-up	312 (22%)	194 (23%)	118 (21%)
Child died during follow-up	34 (2.4%)	20 (2.4%)	14 (2.5%)
Child was rehospitalized at least once during follow-up	115 (8.2%)	71 (8.4%)	44 (7.9%)

¹n (%); Median (IQR)

Table 2. Risk factors for *S. pneumoniae* carriage at hospital discharge

Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
County of recruiting hospital								
Homa Bay	144 (43.8%)	430 (40.3%)	REF	—	—	REF	—	—
Kisii	185 (56.2%)	636 (59.7%)	0.90	0.72, 1.12	0.33	0.88	0.73, 1.06	0.251
Sex								
Male	197 (59.9%)	630 (59.1%)	REF	—	—	REF	—	—
Female	132 (40.1%)	436 (40.9%)	0.98	0.78, 1.22	0.83	0.98	0.81, 1.18	0.849
Age (months)								
0-5	42 (12.8%)	146 (13.7%)	REF	—	—	REF	—	—
6-11	90 (27.4%)	187 (17.5%)	1.45	1.01, 2.10	0.045	1.44	1.05, 1.98	0.052
12-23	122 (37.1%)	282 (26.5%)	1.35	0.95, 1.92	0.092	1.33	0.98, 1.81	0.113
24-59	75 (22.8%)	451 (42.3%)	0.64	0.44, 0.93	0.020	0.63	0.45, 0.88	0.016
Breastfeeding								
Exclusively breastfed	155 (47.1%)	505 (47.4%)	REF	—	—	REF	—	—
Partially breastfed	147 (44.7%)	485 (45.5%)	0.99	0.79, 1.24	0.93	1	0.82, 1.23	0.979
Never breastfed	9 (2.7%)	15 (1.4%)	1.60	0.82, 3.13	0.17	1.58	0.94, 2.68	0.183
Unknown	18 (5.5%)	61 (5.7%)	0.97	0.60, 1.58	0.90	1.1	0.71, 1.72	0.699
Child HIV status (at enrollment) combining unknown categories								
HIV unexposed	270 (82.1%)	920 (86.3%)	REF	—	—	REF	—	—

Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
HIV exposed, uninfected/infection status unknown	40 (12.2%)	106 (9.9%)	1.21	0.87, 1.68	0.27	1.2	0.90, 1.61	0.299
HIV infected	7 (2.1%)	11 (1.0%)	1.71	0.81, 3.63	0.16	1.98	1.03, 3.81	0.076
Uninfected/exposure status unknown	12 (3.6%)	29 (2.7%)	1.29	0.72, 2.30	0.39	1.38	0.84, 2.26	0.279
Stunting (HAZ/LAZ < -2) at enrollment								
HAZ >= -2	247 (75.3%)	828 (78.0%)	REF	—	—	REF	—	—
HAZ <-2	81 (24.7%)	233 (22.0%)	1.12	0.87, 1.44	0.37	1.16	0.93, 1.43	0.263
Underweight (WAZ < -2) at enrollment								
WAZ >= -2	278 (84.8%)	939 (88.1%)	REF	—	—	REF	—	—
WAZ <-2	50 (15.2%)	127 (11.9%)	1.24	0.92, 1.67	0.17	1.14	0.88, 1.47	0.413
Acute malnutrition based on WLZ, MUAC (in 6+ month olds), and/or presence of edema								
None	291 (88.4%)	972 (91.2%)	REF	—	—	REF	—	—
MAM	23 (7.0%)	52 (4.9%)	1.33	0.87, 2.03	0.19	1.19	0.83, 1.71	0.429
SAM	15 (4.6%)	42 (3.9%)	1.14	0.68, 1.92	0.62	0.93	0.59, 1.47	0.795
Has recommended number of PCV doses for this age (with grace of 4 weeks)								
No	30 (9.1%)	66 (6.2%)	REF	—	—	REF	—	—

Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
Yes	299 (90.9%)	994 (93.8%)	0.74	0.51, 1.08	0.12	0.74	0.54, 1.02	0.121
Caregiver reported income (at enrollment)								
Income >= 5000 ksh	96 (29.2%)	358 (33.6%)	REF	—	—	REF	—	—
Income < 5000 ksh	218 (66.3%)	653 (61.3%)	1.18	0.96, 1.46	0.17	1.13	0.92, 1.40	0.322
Household income (at enrollment) above/ below (above/below 5000 ksh or ~\$1/day)								
Income >= 5000 ksh	274 (85.4%)	845 (81.9%)	REF	—	—	REF	—	—
Income < 5000 ksh	38 (11.8%)	155 (15.0%)	0.80	0.59, 1.09	0.21	0.84	0.62, 1.13	0.314
Highest level of education completed by primary caregiver (at enrollment visit)								
Secondary or greater	157 (47.9%)	595 (55.9%)	REF	—	—	REF	—	—
Primary school or less	171 (52.1%)	470 (44.1%)	1.28	1.03, 1.59	0.027	1.27	1.05, 1.54	0.031
Household crowding (>=3 persons per room) at enrollment visit								
No crowding (< 3 persons per room)	270 (82.1%)	854 (80.1%)	REF	—	—	REF	—	—
Crowding (>=3 persons per room)	59 (17.9%)	212 (19.9%)	0.91	0.68, 1.20	0.49	0.85	0.67, 1.09	0.273
No. of children <5 who sleep in the house								

Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
1	187 (56.8%)	649 (60.9%)	REF	—	—	REF	—	—
2	119 (36.2%)	349 (32.7%)	1.14	0.90, 1.43	0.27	1.08	0.88, 1.31	0.538
3+	23 (7.0%)	68 (6.4%)	1.13	0.73, 1.74	0.58	1.03	0.71, 1.49	0.902
No. of other children cared for by the primary caregiver								
0	83 (25.4%)	277 (26.0%)	REF	—	—	REF	—	—
1-2	145 (44.3%)	503 (47.3%)	0.97	0.74, 1.27	0.83	1.03	0.81, 1.30	0.839
3-4	72 (22.0%)	210 (19.7%)	1.11	0.81, 1.52	0.53	1.19	0.91, 1.57	0.282
5+	27 (8.3%)	74 (7.0%)	1.16	0.75, 1.79	0.50	1.38	0.95, 2.00	0.156
Water source (at enrollment visit)								
Improved	266 (80.9%)	884 (83.0%)	REF	—	—	REF	—	—
Unimproved	63 (19.1%)	181 (17.0%)	1.12	0.85, 1.47	0.43	1.12	0.88, 1.42	0.428
Filters, boils, or chlorinates drinking water (among those who do not use bottled)								
No	176 (54.3%)	500 (48.0%)	REF	—	—	REF	—	—
Yes	148 (45.7%)	541 (52.0%)	0.83	0.66, 1.03	0.085	0.78	0.64, 0.96	0.044
Toilet type								
Flush toilet	23 (7.0%)	112 (10.6%)	REF	—	—	REF	—	—
Slab latrine	215 (65.7%)	666 (62.8%)	1.43	0.93, 2.20	0.10	1.37	0.93, 2.03	0.149
Pit latrine (no slab)	77 (23.5%)	241 (22.7%)	1.42	0.89, 2.26	0.14	1.3	0.84, 2.00	0.294

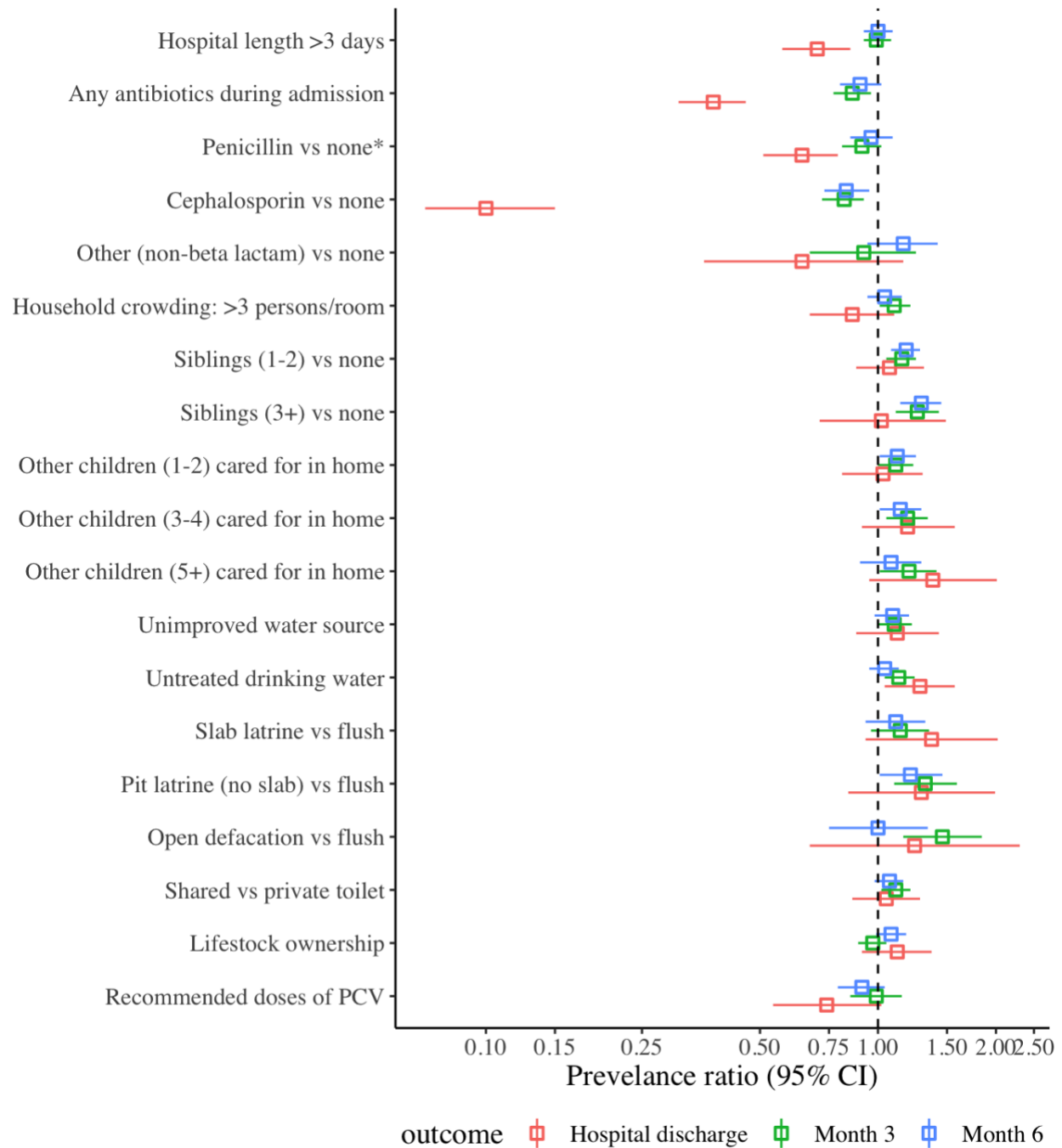
Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
Open defecation	12 (3.7%)	41 (3.9%)	1.33	0.66, 2.67	0.42	1.25	0.67, 2.32	0.543
Shared or Private Toilet								
private toilet	157 (48.2%)	546 (51.5%)	REF	—	—	REF	—	—
shared toilet	157 (48.2%)	473 (44.6%)	1.12	0.92, 1.35	0.33	1.04	0.86, 1.27	0.716
Open Defecation	12 (3.7%)	41 (3.9%)	1.01	0.60, 1.71	0.96	0.98	0.59, 1.64	0.96
Livestock ownership	227 (69.0%)	710 (66.6%)	1.09	0.86, 1.37	0.48	1.12	0.91, 1.37	0.352
Cats	105 (32.0%)	315 (29.7%)	1.09	0.86, 1.37	0.48	1.06	0.87, 1.30	0.614
Dogs	93 (28.3%)	252 (23.8%)	1.19	0.94, 1.51	0.16	1.17	0.95, 1.43	0.209
Discharge diagnosis								
Anemia	44 (14.3%)	133 (13.1%)	1.08	0.79, 1.49	0.62	1.1	0.83, 1.45	0.565
Diarrhea	90 (29.2%)	160 (15.7%)	1.78	1.39, 2.27	<0.001	1.52	1.24, 1.87	0.001
Malaria	80 (26.0%)	253 (24.8%)	1.05	0.81, 1.35	0.72	1.14	0.91, 1.43	0.33
Meningitis	2 (0.6%)	66 (6.5%)	0.12	0.03, 0.49	0.003	0.14	0.03, 0.56	0.006
Pneumonia/Lower respiratory tract infection	121 (39.3%)	315 (30.9%)	1.32	1.05, 1.66	0.017	1.22	0.99, 1.50	0.11
Sepsis	8 (2.6%)	44 (4.3%)	0.65	0.32, 1.32	0.24	0.68	0.36, 1.28	0.282
Sickle cell disease	10 (3.2%)	98 (9.6%)	0.38	0.20, 0.71	0.003	0.41	0.22, 0.75	0.006
Tuberculosis	3 (1.0%)	20 (2.0%)	0.56	0.18, 1.74	0.31	0.51	0.17, 1.51	0.25
Upper respiratory tract infection	21 (6.8%)	115 (11.3%)	0.64	0.41, 1.00	0.049	0.68	0.45, 1.02	0.085
Other	24 (7.8%)	176 (17.3%)	0.48	0.31, 0.72	<0.001	0.54	0.36, 0.79	0.004

Characteristic	Carriage, N = 329 ¹	No carriage, N = 1,066 ¹	PR ²	95% CI ²	p-value	aPR ²	95% CI ²	p-value
Length of hospital stay								
<4 days	201 (61.1%)	535 (50.6%)	REF	—	—	REF	—	—
4+ days	128 (38.9%)	522 (49.4%)	0.72	0.58, 0.90	0.004	0.69	0.57, 0.84	0.001
Any antibiotic used during admission	255 (77.5%)	996 (93.4%)	0.40	0.31, 0.51	<0.001	0.38	0.31, 0.46	<0.001
Type of antibiotic used during admission								
No antibiotics	74 (22.5%)	70 (6.6%)	REF	—	—	REF	—	—
Penicillin (no cephalosporin)	220 (66.9%)	463 (43.4%)	0.63	0.48, 0.82	<0.001	0.64	0.51, 0.79	0.002
Cephalosporin	27 (8.2%)	520 (48.8%)	0.10	0.06, 0.15	<0.001	0.10	0.07, 0.15	<0.001
Other, non-beta-lactam	8 (2.4%)	13 (1.2%)	0.74	0.36, 1.54	0.42	0.64	0.36, 1.16	0.237

¹n (%); Median (IQR)

²PR = Prevalence ratio; aPR = adjusted prevalence ratio, adjusted for age, sex, and county of enrollment; CI = Confidence interval

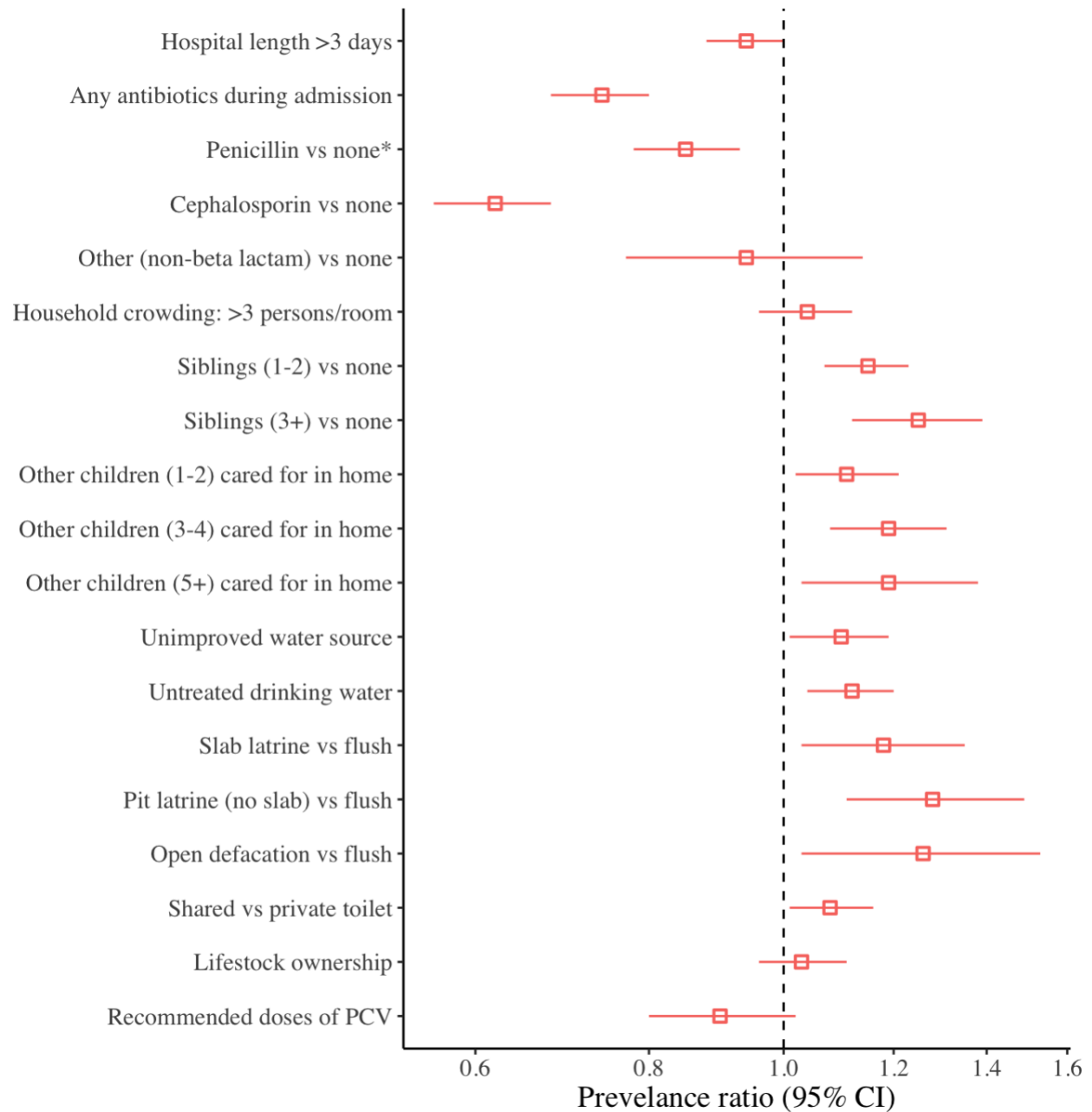
Figure 1. Risk factors for *S. pneumoniae* carriage by timepoint



PCV, pneumococcal conjugate vaccine

Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

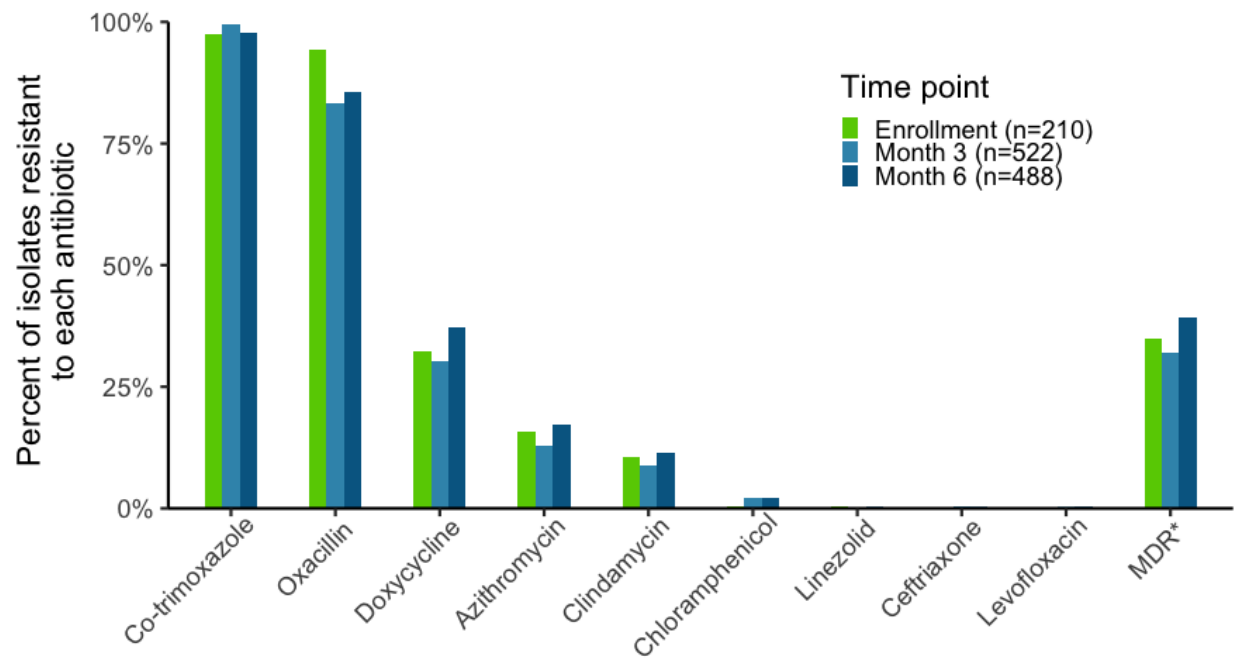
Figure 2. Risk factors for *S. pneumoniae* carriage across all timepoints



PCV, pneumococcal conjugate vaccine

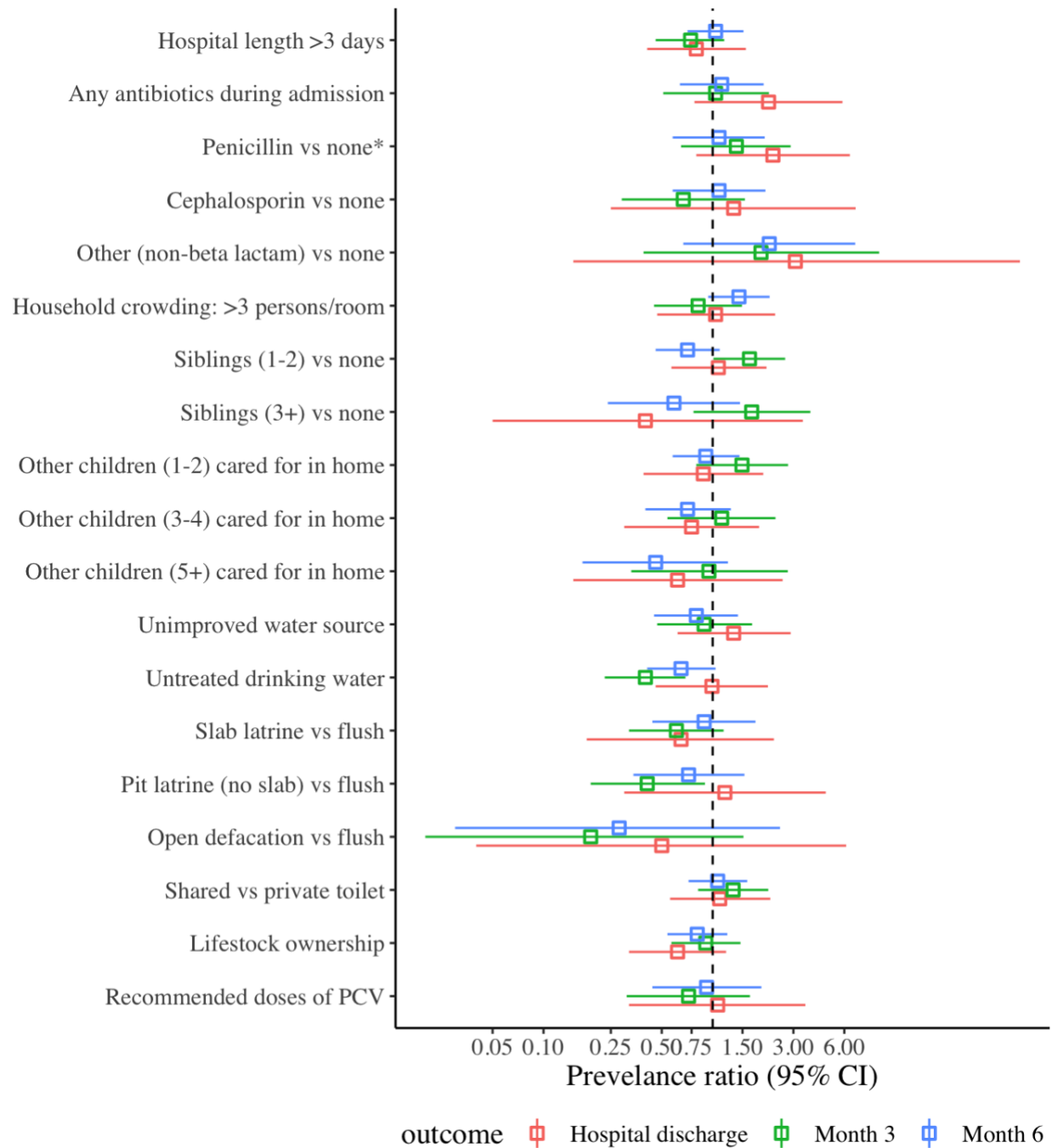
Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

Figure 3. Proportion of isolates resistant to each antimicrobial by timepoint



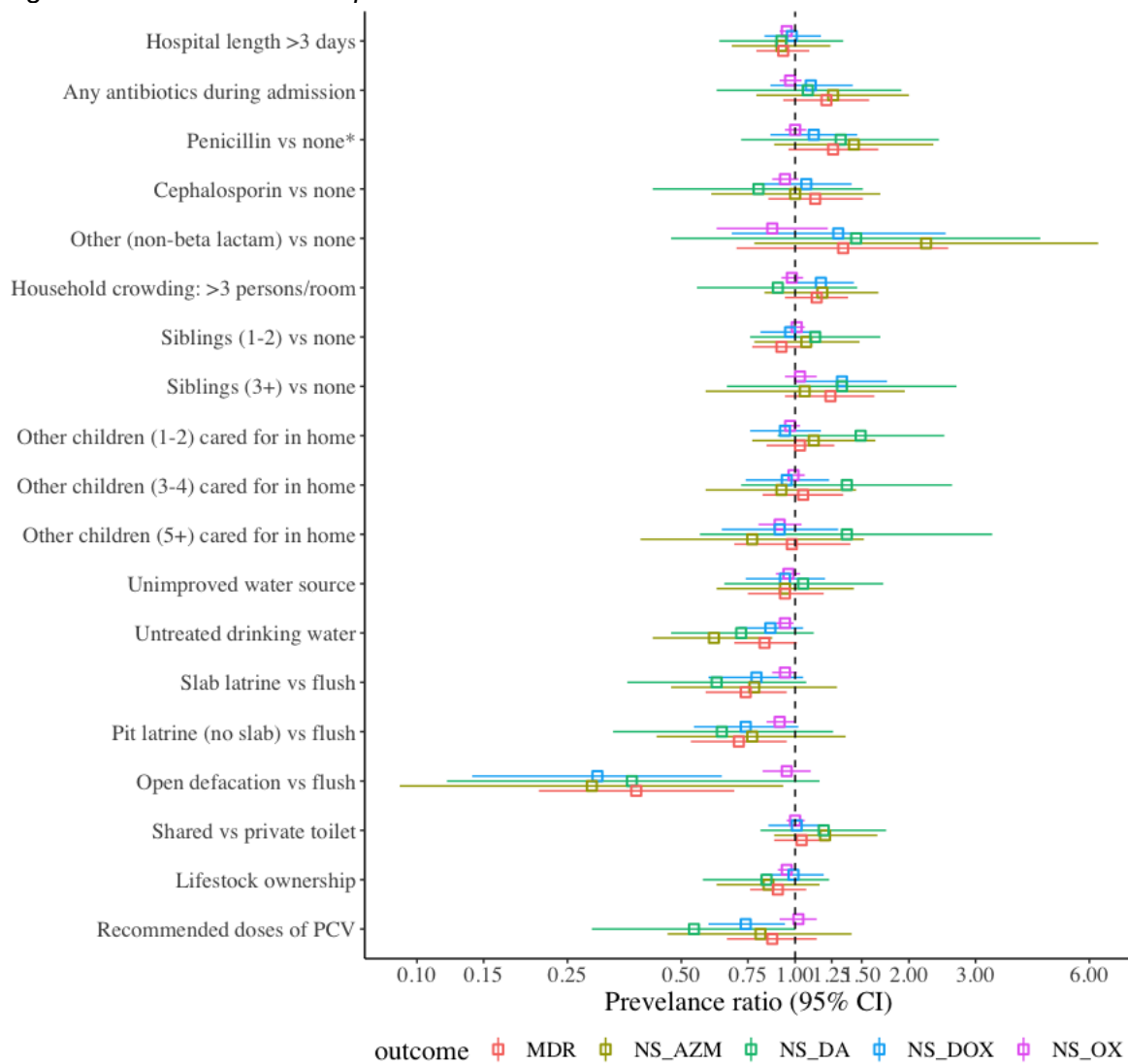
Denominators are the number of carried pneumococci isolated from children in the AMR sub-study at enrollment (n=210), month 3 (n=522), and month 6 (n=488). No resistance to linezolid was detected; Ceftriaxone (n=1); Levofloxacin (n=1)

Figure 4. Risk factors for azithromycin resistance in *S. pneumoniae*, by timepoint



Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

Figure 5. Risk factors for *S. pneumoniae* resistance to each antimicrobial



MDR, multi-drug resistant, NS_AZM, non-susceptible to azithromycin, NS_DA, non-susceptible to clindamycin, NS_DOX, non-susceptible to doxycycline, NS_OX, non-susceptible to penicillin (via oxacillin disc diffusion), PCV, pneumococcal conjugate vaccine
 Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm, and caregiver education (primary school or less)

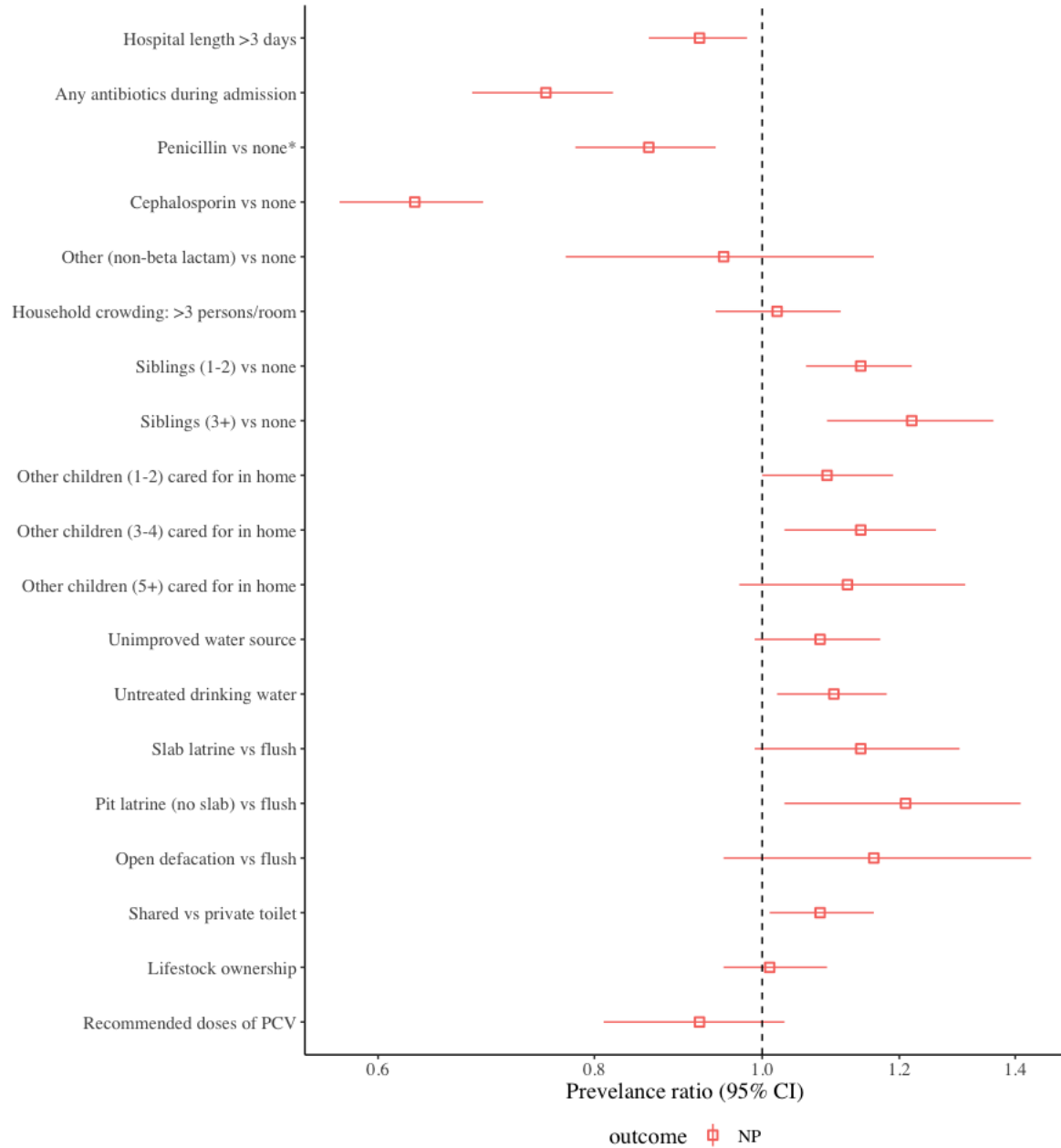
Supplementary Appendix

Table S1. Number of children missing nasopharyngeal swabs at each timepoint by reason, n=1398 children

Reason for missing swab	Enrollment	Month 3	Month 6
STGG out of stock	2		3
Nosebleed	1		1
Child moved			1
Follow-up done over phone/in other location		2	2
Caregiver refused		1	2
Child on oxygen		1	
Obstruction in child's nose		1	
Child died before follow-up visit		23	34
Did not attend follow-up within 30-day window		115	154
Total missing swab	3	146	197

STGG, skim milk–tryptone–glucose–glycerol transport medium

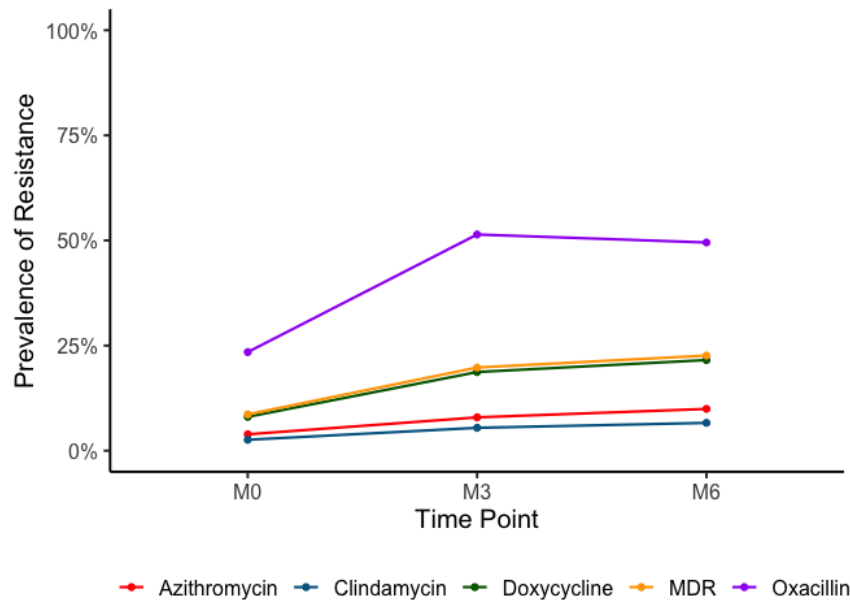
Figure S1. Sensitivity analysis: Risk factors for *S. pneumoniae* carriage across all timepoints, additionally adjusted for caregiver education



PCV, pneumococcal conjugate vaccine

Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, randomization arm, and caregiver education level

Figure S2. The prevalence of *S. pneumoniae* resistant to each antimicrobial in the 6 months following hospital discharge



Footnote: MDR = multi-drug resistant, defined as resistant to three or more antimicrobials tested.

Figure S3. Distribution of resistance counts per antimicrobial, among children with *S. pneumoniae* isolated from at least two timepoints (n=426)

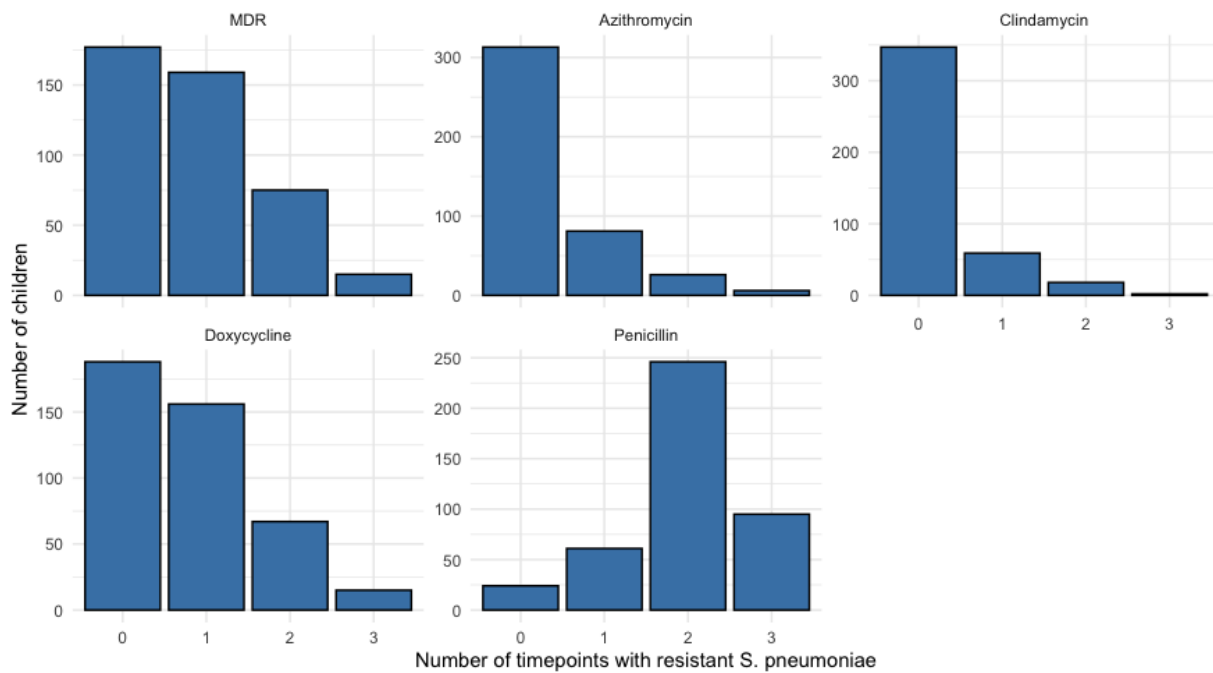
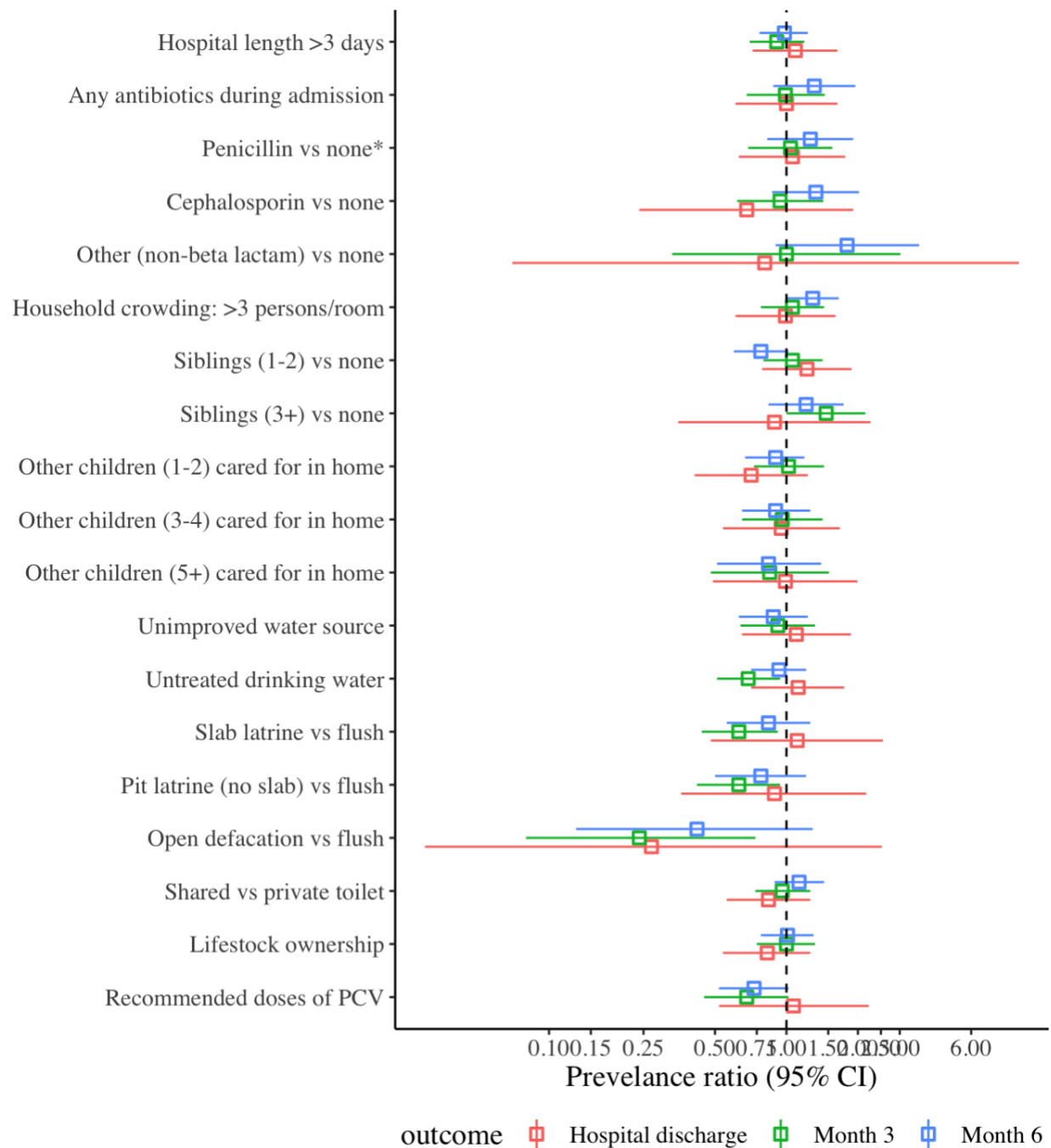


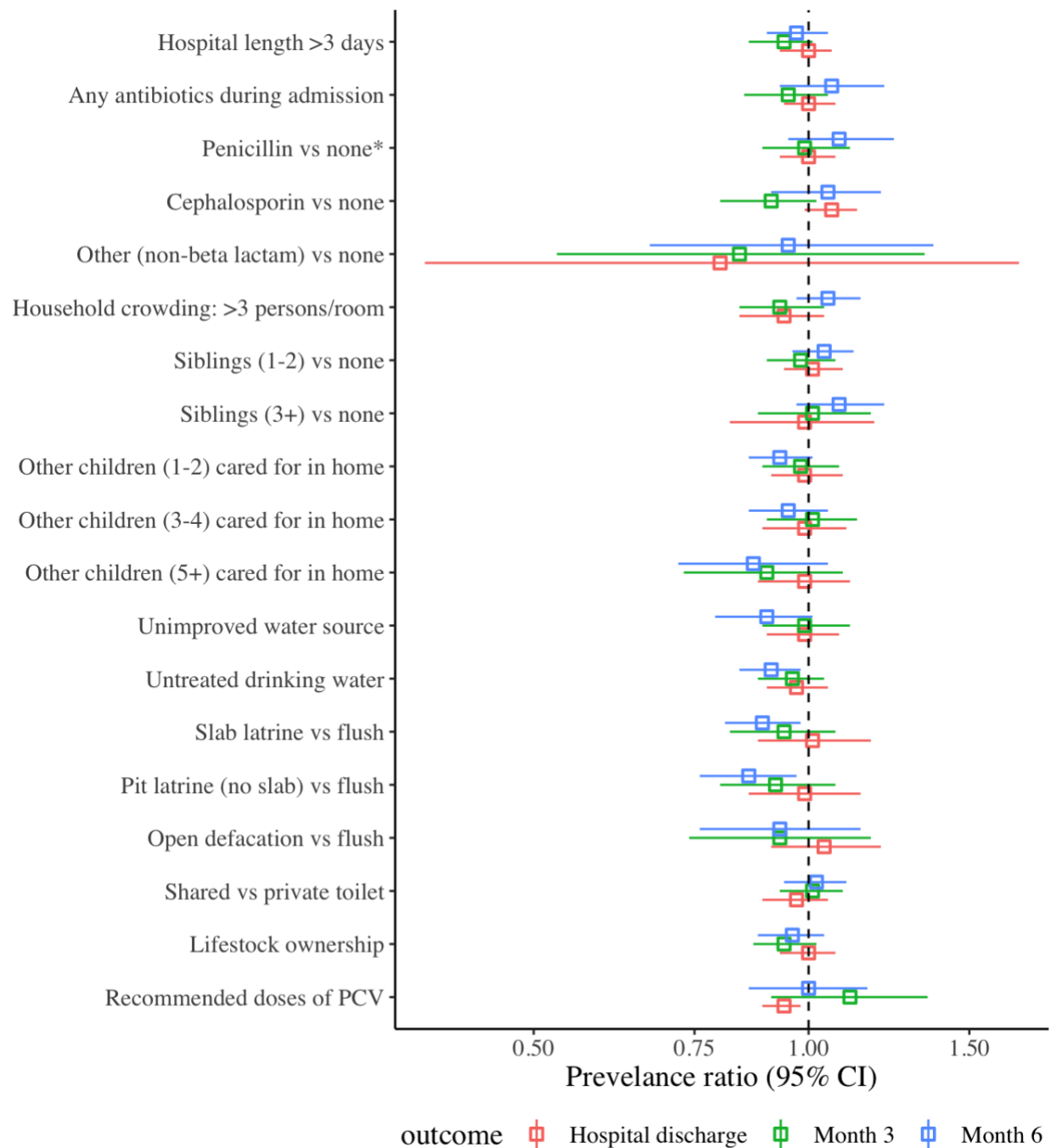
Figure S4. Risk factors for doxycycline resistance in *S. pneumoniae*, by timepoint



PCV, pneumococcal conjugate vaccine

Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

Figure S5. Risk factors for penicillin non-susceptibility* in *S. pneumoniae*, by timepoint

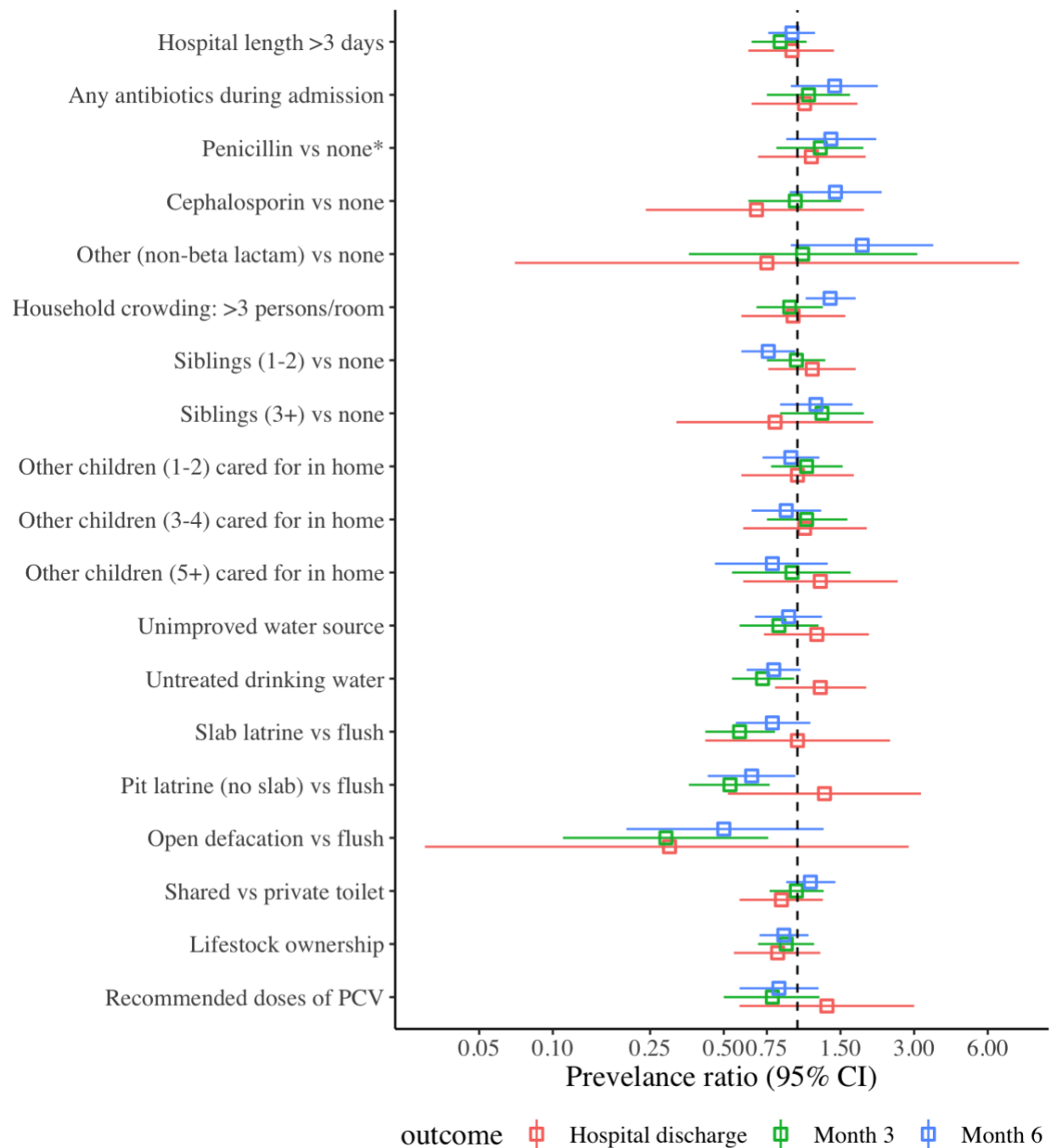


*Assessed via oxacillin disc diffusion

PCV, pneumococcal conjugate vaccine

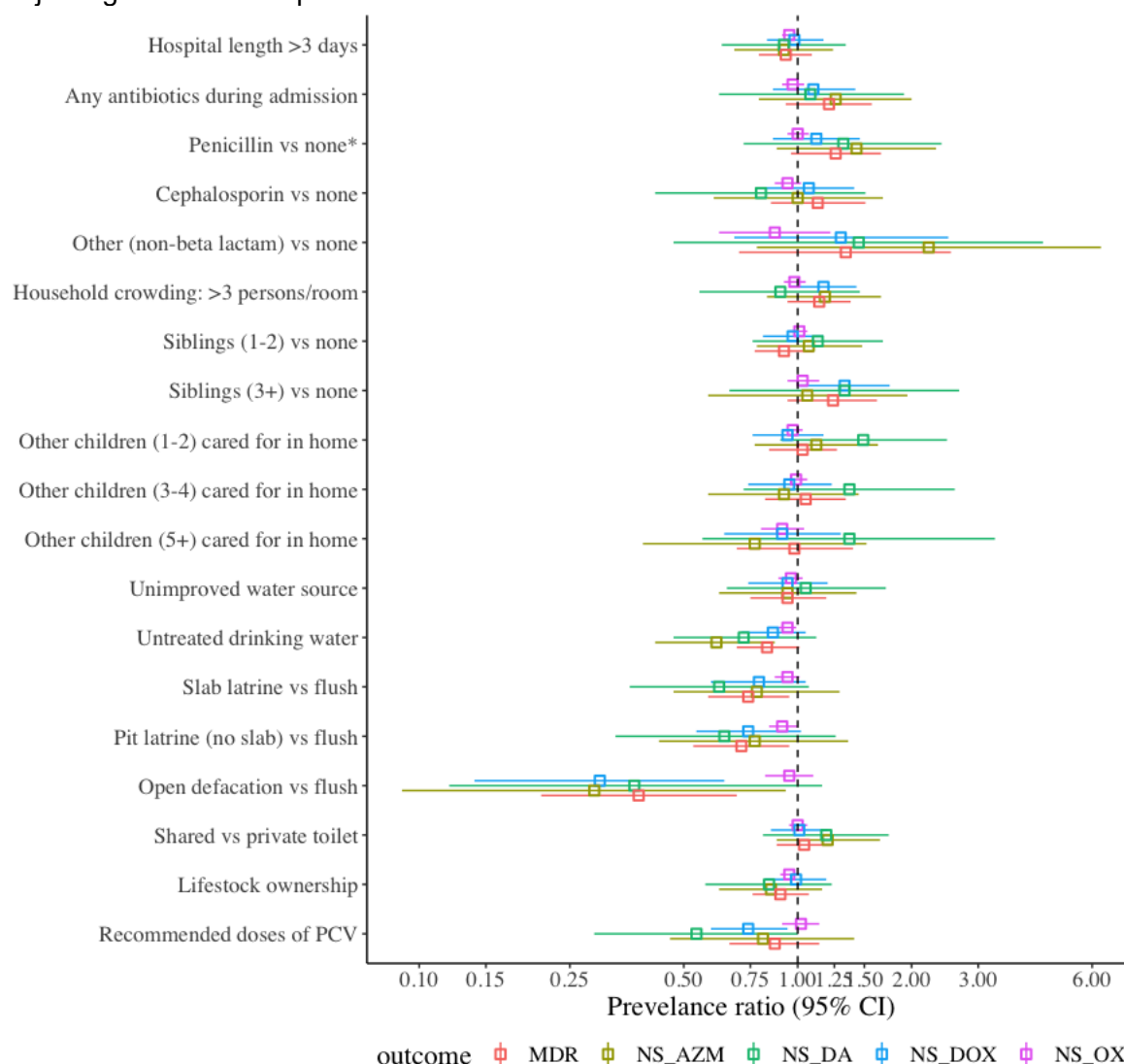
Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

Figure S6. Risk factors for multi-drug resistance (MDR) in *S. pneumoniae*, by timepoint



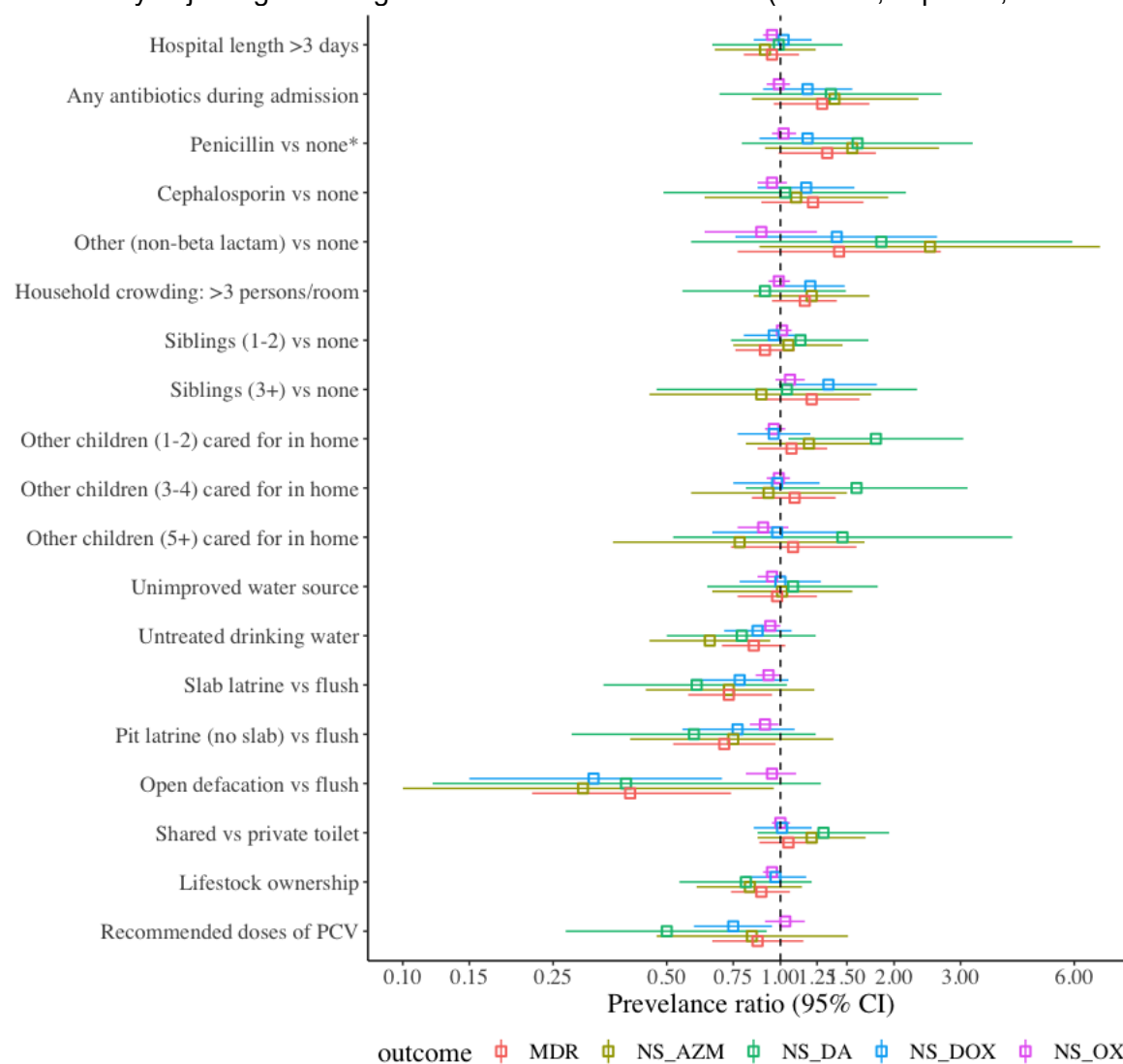
MDR was defined as resistance to three or more antimicrobial classes tested; PCV, pneumococcal conjugate vaccine; PCV, pneumococcal conjugate vaccine
 Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm

Figure S7. Sensitivity analysis: Risk factors for *S. pneumoniae* resistance to each antimicrobial adjusting for additional potential confounders: household income



MDR, multi-drug resistant, NS_AZM, non-susceptible to azithromycin, NS_DA, non-susceptible to clindamycin, NS_DOX, non-susceptible to doxycycline, NS_OX, non-susceptible to penicillin (via oxacillin disc diffusion), PCV, pneumococcal conjugate vaccine
 Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm, and household income (<5000 Kenyan Shillings)

Figure S8. Sensitivity analysis: Risk factors for *S. pneumoniae* resistance to each antimicrobial, additionally adjusting for caregiver education and HIV status (infected, exposed, or neither)



MDR, multi-drug resistant, NS_AZM, non-susceptible to azithromycin, NS_DA, non-susceptible to clindamycin, NS_DOX, non-susceptible to doxycycline, NS_OX, non-susceptible to penicillin (via oxacillin disc diffusion), PCV, pneumococcal conjugate vaccine

Estimates from a GEE model, with Poisson link, adjusted for age, sex, county of enrollment, and randomization arm, caregiver education (primary school or less), and HIV status (infected, exposed, or neither)

Chapter 4: Effect of *Streptococcus pneumoniae* carriage on risk of rehospitalization and death among Kenyan children discharged from hospital

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is a leading cause of morbidity and mortality among young children, responsible for an estimated 973,000 cases of severe disease and 166,000 deaths annually among African children under five years of age.¹ Carriage of *S. pneumoniae* in the nasopharynx is particularly common among children under five, with prevalence estimates ranging from 55% to 85% among children in Kenya.^{18,26} Although carriage is a required precursor for invasive pneumococcal disease,³ and is the source of spread from person-to-person, most individuals who carry the bacterium remain asymptomatic.

Progression from colonization to severe disease – such as pneumonia, meningitis, or septicemia – is influenced by host factors (e.g., underlying medical conditions, preceding respiratory infections, immunization status),⁶³ and bacterial characteristics including serotype, virulence genes, and potentially antimicrobial resistance (AMR). Notably, new acquisition of *S. pneumoniae* is more often implicated in the development of severe pneumococcal disease than prolonged carriage of an existing strain.^{64 65} Asymptomatic carriage can induce a state of mucosal immunity, potentially protecting against severe disease caused by new strains.

Antibiotic exposure can disrupt this balance. While antibiotics may clear susceptible colonizing pneumococcal strains, they can also create ecological space for colonization by new, potentially more virulent strains and/or resistant lineages. In settings of high infectious disease burden, antibiotic use is common, particularly in inpatient pediatric wards where missed bacterial infections could be fatal. Children who survive hospitalization and return home face a particularly high risk of mortality in the 6-months post-discharge, the reasons for which remain unknown.^{29,37,66} Disruption of the commensal microbiota, including *S. pneumoniae*,²⁹ related to inpatient antibiotic exposure may play an important role in understanding post-discharge vulnerabilities.

Although the role of AMR in the progression to pneumococcal disease remains unclear, treatment failure is a growing concern.⁶⁷ Antibiotic exposure is known to select for resistant pneumococcus strains by eliminating susceptible competitors, thereby promoting the persistence and spread of AMR.^{68,69} Moreover, horizontal gene transfer between pneumococci and other pathogens may exacerbate resistance across multiple infectious agents. While hospitalization is a known risk

factor for AMR, it is not yet clear whether post-discharge *S. pneumoniae* carriage—particularly of resistant strains—contributes to increased risk of re-hospitalization or death following discharge. We examined the risk of pneumococcal carriage at discharge on risk of death and rehospitalization in the six months following discharge. A deeper understanding of these relationships is essential for informing clinical management and guiding policy decisions for this vulnerable population.

Methods

We leveraged data from the Toto Bora trial, described in detail in Chapters 2 and 3. Briefly, children aged 1-59 months were enrolled at discharge from hospital in Kisii and Homa Bay in western Kenya and randomized to receive a 5-day course of azithromycin or similar appearing placebo. Children returned with their caregivers for scheduled follow-up visits at 3- and 6-months post-discharge.

For patients that were re-hospitalized, medical records were abstracted by study staff to ascertain antibiotic use and clinical diagnoses. The medical record was considered the primary source for re-hospitalizations when multiple sources (e.g., caregiver interview) were available. Death was ascertained by study staff through community health workers, hospital staff, medical records, or caregiver interview at follow-up visits. Date of death was ascertained by (in order of prioritization) death certificate, hospital record, or caregiver report. Final causes of death were determined by an independent adjudication committee based on medical records, verbal autopsy, caregiver report, hospital records, and death certificates. If a participant failed to return for a scheduled follow-up visit, study staff attempted to make contact via cellphone with primary caregivers. If they could not be reached by phone, study staff followed-up via a home visit. Participants were considered lost-to-follow-up if they did not attend either follow-up visit despite one month of active tracing.

All children enrolled in the trial with a nasopharyngeal swab collected at enrollment were included in our analysis. A random sample of *S. pneumoniae* isolates was selected for antimicrobial susceptibility testing (AST).

The disc diffusion method was used to assess susceptibility to azithromycin, oxacillin (as a proxy for penicillin), doxycycline, levofloxacin, co-trimoxazole, chloramphenicol, clindamycin, and linezolid, while ceftriaxone susceptibility was determined using E-TEST. Classification of isolates as susceptible, intermediate, or resistant was based on the 2024 Clinical and Laboratory

Standards Institute (CLSI) breakpoints for zone diameters or minimum inhibitory concentrations (MICs). Intermediate and resistant isolates were considered non-susceptible.

Statistical analysis

We used Cox proportional hazards regression to compare the cumulative incidence of the combined outcome of all-cause death or first re-hospitalization between the following exposure groups:

- a) children with *S. pneumoniae* carriage at discharge vs. without and
- b) children with *S. pneumoniae* isolated at discharge that was resistant (vs. susceptible) to penicillin, azithromycin, doxycycline, or that was multidrug-resistant (MDR) defined as non-susceptible to three or more antimicrobial classes tested

Secondary analyses were conducted for pneumonia/lower respiratory tract infection (LRTI)-attributable re-hospitalization or death, and for mortality and re-hospitalization as separate endpoints. Children were censored at last follow-up visit (if lost-to-follow-up). Median time to hospitalization or death and median hospital-free survival were compared between *S. pneumoniae* groupings using Kaplan-Meier survival analysis and log-rank tests. The proportional hazards assumption was checked via graphical methods including plotting Schoenfeld residuals over time. We calculated crude and adjusted hazard ratios (HR) and 95% confidence intervals (CI). Associations were considered statistically significant at an alpha of 0.05

Our primary models adjusted for randomization arm and study site as well as a minimal set of *a priori* confounders: age, sex, and caregiver education level. In sensitivity analyses, we adjusted for additional potential cofounders based on the literature and our directed acyclic graph (figure 1): household crowding (≥ 3 persons per room), HIV status (living with HIV, exposed/uninfected, and unexposed), moderate or severe acute malnutrition, discharge diagnosis of diarrhea, exclusive breastfeeding during the first 6 months of life (vs. partial or no breastfeeding), hospitalization duration greater than 3 days, and use of an improved water source, defined by caregiver reporting of water piped to the home or community, a bore hole or tube well, spring water through a pipeline, a lined well with a pump or bucket or bottled water as a primary source of water.

Given our high-dimensional data, we also conducted a sensitivity analysis controlling for confounding by constructing a propensity score for each study participant using a logistic regression model to estimate each participant's probability of carrying *S. pneumoniae*, conditional

on the individual's observed covariates. Based on published literature for potential confounders and our directed acyclic graph (figure 1), we included the following variables in our propensity score: county, age, sex, caregiver education level, exclusive breastfeeding during the first 6 months of life (vs. partial or none), moderate or severe acute malnutrition, household crowding (≥ 3 persons per room), hospitalization duration greater than 3 days, use of an improved water source, household income (>5000 ksh/day), HIV status (living with HIV, exposed/uninfected, and unexposed), number of siblings in the home, treatment of drinking water, and inpatient antibiotic use (none, penicillin, cephalosporin, other).

Overlap of the propensity score among children with and without *S. pneumoniae* and the standardized differences for potential confounders are shown in supplemental figures S1 and S2. We assessed the adequacy of the propensity score by examining covariate distributions between exposure groups after adjustment for the propensity score as a covariate and within quintile strata of the propensity score. Using the Cox model as described previously, we re-estimated the association between carriage and our outcomes adjusting for the propensity score via four methods: adjustment as a continuous variable, adjustment via quintiles of the propensity score (as an indicator variable), adjustment via splines, and inverse probability weighting.

We performed a post-hoc analysis to assess whether azithromycin-resistant pneumococcal carriage modified the effect of azithromycin for prevention of rehospitalization. In our Cox model for risk of rehospitalization, we included an interaction term between randomization arm (azithromycin or placebo) and azithromycin-resistant carriage. We calculated HRs stratified by azithromycin-resistant carriage status (yes/no), and adjusted for *a priori* confounders: county, age, sex, caregiver education, household crowding, HIV status, and acute malnutrition. All analyses were performed in R version 4.0.3

Results

Overall, 1395/1398 (99.8%) participants had a nasopharyngeal swab collected at hospital discharge and were included in our analysis. Reasons for missing swabs included out-of-stock STGG media (n=2) and nosebleed (n=1). Characteristics of the 1395 children included in the *S. pneumoniae* carriage analyses are shown in Table 1. *S. pneumoniae* was isolated from 329/1395 (23.6%) children and 210/329 (63.8%) isolates were randomly selected for AST testing. Resistance was observed to co-trimoxazole (205/210 [97.6%]), oxacillin (198/210 [94.3%]), doxycycline (68/210 [32.4%]), azithromycin (33/210 [15.7%]), clindamycin (22/210 [10.5%]),

chloramphenicol (1/210 [0.5%]) and linezolid (1/210 [0.5%]). 34.8% (73/210) of isolates were MDR.

During the 6-month follow-up, 132/1395 (9.5%) children experienced the combined outcome of death or rehospitalization; 34/1395 (2.4%) children died and 115/1395 (8.2%) were rehospitalized at least once. The incidence of the combined outcome of death or rehospitalization was similar between children with *S. pneumoniae* carriage at discharge (19.9 per 100 child-years) and children without carriage (22.0 per 100 child-years) (adjusted HR 0.84; 95% CI 0.55, 1.28; Table 2). The incidence of death in the 6-months post-discharge was lower among children with *S. pneumoniae* carriage (2.0 per 100 child years) compared to children without carriage (6.4 per 100 child-years) (adjusted HR 0.26; 95% CI 0.08, 0.86). There was no difference observed for the outcome of re-hospitalization alone (Table 2). There were seven deaths attributable to pneumonia or other LRTI and all were among children without pneumococcal carriage at hospital discharge (Table 2). There was no significant difference in the incidence of pneumonia/LRTI-attributable rehospitalization comparing those with and without carriage (adjusted HR 1.15; 95% CI 0.44, 3.02).

In sensitivity analyses, results did not meaningfully differ for the combined outcome of death or re-hospitalization, or for rehospitalization alone (Table 3). The observed inverse association between carriage and risk of all-cause death remained significant when adjusting for additional potential confounders (Table 3). While the effect sizes remained large, our propensity score adjustments (as a continuous, indicator [quintile], or spline variable) resulted in non-significant associations between carriage and death, except for inverse probability weighting, which resulted in a stronger association (adjusted HR 0.20; 95% CI 0.06, 0.66).

We could not evaluate the association of resistance with the primary outcome of death or rehospitalization combined because there were no deaths among the subset of children with *S. pneumoniae* carriage randomly selected for AST. The incidence of all-cause rehospitalization in the 6-months post-discharge was not significantly different comparing children with azithromycin-resistant *S. pneumoniae* (27.0 per 100 child years) and children with azithromycin-sensitive *S. pneumoniae* (17.8 per 100 child-years) (adjusted HR 1.29, 95% CI 0.39, 4.19). Analyses looking at resistance to other antimicrobials were similarly underpowered (Table 4). Among children randomized to azithromycin, the incidence of rehospitalization was 45.5 per 100 child-years in children with azithromycin-resistant pneumococcal carriage whereas it was 12.7 per 100 child-years in children with azithromycin-susceptible carriage (adjusted HR: 5.79; 95%CI: 1.11 30.2); in children randomized to placebo, there was no such difference (adjusted HR: 0.33; 95% CI:

0.04, 2.91) (interaction p-value=0.087, Table 5). Among children with azithromycin-sensitive carriage, the 5-day course of azithromycin was associated with a moderate, though not statistically significant, decrease in risk of rehospitalization (12.7 per 100 child-years) compared to placebo (22.9 per 100 child years; adjusted HR 0.53; 95% CI 0.18, 1.59). We were unable to examine the effect of antibiotic resistance on pneumonia/LRTI-attributable rehospitalization or death because there were few events (a total of 4 pneumonia-attributable re-hospitalizations) among children with pneumococcal carriage at baseline in the AST sample.

Discussion

In this prospective cohort study of children discharged from hospital in Kenya, the prevalence of nasopharyngeal carriage of *S. pneumoniae* at discharge was relatively low compared to studies of similarly aged children in Kenya^{18,26} likely due to high inpatient antibiotic use.⁵⁰ Pneumococcal carriage at discharge was frequently associated with antimicrobial resistance, particularly to co-trimoxazole and β -lactams, however it was not associated with an increased risk of rehospitalization or death in the six-month post-discharge period. In fact, carriage was associated with a significantly lower risk of all-cause mortality, although this association was attenuated in some sensitivity analyses adjusting for confounding.

These findings align with existing evidence suggesting that *S. pneumoniae* carriage—particularly of strains adapted to asymptomatic colonization—may reflect or contribute to mucosal immune protection.⁶⁵ Serotypes commonly associated with non-invasive carriage are typically less virulent and better adapted for long-term persistence in the nasopharynx, in contrast to serotypes that are more often implicated in invasive disease.⁷⁰ Colonization may prime mucosal immune responses and prevent acquisition of more pathogenic strains, which could partially explain the reduced mortality risk observed in our study. The fact that all pneumonia/LRTI-attributable deaths occurred among children without *S. pneumoniae* carriage at discharge further supports the possibility that sustained colonization reflects a relatively benign or even protective state. While some studies have suggested that carriage of *S. pneumoniae* may predispose individuals to respiratory infections such as respiratory syncytial virus (RSV),^{71,72} Brealey *et al.* found little evidence that carriage increased the likelihood of acquisition, but rather that RSV infection “triggered the outgrowth of *S. pneumoniae* from the resident microbiota.”⁷³

While intriguing, the inverse association between pneumococcal carriage and mortality should be interpreted cautiously. First, this may be a chance finding due to small numbers, as only three deaths occurred among children with carriage at discharge. Second, this could reflect selection

bias if children who survived the enrollment hospitalization (and were recruited into the study) are distinct with respect to pneumococcal carriage from those who died prior to discharge (and thus could not be enrolled). Finally, in adjusted models using multiple forms of propensity score control, the association was attenuated and often became non-significant, though effect sizes remained clinically meaningful (adjusted HR ~0.30). These results suggest the possibility of residual confounding—particularly by unmeasured factors such as underlying immune function or microbiome composition—that may influence both carriage and survival. Further investigation into these pathways is warranted.

Our data also support previous observations that antimicrobial resistance is highly prevalent among colonizing *S. pneumoniae* strains in high-burden settings.^{62,74} Nearly all tested isolates were resistant to co-trimoxazole and oxacillin, and over one-third were multidrug-resistant. This is consistent with widespread antibiotic use prior to and during hospitalization, which may select for resistant lineages that persist through treatment. Interestingly, pneumococcal isolates from non-invasive disease cases have been found to harbor more resistance to penicillin, cefotaxime, erythromycin, and tetracycline compared to those isolated from IPD patients.⁷⁵ Serotypes more commonly associated with asymptomatic carriage are also more likely to acquire penicillin-binding protein gene fragments from *Streptococcus mitis*, facilitating resistance development.⁷⁶

In our study, we found no overall difference in the incidence of rehospitalization between children with azithromycin-resistant versus azithromycin-sensitive *S. pneumoniae*, and our ability to evaluate associations between resistance and mortality or pneumonia-related outcomes was limited by small sample sizes and a low number of clinical events. However, among children treated with azithromycin at study onset, those carrying azithromycin-resistant *S. pneumoniae* had a significantly higher risk of rehospitalization compared to those with azithromycin-sensitive carriage. This, alongside a moderate (though non-significant) protective signal for the effect of a 5-day course of azithromycin on risk of rehospitalization among children with azithromycin-sensitive carriage, supports a role for *S. pneumoniae* in azithromycin's mortality-reducing mechanism. These findings are consistent with a recent study evaluating whether macrolide-resistance genes in fecal DNA modified azithromycin's effect in the Toto Bora trial. Mogeni *et al.* found 5-day treatment with azithromycin to be associated with a 35% decrease in the risk of death or rehospitalization among children without the *mef(A)* gene, which commonly confers resistance in streptococci.⁷⁷ The adverse impact of azithromycin on those with azithromycin-resistant bacteria may reflect expansion of resistance within the microbiota, leading to bacterial overgrowth and increased risk of invasive infection.

This study is subject to several limitations not previously discussed. First, serotyping data were not available, precluding an evaluation of serotype-specific risk, and we were unable to distinguish between persistent and newly acquired colonization. Second, only one isolate was selected for antimicrobial susceptibility testing for each child, so we are unable to detect simultaneous carriage of multiple strains, which has been recognized via deep sequencing to occur in a third of infants.⁶⁹ Third, we relied on clinical diagnosis for lower respiratory tract infections and pneumonia, as data from chest x-rays or culture were not available. Lastly, our AMR analyses were underpowered due to lower-than-expected prevalence of *S. pneumoniae*, lower-than-expected resistance to azithromycin, and higher than expected resistance to penicillin, restricting our ability to draw definitive conclusions about the clinical impact of specific resistance patterns.

Our findings have several important implications. First, carriage of *S. pneumoniae* at hospital discharge does not appear to signal heightened vulnerability in the post-discharge period and may in fact be associated with improved survival. Second, given the persistently high post-discharge mortality observed among children without *S. pneumoniae* carriage, this group may warrant particular attention in future interventions targeting vulnerable populations.⁷⁸ Lastly, azithromycin resistance correlated with worse outcomes in our cohort for children treated with azithromycin. These findings highlight the importance of appropriate treatment and suggest a role for *S. pneumoniae* in azithromycin's mortality-reducing mechanism.

Future research should aim to characterize the longitudinal dynamics of pneumococcal colonization and AMR in the post-discharge setting, ideally incorporating molecular typing. The potential role of *S. pneumoniae* in empiric azithromycin effect mechanisms needs to be confirmed in other populations, such as community settings with lower antibiotic use. Expanding sample sizes for resistance outcome analyses and incorporating metagenomic approaches could further elucidate the role of *S. pneumoniae* within the broader respiratory microbiome, and its implications for child health.

Acknowledgements

We would like to thank the children and their caregivers who participated in the Toto Bora study and the clinical and laboratory staff at each study site. We are thankful to Paul Ndungu, Gerald Okeyo and Hannah Atlas for their support in study coordination.

Tables and Figures

Figure 1. Directed acyclic graph depicting relationships between *S. pneumoniae* carriage at hospital discharge and re-hospitalization/death

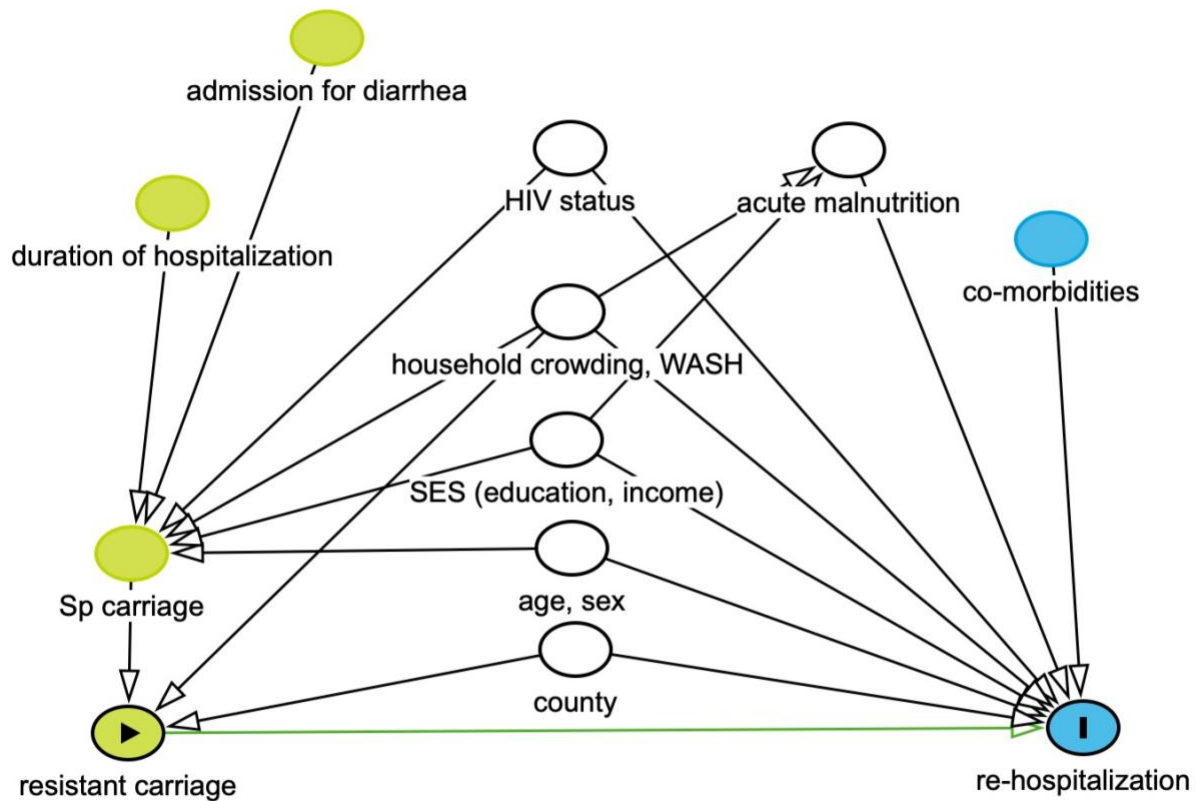


Table 1. Characteristics of study participants

Characteristic	Overall, N = 1,395¹	Carriage, N = 329¹	No carriage, N = 1,066¹
Randomization arm			
Placebo	694 (49.7%)	171 (52.0%)	523 (49.1%)
AZM	701 (50.3%)	158 (48.0%)	543 (50.9%)
County of recruiting hospital			
Homa Bay	574 (41.1%)	144 (43.8%)	430 (40.3%)
Kisii	821 (58.9%)	185 (56.2%)	636 (59.7%)
Sex (enrollment)			
Male	827 (59.3%)	197 (59.9%)	630 (59.1%)
Female	568 (40.7%)	132 (40.1%)	436 (40.9%)
Child age at enrollment (month)			
0-5	188 (13.5%)	42 (12.8%)	146 (13.7%)
6-11	277 (19.9%)	90 (27.4%)	187 (17.5%)
12-23	404 (29.0%)	122 (37.1%)	282 (26.5%)
24-59	526 (37.7%)	75 (22.8%)	451 (42.3%)
Age at enrollment	18 (9, 32)	14 (9, 22)	20 (9, 34)
Highest level of education completed by primary caregiver (at enrollment visit)			
Secondary or greater	752 (54.0%)	157 (47.9%)	595 (55.9%)
Primary school or less	641 (46.0%)	171 (52.1%)	470 (44.1%)
Caregiver reported income (at enrollment)			
Income >= 5000 ksh	454 (32.5%)	96 (29.2%)	358 (33.6%)
Income < 5000 ksh	871 (62.4%)	218 (66.3%)	653 (61.3%)
Unknown or refuse to answer	70 (5.0%)	15 (4.6%)	55 (5.2%)
Household income (at enrollment) above/ below (above/below 5000 ksh or ~\$1/day)			

Characteristic	Overall, N = 1,395¹	Carriage, N = 329¹	No carriage, N = 1,066¹
Income >= 5000 ksh	1,119 (82.7%)	274 (85.4%)	845 (81.9%)
Income < 5000 ksh	193 (14.3%)	38 (11.8%)	155 (15.0%)
Unknown or refuse to answer	41 (3.0%)	9 (2.8%)	32 (3.1%)
Household crowding (>=3 persons per room) at enrollment visit	271 (19.4%)	59 (17.9%)	212 (19.9%)
Number of children under 5 years sleeping in the home			
1	836 (59.9%)	187 (56.8%)	649 (60.9%)
2	468 (33.5%)	119 (36.2%)	349 (32.7%)
3+	91 (6.5%)	23 (7.0%)	68 (6.4%)
Number of other children under 5 years cared for by the primary caregiver			
0	360 (25.9%)	83 (25.4%)	277 (26.0%)
1-2	648 (46.6%)	145 (44.3%)	503 (47.3%)
3-4	282 (20.3%)	72 (22.0%)	210 (19.7%)
5+	101 (7.3%)	27 (8.3%)	74 (7.0%)
Water source			
Improved	1,150 (82.5%)	266 (80.9%)	884 (83.0%)
Unimproved	244 (17.5%)	63 (19.1%)	181 (17.0%)
Water treatment			
Filters, boils, chlorinates	689 (50.5%)	148 (45.7%)	541 (52.0%)
Untreated	676 (49.5%)	176 (54.3%)	500 (48.0%)
Toilet type			
Flush toilet	135 (9.7%)	23 (7.0%)	112 (10.6%)
Slab latrine	881 (63.5%)	215 (65.7%)	666 (62.8%)
Pit latrine (no slab)	318 (22.9%)	77 (23.5%)	241 (22.7%)
Open defecation	53 (3.8%)	12 (3.7%)	41 (3.9%)

Characteristic	Overall, N = 1,395¹	Carriage, N = 329¹	No carriage, N = 1,066¹
Shared Toilet			
Private	703 (50.7%)	157 (48.2%)	546 (51.5%)
Shared	630 (45.5%)	157 (48.2%)	473 (44.6%)
Open defecation	53 (3.8%)	12 (3.7%)	41 (3.9%)
Breastfeeding			
Exclusively breastfed	660 (47.3%)	155 (47.1%)	505 (47.4%)
Partially breastfed	632 (45.3%)	147 (44.7%)	485 (45.5%)
Never breastfed	24 (1.7%)	9 (2.7%)	15 (1.4%)
Unknown	79 (5.7%)	18 (5.5%)	61 (5.7%)
Child HIV status (at enrollment) combining unknown categories			
HIV unexposed	1,190 (85.3%)	270 (82.1%)	920 (86.3%)
HIV exposed, uninfected/infection status unknown	146 (10.5%)	40 (12.2%)	106 (9.9%)
HIV infected	18 (1.3%)	7 (2.1%)	11 (1.0%)
Uninfected/exposure status unknown	41 (2.9%)	12 (3.6%)	29 (2.7%)
Stunting (HAZ/LAZ < -2) at enrollment			
HAZ >= -2	1,075 (77.4%)	247 (75.3%)	828 (78.0%)
HAZ < -2	314 (22.6%)	81 (24.7%)	233 (22.0%)
Underweight (WAZ < -2) at enrollment			
WAZ >= -2	1,217 (87.3%)	278 (84.8%)	939 (88.1%)
WAZ < -2	177 (12.7%)	50 (15.2%)	127 (11.9%)
Acute malnutrition based on WLZ, MUAC (in 6+ month olds), and/or presence of edema			
None	1,263 (90.5%)	291 (88.4%)	972 (91.2%)

Characteristic	Overall, N = 1,395¹	Carriage, N = 329¹	No carriage, N = 1,066¹
MAM	75 (5.4%)	23 (7.0%)	52 (4.9%)
SAM	57 (4.1%)	15 (4.6%)	42 (3.9%)
Has recommended number of vaccines for this age (with grace of 4 weeks)	1,293 (93.1%)	299 (90.9%)	994 (93.8%)
Hospital length greater than 3 days (enrollment)	650 (46.9%)	128 (38.9%)	522 (49.4%)
Any antibiotic used during admission	1,251 (89.7%)	255 (77.5%)	996 (93.4%)
Type of antibiotic used during admission			
Any Macrolide	31 (2.2%)	1 (0.3%)	30 (2.8%)
Penicillin	837 (60.0%)	227 (69.0%)	610 (57.2%)
Amoxicillin	41 (2.9%)	11 (3.3%)	30 (2.8%)
Ampicillin	2 (0.1%)	0 (0.0%)	2 (0.2%)
Augmentin/co-amoxiclav	3 (0.2%)	0 (0.0%)	3 (0.3%)
Cefuroxime	16 (1.1%)	1 (0.3%)	15 (1.4%)
Ceftriaxone	538 (38.6%)	26 (7.9%)	512 (48.0%)
Tetracycline	6 (0.4%)	1 (0.3%)	5 (0.5%)
Gentamicin	755 (54.1%)	205 (62.3%)	550 (51.6%)
Ciprofloxacin	13 (0.9%)	2 (0.6%)	11 (1.0%)
Metronidazole	73 (5.2%)	17 (5.2%)	56 (5.3%)
Beta-lactam category of antibiotic used during admission ²			
No antibiotic	144 (10.3%)	74 (22.5%)	70 (6.6%)
Penicillin (no cephalosporin)	683 (49.0%)	220 (66.9%)	463 (43.4%)
Any cephalosporin	547 (39.2%)	27 (8.2%)	520 (48.8%)
Other, non-beta-lactam	21 (1.5%)	8 (2.4%)	13 (1.2%)
Discharge diagnosis			

Characteristic	Overall, N = 1,395¹	Carriage, N = 329¹	No carriage, N = 1,066¹
Anemia	177 (13.3%)	44 (14.3%)	133 (13.1%)
Diarrhea	250 (18.8%)	90 (29.2%)	160 (15.7%)
Malaria	333 (25.1%)	80 (26.0%)	253 (24.8%)
Meningitis	68 (5.1%)	2 (0.6%)	66 (6.5%)
Pneumonia	436 (32.9%)	121 (39.3%)	315 (30.9%)
Sepsis	52 (3.9%)	8 (2.6%)	44 (4.3%)
Sickle cell disease	108 (8.1%)	10 (3.2%)	98 (9.6%)
Tuberculosis	23 (1.7%)	3 (1.0%)	20 (2.0%)
URTI	136 (10.2%)	21 (6.8%)	115 (11.3%)
Other	200 (15.1%)	24 (7.8%)	176 (17.3%)
Any antibiotic prescribed at discharge	865 (62.0%)	190 (57.8%)	675 (63.3%)

¹N (%) or median (IQR)

²Mutually exclusive categories: “Any cephalosporin” includes all children who had ceftriaxone or cefuroxime; “Penicillin” includes penicillin, amoxicillin, ampicillin and Augmentin, but no ceftriaxone or cefuroxime; “Other, non-beta-lactam” includes children who did not have any of these antibiotics.

Table 2. Effect of *S. pneumoniae* carriage at discharge on time to death or first rehospitalization during 6-month follow-up (n=1395)

Outcome	<i>S. pneumoniae</i> carriage + (n=329)			No <i>S. pneumoniae</i> carriage (n=1066)			Hazard ratio (95% CI)	P value
	N	Child-years of follow-up	Incidence rate, per 100 child years	N	Child-years of follow-up	Incidence rate, per 100 child years		
All-cause								
Death or rehospitalization	29	146	19.9	103	498	22.0	0.84 (0.55, 1.28)	0.42
Death	3	152	2.0	31	487	6.4	0.26 (0.08, 0.86)	0.03
First-rehospitalization	27	146	18.5	88	469	18.8	0.93 (0.60, 1.44)	0.73
LRTI-attributable								
Death or rehospitalization	7	146	4.8	21	480	4.2	0.92 (0.36, 2.34)	0.86
Death	0	152	0.0	7	487	1.4	undefined	
First-rehospitalization	7	146	4.8	17	469	3.6	1.15 (0.44, 3.02)	0.77

CI, confidence interval; LRTI, lower respiratory tract infection/pneumonia

Hazard ratios from Cox proportional hazard regression models adjusted for county, age, sex, and caregiver education.

Table 3. Sensitivity analysis: Effect of *S. pneumoniae* carriage at discharge on time to all-cause death or first rehospitalization during 6-month follow-up, by confounder adjustment method

Model	Death or rehospitalization		Death		Rehospitalization	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Crude (n=1395)	0.91 (0.60, 1.37)	0.638	0.31(0.09, 1.02)	0.053	0.99 (0.64, 1.52)	0.951
Min. adjusted ¹ (n=1393)	0.84 (0.55, 1.28)	0.420	0.26 (0.08, 0.86)	0.030	0.93 (0.60, 1.44)	0.731
More adjusted ² (n=1393)	0.82 (0.54, 1.25)	0.366	0.24 (0.07, 0.79)	0.019	0.91 (0.59, 1.42)	0.690
Fully adjusted ³ (n=1321)	0.99 (0.64, 1.53)	0.971	0.29 (0.09, 0.99)	0.049	1.06 (0.67, 1.67)	0.817
Crude, PS subset (n=1242)	0.93 (0.60, 1.44)	0.746	0.38 (0.12, 1.26)	0.114	0.99 (0.63, 1.57)	0.974
PS adjusted (n=1242)	0.97 (0.60, 1.57)	0.909	0.34 (0.10, 1.20)	0.095	0.99 (0.63, 1.57)	0.974
PS quintiles (n=1242)	0.95 (0.59, 1.54)	0.850	0.35 (0.10, 1.22)	0.100	1.06 (0.64, 1.75)	0.829
PS splines (n=1242)	0.99 (0.62, 1.6)	0.987	0.33 (0.09, 1.2)	0.095	1.08 (0.65, 1.8)	0.750
IPW by PS (n=1242)	0.90 (0.50, 1.62)	0.734	0.20 (0.06, 0.66)	0.009	1.0 (0.54, 1.837)	1.000

HR, Hazard ratio; CI, confidence interval; PS, propensity score; IPW, inverse probability weighting. Bolded values were statistically significant at alpha 0.05.

¹Primary model: adjusted for age, sex, enrollment site, randomization arm, and caregiver education.

²Adjusted for age, sex, enrollment site, randomization arm, caregiver education, household crowding, HIV status, and acute malnutrition

³Adjusted for age, sex, enrollment site, randomization arm, and caregiver education, household crowding, HIV status, acute malnutrition, hospital length, exclusive breastfeeding, discharge diagnosis of diarrhea

Table 4. Effect of antibiotic resistance in *S. pneumoniae* on time-to-first rehospitalization (n=210)

	Antibiotic-resistant carriage			Antibiotic-sensitive carriage				
Antibiotic tested	events/ N	Child-years of follow-up	Incidence rate, per 100 child years	events /N	Child-years of follow-up	Incidence rate, per 100 child years	Hazard ratio (95% CI)	P-value
Azithromycin	4/33	14.8	27.0	14/177	78.6	17.8	1.29. (0.39, 4.19)	0.667
Oxacillin	18/198	87.8	20.5	0/12	5.66	0.0	undefined	
Doxycycline	4/68	30.4	13.2	14/142	63.0	22.2	0.50 (0.16, 1.60)	0.244
MDR ¹	4/73	33	12.1	14/137	60.4	23.2	0.45 (0.14, 1.44)	0.179

Incidence rate of first re-hospitalization

Hazard ratios from Cox proportional hazard regression models adjusted for county, age, sex, caregiver education, household crowding, HIV status, and acute malnutrition

MDR, multidrug-resistant, defined as resistance to three or more antimicrobial classes tested.

¹For MDR, the incidence of hospitalization among “antibiotic-sensitive carriage” includes children with non-resistant strains and those with *S. pneumoniae* resistant to fewer than 3 antimicrobial classes

Table 5. Impact of azithromycin-resistant pneumococcal carriage on the effect of azithromycin (vs placebo) for prevention of death or rehospitalization: post-hoc analysis

Randomization arm	Azithromycin-resistant carriage (n=33)			Azithromycin-sensitive carriage (n=177)			Hazard ratio (95% CI)	P-value
	Events /N	Child-years of follow-up	Incidence rate, per 100 child years	Events /N	Child-years of follow-up	Incidence rate, per 100 child years		
Azithromycin-treated children (n=104)	3/15	6.60	45.5	5/89	39.36	12.7	5.79 (1.11, 30.17)	0.037
Placebo-treated children (n=106)	1/18	8.22	12.2	9/88	39.24	22.9	0.33 (0.04, 2.91)	0.318

Hazard ratios from Cox proportional hazards regression models stratified by randomization arm (azithromycin or placebo), adjusted for county, age, sex, caregiver education, household crowding, HIV status, and acute malnutrition.

Note: In the combined (unstratified) model, the p-value for the interaction between randomization arm*azithromycin resistance p=0.087

Supplementary Appendix

Figure S1. Overlap in propensity scores by exposure (*S. pneumoniae* carriage) status

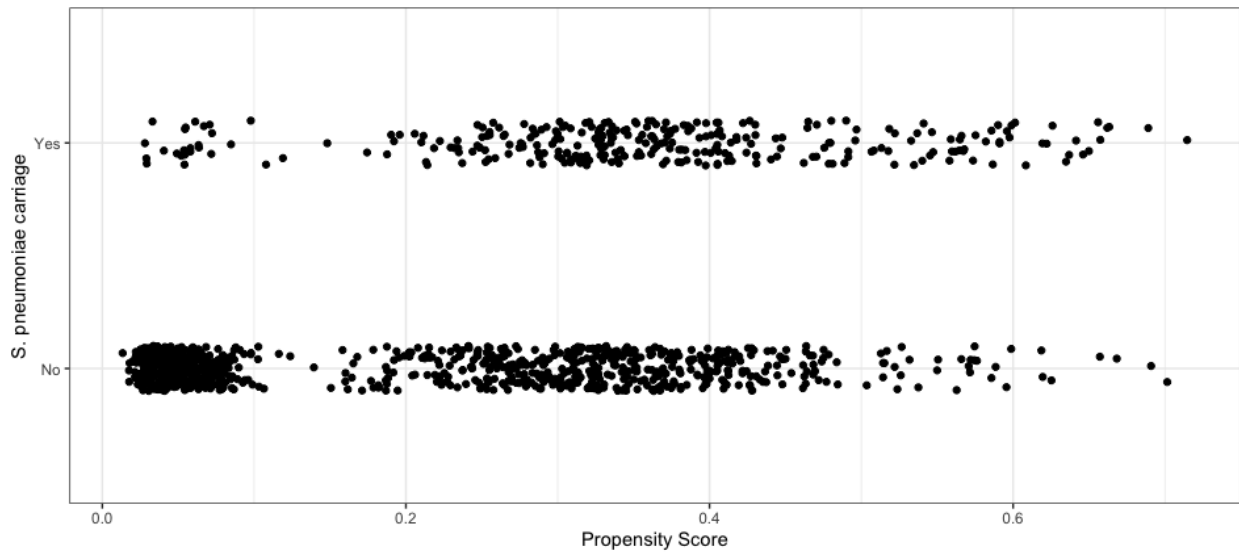
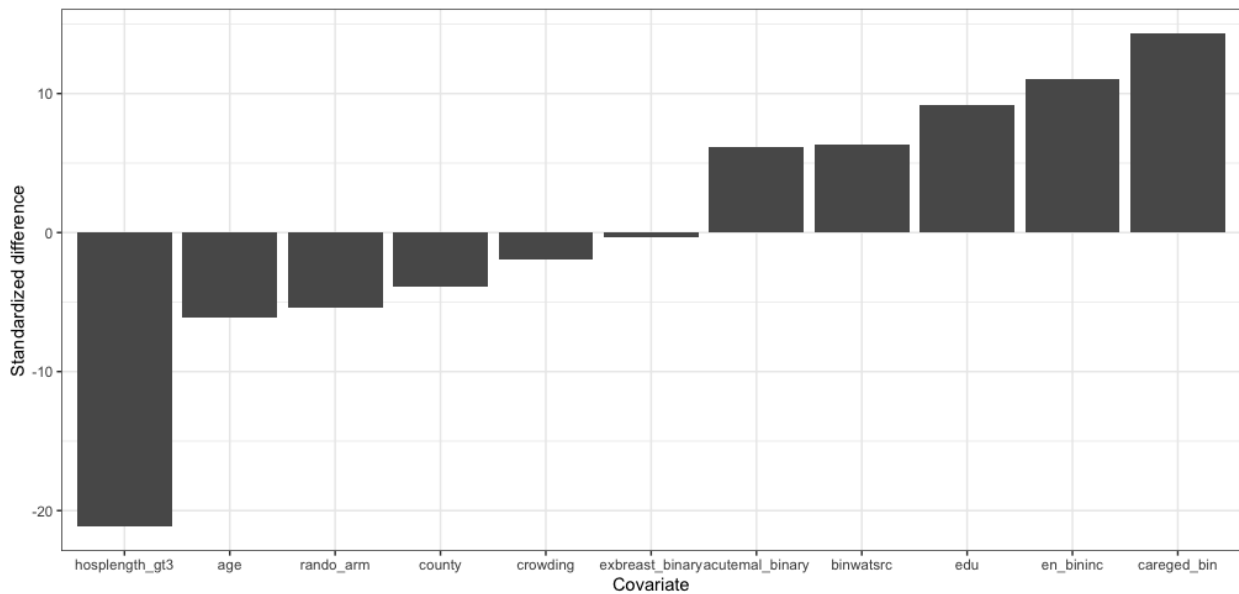


Figure S2. Distribution of standardized differences of confounders between children with and without pneumococcal carriage at discharge (prior to propensity score confounder control)



Chapter 5: Conclusion

This dissertation explored the epidemiology of carriage and antimicrobial resistance (AMR) in *Streptococcus pneumoniae* among Kenyan children during the critical six-month period following hospital discharge. Drawing on longitudinal data from the Toto Bora trial, the three studies presented here provide new insights into the effects of azithromycin treatment, the environmental and clinical determinants of pneumococcal carriage and resistance, and the prognostic significance of colonization in the post-discharge period. Together, these studies provide a nuanced understanding of pneumococcal dynamics in a high-risk pediatric population and offer evidence for policymakers and clinicians considering the empiric use of azithromycin; water, sanitation, and hygiene (WASH) interventions; and post-discharge care strategies.

Chapter 2 assessed whether a 5-day course of azithromycin at discharge influenced pneumococcal carriage or resistance. Despite high adherence and a well-powered design, the intervention did not significantly alter the prevalence of carriage or the proportion of resistant isolates at 3- or 6-months post-discharge. These findings suggest that in settings with high baseline antibiotic exposure—where nearly 90% of children received antibiotics during hospitalization—the marginal impact of additional azithromycin may be limited. This contrasts with findings from mass drug administration (MDA) trials, where community-wide use of azithromycin has been associated with both mortality reduction and increased resistance.^{31,32} The lack of effect observed here may reflect the transient nature of azithromycin’s selective pressure and the rapid reacquisition of community strains following discharge.⁴⁹ These findings are relevant for clinicians and policy makers from similar settings considering post-discharge interventions for mortality prevention: azithromycin-resistance may be of lesser concern when azithromycin is targeted to high-risk groups compared to community-wide distribution.

Chapter 3 identified key risk factors for pneumococcal carriage and resistance over time. While inpatient antibiotic use and longer hospital stays were associated with reduced carriage at discharge, these associations diminished by 3 and 6 months, when household-level exposures became more influential. Children living in homes with unimproved water sources, untreated drinking water, and shared sanitation facilities were more likely to carry pneumococcus. Resistance to doxycycline was more common among children with multiple young siblings, while receipt of the recommended doses of pneumococcal conjugate vaccine (PCV) was associated with lower resistance to doxycycline and clindamycin. These findings highlight the importance of

environmental determinants in shaping post-discharge colonization and support the role of PCVs in mitigating AMR.

Chapter 4 examined whether pneumococcal carriage at discharge was associated with adverse clinical outcomes. Carriage was not associated with increased risk of rehospitalization or death. In fact, children who carried *S. pneumoniae* at discharge had a significantly lower risk of all-cause mortality, even after adjusting for key confounders, though the number of events was small. This finding may reflect the immunological benefits of sustained colonization with less virulent strains, which can induce mucosal immunity and potentially protect against acquisition of more pathogenic serotypes.^{3,65} Azithromycin-resistant pneumococcal carriage correlated with worse outcomes in our cohort for children treated with azithromycin. Further, azithromycin treatment was associated with better outcomes among those with azithromycin-sensitive carriage. These findings highlight the importance of appropriate antibiotic therapy and suggest a role for *S. pneumoniae* in azithromycin's mortality-reducing mechanism.

Implications for Public Health

The findings from this dissertation have several important implications for public health, particularly in low-resource settings:

1. **Azithromycin mortality mechanisms:** The lack of an observed effect of azithromycin on pneumococcal carriage or AMR is consistent with the absence of an effect on mortality and rehospitalization in the primary trial and suggests that routine post-discharge antibiotic prophylaxis may not be warranted in high antibiotic-use settings. Our findings support the role of *S. pneumoniae* in azithromycin's previously observed mortality-reducing effects. Policymakers should consider the local context of antibiotic exposure and resistance patterns when evaluating such interventions.
2. **Strengthening WASH infrastructure:** The strong association between household environmental conditions and pneumococcal carriage underscores the need for integrated approaches to AMR prevention. Investments in clean water, sanitation, and hygiene (WASH) infrastructure could reduce transmission of resistant pathogens and improve child health outcomes beyond the hospital setting.²¹
3. **Leveraging vaccines for AMR control:** The protective association between PCV and reduced resistance to certain antibiotics reinforces the broader role of vaccines in AMR

mitigation. Sustaining high PCV coverage and monitoring for serotype replacement remain essential components of national immunization programs.⁵⁸

4. **Refining post-discharge risk stratification:** The finding that pneumococcal carriage at discharge was not predictive of adverse outcomes challenges its utility as a clinical marker of vulnerability. Future efforts to identify high-risk children should incorporate a broader range of clinical, environmental, and social indicators.
5. **Enhancing AMR surveillance:** The high prevalence of multidrug-resistant pneumococci at discharge and throughout the 6 months after highlights the need for robust AMR surveillance systems in pediatric inpatient settings. These systems should be linked to antimicrobial stewardship programs and community-level interventions to reduce disease transmission and unnecessary antibiotic use.

Future Directions

Future research should build on these findings by incorporating pneumococcal serotype data. Linking serotypes to antimicrobial resistance and clinical outcomes is essential for evaluating current vaccines and guiding the expansion of vaccine coverage to include emerging high-risk serotypes. To better understand azithromycin's mortality-reducing mechanism, metagenomic approaches should be used to explore its impact on the microbiome and immune modulation. These techniques could also clarify whether the *mef(A)* resistance gene found in fecal DNA is associated with *S. pneumoniae*. Further studies should evaluate optimal timing and dosing of azithromycin for post-discharge mortality prevention, ideally incorporating a washout period and potentially using a monthly regimen during the six-month vulnerability period following hospitalization. Finally, the lack of tools to identify children at risk of post-discharge mortality remains a major barrier. Recent efforts to develop predictive algorithms should be validated in Kenya and other settings and integrated into hospital discharge workflows to improve post-discharge outcomes.

Conclusion

This body of work contributes to a deeper understanding of pneumococcal epidemiology in the post-discharge period—a time of heightened vulnerability for children in low-resource settings. The findings emphasize the importance of context-specific strategies that address both clinical and environmental drivers of AMR. By integrating microbiological surveillance, vaccine policy, and WASH interventions, health systems can more effectively reduce the burden of

pneumococcal disease and curb the spread of antimicrobial resistance. Future research should build on these findings by incorporating molecular typing, expanding AMR surveillance, and evaluating the long-term impact of integrated interventions on child health and AMR.

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