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# Impact of Intermittent Preventive Treatment in Pregnancy with Azithromycin-Containing Regimens on Maternal Nasopharyngeal Carriage and Antibiotic Sensitivity of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*: a Cross-Sectional Survey at Delivery

**Holger W. Unger,[a,b] Celestine Aho,[b] Maria Ome-Kaius,[b] Regina A. Wangnapi,[b] Alexandra J. Umbers,[a,b] Wanda Jack,[b] Alice Lafana,[b] Audrey Michael,[b†] Sarah Hanieh,[a] Peter Siba,[b] Ivo Mueller,[c,d,e] Andrew R. Greenhill,[b,f] Stephen J. Rogerson[a]**

Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Parkville, Australia[a]; Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea[b]; Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia[c]; Barcelona Centre for International Health Research (CRESIB), Hospital Clínic-Universitat de Barcelona, Barcelona, Spain[d]; Department of Medical Biology, The University of Melbourne, Parkville, Australia[e]; School of Applied and Biomedical Sciences, Federation University, Churchill, Australia[f]

**Sulfadoxine-pyrimethamine (SP) plus azithromycin (AZ) (SPAZ) has the potential for intermittent preventive treatment of malaria in pregnancy (IPTp), but its use could increase circulation of antibiotic-resistant bacteria associated with severe pediatric infections. We evaluated the effect of monthly SPAZ-IPTp compared to a single course of SP plus chloroquine (SPCQ) on maternal nasopharyngeal carriage and antibiotic susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* at delivery among 854 women participating in a randomized controlled trial in Papua New Guinea. Serotyping was performed, and antibiotic susceptibility was evaluated by disk diffusion and Etest. Potential risk factors for carriage were examined. Nasopharyngeal carriage at delivery of *S. pneumoniae* (SPAZ, 7.2% [30/418], versus SPCQ, 19.3% [84/436]; $P < 0.001$) and *H. influenzae* (2.9% [12/418] versus 6.0% [26/436], $P = 0.028$), but not *S. aureus*, was significantly reduced among women who had received SPAZ-IPTp. The number of macrolide-resistant pneumococcal isolates was small but increased in the SPAZ group (13.3% [4/30], versus SPCQ, 2.2% [2/91]; $P = 0.033$). The proportions of isolates with serotypes covered by the 13-valent pneumococcal conjugate vaccine were similar (SPAZ, 10.3% [3/29], versus SPCQ, 17.6% [16/91]; $P = 0.352$). Although macrolide-resistant isolates were rare, they were more commonly detected in women who had received SPAZ-IPTp, despite the significant reduction of maternal carriage of *S. pneumoniae* and *H. influenzae* observed in this group. Future studies on SPAZ-IPTp should evaluate carriage and persistence of macrolide-resistant *S. pneumoniae* and other pathogenic bacteria in both mothers and infants and assess the clinical significance of their circulation.**

Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) can prevent low birth weight (LBW; <2,500 g) and reduces infant morbidity and mortality in resource-limited countries (1, 2). However, high-level resistance of *Plasmodium falciparum* to SP, recently observed in East Africa, may render IPTp-SP clinically inefficacious (3–5). The development and evaluation of new IPTp regimens are therefore of utmost importance (6, 7).

Azithromycin (AZ) is a broad-spectrum macrolide (azalide) antibiotic with favorable antimalarial properties and a good safety profile in pregnancy (8, 9). IPTp with SPAZ prevented LBW and preterm delivery in two clinical trials (10, 11), and confounding may in part explain the observation of no effect in the third (6, 12); this warrants its further evaluation as a potential alternative to SP-IPTp.

An integral part of such evaluation is to screen for adverse consequences, including the selection of drug-resistant *Streptococcus pneumoniae* (13), a principal cause of meningitis and respiratory infections in infants (14). Mass administration (MDA) of AZ to children has been associated with increased nasopharyngeal carriage of macrolide-resistant pneumococci, particularly when MDA was given at 6- to 12-monthly intervals (15–20). However, this is not a unanimous finding (21) and may in part depend on the prevalence of resistant isolates at baseline (15–18). Longitudinal studies of carriage and resistance patterns indicate that in the event of MDA-driven temporary expansion of macrolide-resistant isolates, a decrease in their prevalence tends to occur 12 to 24 months after the most recent treatment (19, 20).

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Address correspondence to Stephen J. Rogerson, sroger@unimelb.edu.au.

† Deceased.

H.W.U. and C.A. contributed equally to this work.

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In addition to macrolide resistance, MDA has also been associated with selection of β-lactam-resistant pneumococci (22) and causes serotype replacement, which is probably temporary (15); the consequences for clinical response in severe pediatric infections remain to be elucidated (20). Furthermore, it is unclear whether MDA preferentially selects for nonvaccine serotypes, whose role in severe infection requires further investigation (15). Such potential drawbacks will need to be carefully weighed against potential benefits of MDA, or SPAZ-IPTp, which include reductions in child and adult morbidity and mortality (23). Also of interest is the impact of AZ administration on carriage and resistance patterns in other important pathogenic bacteria, e.g., *Staphylococcus aureus* and *Haemophilus influenzae*. Frequent or long-term administration of AZ can alter the carriage of these two organisms (24), and *S. aureus* commonly displays antimicrobial resistance.

As such, we studied the impact of IPTp with SPAZ on maternal nasopharyngeal carriage and antibiotic susceptibility of *S. pneumoniae*, *H. influenzae*, and *S. aureus* at delivery in Papua New Guinea (PNG).

## MATERIALS AND METHODS

**Setting.** Between 2009 and 2012, pregnant women in antenatal clinics in Madang Province, PNG, were recruited to a randomized controlled trial ("IPTp trial") investigating the safety and efficacy of IPTp with AZ (2 tablets [500 mg] twice daily for 2 days; Pfizer, USA) and SP (3 tablets [500/25 mg]; Micro Labs Ltd., India) given up to three times to prevent LBW (clinical trial registration no. NCT01136850) (11). The control arm consisted of SP (3 tablets, 500/25 mg) plus chloroquine (CQ) (3 or 4 150-mg tablets, daily for 3 days; Medopharm, India) given at first antenatal visit, followed by two courses of placebo (J. Bonal S.A., Spain). The first dose of each course was taken under direct supervision by clinical staff, with the remainder to be taken at home. Patient-reported adherence to subsequent doses of each course was high (~98%) (11).

In PNG, macrolide use is confined to management of preterm premature rupture of membranes and sexually transmitted infections (STIs) (25), which are common (26). *H. influenzae* type b (Hib) vaccination, but not pneumococcal vaccine, has been routinely offered since 2008 (27).

Maternal pneumococcal carriage data for PNG are lacking. Carriage is common in infants in the PNG highlands (28, 29). Pneumococcal isolates from children presenting with bacterial meningitis to the Madang Provincial Hospital (2006 to 2010) were sensitive to erythromycin (E) and ceftriaxone, yet some exhibited reduced or no susceptibility to penicillin, chloramphenicol (CHL), co-trimoxazole (COT), and tetracycline (TET) (L. Manning, personal communication) (30). Drug resistance was very common among invasive *H. influenzae* isolates, prompting a change in PNG national treatment policy (31). The same study identified nonvaccine isolates of *S. pneumoniae* as a cause of severe pediatric illness: two invasive pneumococcal isolates were nonserotypeable (NST), and nine and six were not covered by the 13-valent pneumococcal conjugate vaccine (PCV-13) and the 23-valent pneumococcal polysaccharide vaccine (PPV-23), respectively (L. Manning, personal communication). Previous and ongoing surveillance of pediatric meningitis cases in the highlands of PNG reveals the important role of non-PCV-13 strains in disease: approximately one-third of culture-confirmed cases of pneumococcal meningitis were caused by nonvaccine types 2, 24, and 46 (A. R. Greenhill, S. Phuanukoonnon, A. Michael, M. Yoannes, T. Orami, H. Smith, D. Murphy, C. Blyth, J. Reeder, P. Siba, W. Pomat, and D. Lehmann, submitted for publication). *S. pneumoniae* and Hib were the most important bacteria causing meningitis. While resistance to penicillin was common in *S. pneumoniae* (21.5% of isolates), none of the isolates tested was resistant to erythromycin (Greenhill et al., submitted). Methicillin-resistant *S. aureus* was observed among septic surgical patients at the referral hospital in the study area (32).

**TABLE 1** Characteristics of study participants, by treatment groups

| Characteristic | No. (%) SPCQ ($n = 436$) | No. (%) SPAZ ($n = 418$) | *P* |
|---|---|---|---|
| Age, yr | | | |
| <20 | 67 (15.4) | 90 (21.5) | 0.061 |
| ≥20 and <30 | 285 (65.4) | 258 (61.7) | |
| ≥30 | 84 (19.3) | 70 (16.8) | |
| Fundal ht at enrollment (cm) | | | |
| <20 | 94 (21.6) | 110 (26.3) | 0.103 |
| ≥20 and <27 | 342 (78.4) | 308 (73.7) | |
| Undernourished[a] | 129 (29.6) | 111 (26.6) | 0.324 |
| Primigravida | 200 (45.9) | 224 (53.6) | 0.024 |
| Residing in periurban settlement | 91 (20.9) | 87 (20.8) | 0.983 |
| Smoker | 84 (19.3) | 93 (22.3) | 0.282 |
| User of areca nut | 355 (81.4) | 338 (81.3) | 0.834 |
| Literate | 391 (90.6) | 378 (90.4) | 0.934 |
| Sampling period | | | |
| Phase 1 (dates) | 302 (69.3) | 279 (66.8) | |
| Phase 2 (dates) | 134 (30.7) | 139 (33.3) | 0.430 |
| Recent antibiotic use (<30 days)[b] | 53 (12.2) | 53 (12.7) | 0.817 |
| Antibiotic use (any) during pregnancy[b] | 105 (24.1) | 99 (23.7) | 0.891 |

[a] Defined as a mid-upper-arm circumference of <23 cm at first antenatal visit.
[b] Excluding trial drugs, ≥1 course during time period and until and including at the time of sampling.

**Study population.** All pregnant women participating in the IPTp trial were eligible for inclusion at baseline in the present study (11). Eligibility criteria for the original trial are described in detail elsewhere (11). Women were randomized to treatment, and demographic and clinical information, including additional antibiotic use, were collected at enrollment, monthly treatment visits ($n = 2$), morbidity visits, and delivery. The protocol was approved by the PNG Institute of Medical Research Institutional Review Board, the PNG Medical Research Advisory Council, and the Melbourne Health Human Research Ethics Committee. All women provided written informed consent for inclusion in the original trial and the bacteriology study.

**Sample collection.** In this cross-sectional survey, samples were collected at delivery from trial participants from January 2010 to April 2011 (phase 1) and from February to October 2012 (phase 2) (Table 1). Sampling periods were dictated by staff availability, and swab collection was opportunistic and undertaken only when collection of main trial data and samples permitted. A sterile swab (Medical Wire & Equipment, Corsham, United Kingdom) was inserted into the posterior nasopharynx, in accordance with standard methodology (33). Swab tips were clipped into cryovials containing 1 ml of skim milk-tryptone-glucose-glycerin medium (STGG), stored at −20°C, and transported frozen to the bacteriology laboratory (Goroka, PNG), where they were stored at −70°C until processed (34).

**Laboratory investigations.** All laboratory staff were blinded to treatment. For isolation of *S. pneumoniae*, *H. influenzae*, and *S. aureus*, specimens were inoculated onto horse blood agar (HBA), chocolate agar (CA), gentamicin blood agar (GBA) (5% horse blood and 5 μl/ml gentamicin), and bacitracin chocolate agar (BCA) (Oxoid, Thermofisher, Australia) and incubated for 24 to 48 h at 37°C in 5% $CO_2$.

Colonies of *S. pneumoniae* were distinguished from other alpha-hemolytic streptococci using optochin disk inhibition (Oxoid, Thermofisher, Australia), complemented by a positive bile solubility test (10% sodium desoxycholate). For each carrier, two colonies were serotyped by

**TABLE 2** Effect of IPTp with SPAZ on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* at delivery

| Species and treatment group (*n*) | No. (%) of carriers | Crude OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|
| *Streptococcus pneumoniae* | | | |
| SPCQ (436) | 84 (19.3) | 1.0 | 1.0 |
| SPAZ (418) | 30 (7.2) | 0.32 (0.21, 0.51) | 0.32 (0.21, 0.51)[a] |
| *Haemophilus influenzae* | | | |
| SPCQ (436) | 26 (6.0) | 1.0 | 1.0 |
| SPAZ (418) | 12 (2.9) | 0.47 (0.21, 0.97) | 0.46 (0.23, 0.93)[b] |
| *Staphylococcus aureus* | | | |
| SPCQ (436) | 105 (24.1) | 1.0 | 1.0 |
| SPAZ (418) | 99 (23.7) | 0.98 (0.71, 1.34) | 0.99 (0.72, 1.36)[c] |

[a] Adjusted for period of sampling, area of residence, and areca nut consumption.
[b] Adjusted for period of sampling and partner's income generation status.
[c] Adjusted for period of sampling and women's income generation status.

Quellung reaction with pooled and individual serotype-specific pneumococcal antisera (SSI Diagnostics, Copenhagen, Denmark).

Antibiotic susceptibility testing was performed for one of two serotyped isolates per carrier if both were of the same serotype/serogroup and for both if they differed. Sensitivities of pneumococcal isolates to AZ, ceftriaxone, chloramphenicol (CHL), co-trimoxazole (COT), erythromycin (E), oxacillin (OX), and tetracycline (TET) were assessed by disk diffusion (Oxoid, Thermofisher, Australia) (35). MICs for *S. pneumoniae* isolates with intermediate or full resistance by disk diffusion were evaluated on Mueller-Hinton medium with 5% lysed horse blood using Etest strips (AZ, CHL, COT, penicillin, and TET) (AB Biodisk, Solna, Sweden). For AZ, equivalent MIC breakpoints of ≤0.5 μg/ml and ≥2 μg/ml were defined as sensitive and resistant, respectively (35).

Growth on CA and/or BCA, but not HBA, was used to identify colonies with morphological characteristics consistent with *H. influenzae*. Strains were confirmed through growth only in the presence of both X and V factors (Oxoid, Thermofisher, Australia). A chromogenic cephalosporin disk was used to test isolates for β-lactamase production (BD, Franklin Lakes, NJ, USA). Serotyping for *H. influenzae* was not performed. *S. aureus* was identified through DNase and direct tube coagulase testing. Confirmed *S. aureus* isolates were tested for antibiotic susceptibility (ampicillin, AZ, COT, CHL, E, gentamicin, OX, and TET) by disk diffusion (Oxoid, Thermofisher, Australia) (35).

**Statistical analysis.** Maternal nasopharyngeal pneumococcal colonization was estimated at 20% (36, 37). To show a 40% reduction in *S. pneumoniae* carriage in women who received SPAZ (significance level, 0.05; power of 90%), a minimum of 383 specimens were required from each arm. Because opportunity sampling was applied, an additional 10% of the calculated total number of specimens were collected.

Primary outcome measures included (i) nasopharyngeal carriage, defined as the proportion of women with a positive culture; (ii) the proportion of serotypes/serogroups among the total of distinct isolates; and (iii) antibiotic susceptibility, characterized as the proportion of isolates showing intermediate or full resistance to a given antibiotic by disk diffusion.

Univariate comparisons of variables were performed using the chi-square test or Fisher's exact test for categorical data, the Student *t* test for parametric data, and the Mann-Whitney U test for nonparametric data. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for categorical outcomes. *P* values at <0.05 were considered statistically significant. All factors with a tendency for association with, e.g., pneumococcal carriage when analyzed univariately (defined as a *P* value of <0.10) were included in a multivariable logistic regression model which then used a backward stepwise elimination model selection procedure.

**TABLE 3** Factors associated with nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* in Madang Province, Papua New Guinea, by crude analysis

| Species and characteristic | Total | *n* (%) | OR (95% CI) | *P* |
|---|---|---|---|---|
| *Streptococcus pneumoniae* | | | | |
| Sampling period | | | | |
| Phase 1 | 581 | 95 (16.4) | 1.00 | |
| Phase 2 | 273 | 19 (7.0) | 0.38 (0.23, 0.64) | <0.001 |
| Lives in periurban settlement | | | | |
| No | 671 | 80 (11.9) | 1.00 | |
| Yes | 178 | 33 (18.5) | 1.68 (1.08, 2.63) | 0.021 |
| Consumes areca nut | | | | |
| No | 161 | 11 (6.8) | 1.00 | |
| Yes | 693 | 103 (14.9) | 2.38 (1.24, 4.56) | 0.007 |
| Woman generates income | | | | |
| No | 433 | 71 (16.4) | 1.00 | |
| Yes | 421 | 43 (10.2) | 0.58 (0.39, 0.87) | 0.008 |
| *Haemophilus influenzae* | | | | |
| Sampling period | | | | |
| Phase 1 | 581 | 36 (6.2) | 1.00 | |
| Phase 2 | 273 | 0.7 (2) | 0.11 (0.02, 0.47) | <0.001 |
| Partner generates income | | | | |
| No | 280 | 21 (7.5) | 1.00 | |
| Yes | 574 | 17 (3.0) | 0.38 (0.20, 0.73) | 0.003 |
| *Staphylococcus aureus* | | | | |
| Sampling period | | | | |
| Phase 1 | 581 | 162 (27.9) | 1.00 | |
| Phase 2 | 273 | 42 (15.4) | 0.47 (0.32, 0.69) | <0.001 |
| Woman generates income | | | | |
| No | 433 | 121 (27.9) | 1.00 | |
| Yes | 421 | 83 (19.7) | (0.46, 0.87) | 0.005 |

## RESULTS

**Baseline characteristics.** Of 2,793 women randomized to treatment in the original trial, 2,775 were eligible for inclusion at baseline. Among them, 50 participants were withdrawn before delivery, 37 did not receive study medications, and 454 were lost to follow-up, resulting in 2,234 women with pregnancy outcome follow-up. Nasopharyngeal swabs were collected and cultured for 857 participants, using opportunity sampling. Three study participants were subsequently excluded for treatment crossover, leaving 854 participants for inclusion in the present analysis. Of them, 418 (49.0%) received ≥1 treatment course of SPAZ (mean, 2.8; median, 3; range, 1 to 3). Women who had received SPAZ were more likely to be primigravid (SPAZ, 53.6%, versus SPCQ, 45.9%; $P = 0.024$). Other participant characteristics were similar between treatment groups (Table 1). The mean (± standard deviation) time difference between last study treatment visit and swab collection was 53 ± 30 days (median, 49 days).

**Impact of IPTp with SPAZ on nasopharyngeal carriage of *S. pneumoniae*.** Overall, specimens from 114 women (13.4%) yielded 126 isolates of *S. pneumoniae*. Carriage rates were significantly lower in the intervention group (SPAZ, 7.2%, versus SPCQ, 19.3%; OR [95% CI], 0.32 [0.21 to 0.51]; $P < 0.001$) (Table 2). Factors associated with reduced carriage included late sample period, not residing in a periurban settlement, not chewing areca nut, and women pursuing an income-generating activity (Table 3). When adjusted for these factors, the relationship between

**TABLE 4** Antibiotic susceptibility testing of maternal nasopharyngeal *Streptococcus pneumoniae* isolates collected at delivery following a single course of SP and chloroquine or IPTp with sulfadoxine-pyrimethamine (SP) and azithromycin (AZ)[f]

| Antibiotic | SPCQ ($n = 91$) | | | | SPAZ ($n = 30$) | | | | $P$[e] |
|---|---|---|---|---|---|---|---|---|---|
| | No. of isolates (% of total tested) | | | Median MIC, μg/ml (IQR) | No. of isolates (% of total tested) | | | Median MIC, μg/ml (IQR) | |
| | S | I | R | | S | I | R | | |
| Azithromycin | 89 (97.8) | 0 | 2 (2.2) | 12[b] | 26 (86.7) | 0 | 4 (6.7) | 70 (7.5, 192) | **0.033** |
| Ceftriaxone | 88 (96.7) | 2 (2.2)[d] | 1 (1.1)[d] | | 30 (100.0) | 0 | 0 | | 0.422 |
| Chloramphenicol | 90 (98.9) | 1 (1.1) | 0 | 2 | 30 (100.0) | 0 | 0 | | 0.752 |
| Co-trimoxazole | 52 (57.1) | 5 (5.4) | 34 (37.4) | 6 (1.25, 12)[b] | 18 (60.0) | 0 | 12 (40.0) | 4 (2.5–7) | 0.783 |
| Penicillin[a] | 35 (38.5) | 11 (12.1) | 45 (49.5) | 0.25 (0.125, 0.5)[b] | 14 (46.7) | 3 (10.0) | 13 (43.3) | 0.25 (0.125, 0.38) | 0.427 |
| Tetracycline | 84 (92.3) | 1 (1.1) | 6 (6.6) | 24 (8.125, 40)[c] | 26 (86.7) | 3 (10.0) | 1 (3.3) | 32 (24, 32) | 0.427 |
| MDR(2) | 54 (59.3) | | 40 (44.0) | | 18 (60.0) | | 12 (40.0) | | 0.949 |
| MDR(3) | 89 (97.8) | | 2 (2.2) | | 26 (86.7) | | 4 (13.3) | | **0.033** |

[a] Kirby-Bauer disk diffusion with oxacillin, Etest with penicillin.
[b] Missing MIC data for one isolate.
[c] Missing MIC data for 2 isolates.
[d] All isolates showed reduced sensitivity to oxacillin by disk diffusion; one isolate had a penicillin MIC of 0.047 μg/ml.
[e] $P$ values of <0.05 are marked in bold.
[f] Abbreviations: S, sensitive; I, intermediate; R, resistant; IQR, interquartile range; MDR(2), multidrug resistant—reduced sensitivity to ≥2 antibiotic classes tested by disk diffusion; MDR(3), multidrug resistant—reduced sensitivity to ≥3 antibiotic classes tested by disk diffusion.

SPAZ and reduced pneumococcal carriage remained significant (Table 2). Reduction in carriage in the intervention arm was noted in each sampling period (phase 1, SPAZ, 8.6% [24/279], versus SPCQ, 23.5% [71/302]; $P < 0.001$; phase 2, SPAZ, 4.3% [6/139], versus SPCQ, 9.7% [13/134]; $P = 0.098$). There were no statistically significant associations between carriage and the length of time since last study treatment, reported or documented use of other antibiotics in pregnancy, season (overall and by treatment arms), or other participant characteristics (Table 1). In the intervention arm, there was a tendency for less frequent pneumococcal carriage among women who had ≥2 study treatments compared to a single treatment (6.7% [27/404] versus 21.4% [3/14]; $P = 0.071$), but the number of women who received only one course of SPAZ was small.

**Frequency and distribution of serotypes by treatment arms.** Of 114 women with pneumococcal carriage, serotype data were available for 108; 120 distinct isolates were detected (91 and 29 isolates in SPCQ and SPAZ groups, respectively) (see Table S1 in the supplemental material). Twelve women (10 for SPCQ, 2 for SPAZ) carried two serotypes. Fifty-six isolates were nonserotypeable (NST), accounting for a large proportion of isolates in both groups (SPAZ, 41.4% [12/29], versus SPCQ, 48.4% [44/91]; $P = 0.512$). Proportions of isolates with serotypes/serogroups covered by the 13-valent pneumococcal conjugate vaccine (PCV-13) or the 23-valent pneumococcal polysaccharide vaccine (PPV-23) were low and did not differ significantly between treatment groups (PCV-13: SPAZ, 10.3% [3/29], versus SPCQ, 17.6% [16/91], $P = 0.559$; PPV-23: SPAZ, 24.1% [7/29], versus SPCQ, 20.9% [19/91], $P = 0.711$). The most common serotypeable pneumococci were of serotypes 6A and 10; their proportion did not differ significantly by treatment arm (serotype 6A: SPAZ, 3.5% [1/29], versus 9.9% [9/91], $P = 0.448$; serotype 10: SPAZ, 6.9% [2/29], versus 5.5% [5/91], $P = 0.675$) (see Table S1 in the supplemental material).

**Pneumococcal antibiotic susceptibility following IPTp with SPAZ.** In the SPCQ and SPAZ groups, 2 of 91 and 4 of 30 pneumococcal isolates, respectively, that underwent antibiotic sensitivity testing exhibited resistance to macrolides ($P = 0.033$) (Table 4). None of the women with macrolide-resistant isolates had documentation of additional macrolide use in the index pregnancy. Macrolide-resistant isolates were the only isolates exhibiting reduced susceptibility to ≥3 different antibiotic classes on disk diffusion; all remained sensitive to chloramphenicol (see Table S2 in the supplemental material).

The proportion of β-lactam- and co-trimoxazole-resistant pneumococcal isolates was high and did not differ by treatment arm (β-lactam: SPAZ, 53.3% [16/30], versus SPCQ, 61.5% [56/91], $P = 0.427$; co-trimoxazole: SPAZ, 40.0% [12/30], versus SPCQ, 42.9% [39/91], $P = 0.783$) (Table 4).

**Impact of SPAZ on nasopharyngeal carriage and antibiotic susceptibility of *H. influenzae* and *S. aureus*.** Nasopharyngeal carriage of *H. influenzae* was less frequent among women who had received SPAZ (2.9% [12/418], versus SPCQ, 6.0% [26/436]; $P = 0.028$) (Table 2), but carriage of *S. aureus* was similar ($P = 0.892$; Table 2). The proportion of AZ-resistant *S. aureus* isolates was significantly larger in the SPAZ group (79.6% [78/98], versus SPCQ, 14.4% [15/104]; $P < 0.001$), as was the number of isolates resistant to chloramphenicol (SPAZ, 11.2% [11/98], versus SPCQ, 2.9% [3/104]; $P = 0.019$) and resistant to ≥2 antibiotic classes (SPAZ, 19.4% [18/98], versus SPCQ, 3.8% [4/104]; $P < 0.001$) (see Table S3 in the supplemental material). Four of 98 and two of 104 isolates exhibited reduced sensitivity to both macrolides and oxacillin in SPCQ and SPAZ groups, respectively. Resistance to gentamicin, co-trimoxazole, and tetracycline was absent or rare (see Table S3 in the supplemental material).

## DISCUSSION

IPTp with SPAZ significantly reduced maternal nasopharyngeal carriage of *S. pneumoniae* and *H. influenzae* at delivery. Macrolide-resistant pneumococci were uncommon overall but occurred more frequently among women who had received SPAZ. Many pneumococcal isolates were resistant to β-lactams and co-trimoxazole, and most were NST and of nonvaccine type. SPAZ had no effect on nasopharyngeal colonization with *S. aureus* but increased the proportion of macrolide- and chloramphenicol-resistant staphylococci.

Pneumococcal nasopharyngeal carriage rates in women who received SPCQ were similar to those in other low-resource environments (36, 37). Macrolide-resistant pneumococci were occasionally isolated from women in the SPCQ group: the use of AZ for syndromic management of bacterial STIs (25) and/or antibiotic cross-class resistance may explain their circulation in the study area. The increase in the proportion of macrolide-resistant pneumococci observed in the SPAZ group may be primarily due to selection of such circulating drug-resistant isolates, rather than induction of drug resistance *de novo* (15, 18). Reassuringly, pneumococci recently isolated from local children with acute bacterial meningitis remained macrolide sensitive (L. Manning, personal communication) (30).

The marked reduction in pneumococcal carriage in the SPAZ group has not been observed in all MDA studies (20). SPAZ-IPTp is administered to a comparatively small fraction of the community, as opposed to MDA, which involves the simultaneous treatment with lower dose of AZ (20 mg/kg of body weight; maximum, 1,000 mg) of many individuals at a single time point. The lower dosage and widespread administration may lead to increasing community "baseline" resistance levels. MDA may need to be undertaken annually or more regularly. IPTp is given in monthly intervals from quickening until delivery but will then not be required until the subsequent pregnancy, ≥24 months in breastfeeding mothers (38). With an intermission of ~2 years, macrolide-resistant isolates may have been eliminated as a result of the cost associated with drug resistance (39). By comparison, MDA repeated in short intervals may increase the risk of antimicrobial resistance (20), although evidence remains inconclusive (21).

The proportion of isolates of nonvaccine serotypes was high, and many were nonserotypeable: similar observations have been made in pregnant women in The Gambia and Thailand (36, 37). Both amoxicillin and co-trimoxazole are widely available in PNG, while SP was until recently a first-line antimalarial (40). Although penicillin resistance in *S. pneumoniae* may be short-lived (41), the high number of β-lactam- and co-trimoxazole-resistant pneumococci and the presence of ceftriaxone-resistant isolates in the control arm raise concerns. Elimination of unnecessary antibiotic use and careful monitoring of ceftriaxone resistance are required: ceftriaxone is a first-line treatment for acute bacterial meningitis in PNG (27). Pneumococcal carriage was higher among regular consumers of areca (betel) nuts, which in PNG are usually chewed together with the betel pepper fruit and lime. The latter is frequently shared between chewers; this, and mucosal inflammation secondary to chewing, may facilitate pneumococcal colonization. Chewing has already been associated with tuberculosis (42).

SPAZ reduced the proportion of women with nasopharyngeal carriage of *H. influenzae* but did not affect carriage of *S. aureus*. The significance of the increased proportion of macrolide- and chloramphenicol-resistant *S. aureus* isolates is unclear, but resistance monitoring for a range of bacteria will be required should IPTp with SPAZ be implemented. A small number of isolates were resistant to both oxacillin and macrolides: this points toward circulation of low levels of community-acquired methicillin-resistant *S. aureus* (MRSA) (32).

This is the first study to assess the impact of IPTp with SPAZ on nasopharyngeal bacterial carriage and antibiotic susceptibility. Our appropriate sample size, detection of three key bacterial species (with comprehensive antimicrobial susceptibility testing for two), pneumococcal serotyping, access to a large clinical and demographic data set, and availability of a nonmacrolide control group all contribute to a robust experimental design. Nonetheless, experimental limitations exist. Nasopharyngeal carriage was assessed at a single time point only (cross-sectional survey at delivery), and nasopharyngeal carriage characteristics before treatment or after, e.g., 6 to 12 months following treatment, are unknown; this has implications at several levels. Pneumococcal carriage and susceptibility patterns at delivery in the SPCQ group may mirror the general, pretreatment population of pregnant women; however, receipt of a single course of SPCQ could affect bacterial colonization and antibiotic sensitivities (40). Single-point sampling precluded the evaluation of both recolonization rates following treatment or after delivery and long-term effects of SPAZ on persistence of macrolide-resistant pneumococci and serotype replacement that were observed in MDA studies (15, 19, 20). Paired mother-infant nasopharyngeal specimens could have demonstrated the rates of mother-to-infant transfer of specific isolates from mother to infant; data from The Gambia suggest that the population-attributable fraction of infant carriage due to maternal carriage is low (9.5%) (36). Lastly, a freezer failure resulted in the loss of a small set of isolates for serotyping and/or antibiotic susceptibility testing. Additionally, we may not have detected/measured all potential confounders for our outcomes.

Future studies of IPTp with SPAZ, as well as its potential implementation, need to be coupled with antimicrobial surveillance as its administration carries the risk of selecting drug-resistant bacterial isolates.

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