Effect of presumptive co-trimoxazole prophylaxis on pneumococcal colonization rates, seroepidemiology and antibiotic resistance in Zambian infants: a longitudinal cohort study

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Objective To ascertain the microbiological consequences of WHO's recommendation for presumptive co-trimoxazole prophylaxis for infants with perinatal HIV exposure.

Methods Using a longitudinal cohort design, we followed HIV-exposed and HIV-unexposed infants trimonthly for up to 18 months per infant. HIV-exposed infants received daily co-trimoxazole prophylaxis from 6 weeks to ≥ 12 months of age. Using *Streptococcus pneumoniae* as our sentinel pathogen, we measured how co-trimoxazole altered nasopharyngeal colonization, pneumococcal resistance to antibiotics and serotype distribution as a function of co-trimoxazole exposure.

Findings From 260 infants followed for 3096 patient-months, we detected pneumococci in 360/1394 (25.8%) samples. HIV-exposed infants were colonized more frequently than HIV-unexposed infants (risk ratio, RR: 1.4; 95% confidence interval, CI: 1.0–1.9, $P = 0.04$). Co-trimoxazole prophylaxis reduced colonization by ca 7% but increased the risk of colonization with co-trimoxazole-resistant pneumococci within 6 weeks of starting prophylaxis (RR: 3.2; 95% CI: 1.3–7.8, $P = 0.04$). Prophylaxis with co-trimoxazole led to a small but statistically significant increase of nasopharyngeal colonization with pneumococci not susceptible to clindamycin (RR: 1.6; 95% CI: 1.0–2.6, $P = 0.04$) but did not increase the risk of non-susceptibility to penicillin (RR: 1.1; 95% CI: 0.7–1.7), erythromycin (RR: 1.0; 95% CI: 0.6–1.7), tetracycline (RR: 0.9; 95% CI: 0.6–1.5) or chloramphenicol (RR: 0.8; 95% CI: 0.3–2.3). Co-trimoxazole prophylaxis did not cause the prevailing pneumococcal serotypes to differ from those that are targeted by the 7-valent conjugate pneumococcal vaccine (RR: 1.0; 95% CI: 0.7–1.6).

Conclusion Co-trimoxazole prophylaxis modestly suppresses pneumococcal colonization but accelerates infant acquisition of co-trimoxazole- and clindamycin-resistant pneumococci. Co-trimoxazole prophylaxis appears unlikely to compromise the future efficacy of conjugate vaccines.

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Une traduction en français de ce résumé figure à la fin de l'article. *Al final del artículo se facilita una traducción al español.* الترجمة العربية لهذه الخلاصة في نهاية النص الكامل لهذه المقالة.

Introduction

In 2000, the WHO and Joint United Nations Programme on HIV/AIDS (UNAIDS) secretariats recommended that infants in resource-poor settings with perinatal HIV exposure from an infected mother should receive co-trimoxazole (trimethoprim-sulfamethoxazole) prophylaxis presumptively.[1,2] Co-trimoxazole prophylaxis would continue until two conditions are satisfied: (i) the child has been fully weaned and is no longer being exposed to maternal HIV; and (ii) the child can be proven uninfected with HIV. In resource-poor settings, presumptive prophylaxis would be necessary for at least a year in most cases. While WHO's policy is intended to protect the subset of HIV-exposed infants who are (or become) HIV-infected in their first year, the majority of infants so targeted will escape HIV infection.[3]

In an earlier commentary on this policy,[4] we drew attention to multiple potential adverse consequences of presumptive co-trimoxazole prophylaxis, with a particular focus on antimicrobial resistance. To better understand how co-trimoxazole prophylaxis affects microbial colonization and resistance rates, we implemented the co-Trimoxazole in Zambian Infants (TZI) project, a longitudinal cohort study designed to measure selected microbiological consequences of WHO's policy. We selected *Streptococcus pneumoniae* as our sentinel pathogen for several reasons. First, the pneumococcus is a leading cause of morbidity/mortality among infants worldwide. Second, drug-resistant pneumococci are increasingly common and of particular public health concern. Third, multiple aspects of pneumococcal epidemiology can be assessed conveniently via nasopharyngeal colonization surveys. Lastly, the

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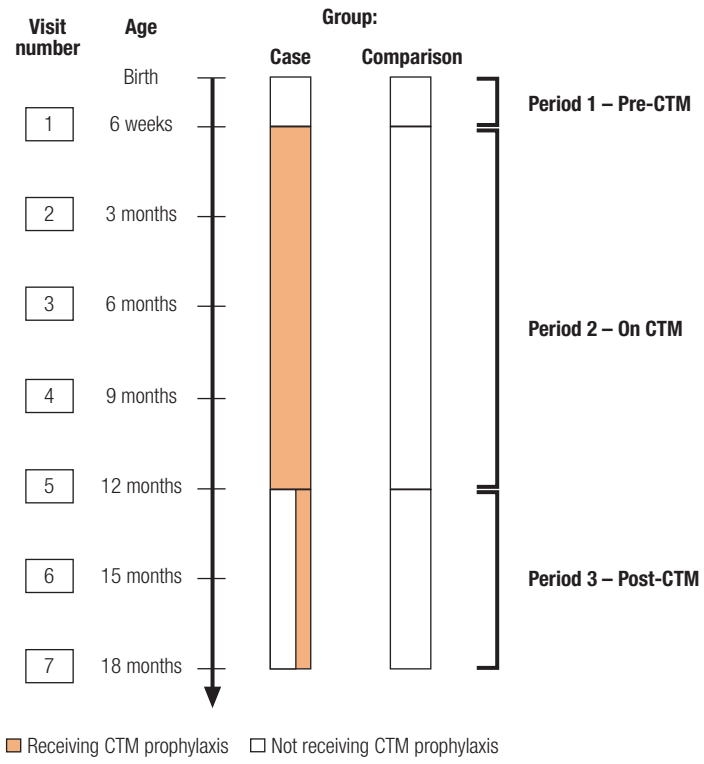
effectiveness of antibiotics and vaccines, our two main tools for combating pneumococcal disease, could both be degraded by widespread presumptive co-trimoxazole prophylaxis. Exposure to sulfonamides is presumably the dominant force behind the emergence of co-trimoxazole-resistant pneumococci and might induce cross-resistance to other antibiotics.[5–8] Moreover, because antibiotic resistance is often linked with specific serotypes,[9–12] exposure to co-trimoxazole might shift the prevailing pneumococcal serotypes away from those represented by the 7-valent conjugate pneumococcal vaccine.

Hence, this analysis addressed the following questions:

- Does co-trimoxazole prophylaxis alter nasopharyngeal pneumococcal colonization rates?
- Does co-trimoxazole prophylaxis induce co-trimoxazole-resistant pneumococci and, if so, how quickly?
- Does co-trimoxazole prophylaxis induce cross-resistance to other antibiotic classes?
- Does co-trimoxazole exposure alter the distribution of pneumococcal serotypes away from 7-valent vaccine strains?

Fig. 1. **Design of longitudinal cohort study of co-trimoxazole prophylaxis among Zambian infants**



CTM, co-trimoxazole; TZI, co-trimoxazole in Zambian Infants.

Methods

Study overview

The TZI project was a two-arm longitudinal cohort study whose principal objective was to measure the microbiological consequences of implementing WHO guidelines[2] for presumptive co-trimoxazole prophylaxis on pneumococcal colonization, drug resistance and seroepidemiology. The project was conducted at three antenatal clinics in Ndola, Zambia. Ndola is Zambia's third-largest city, with most of its inhabitants living below the poverty level in periurban compounds. All mother/infant pairs were enrolled at the study clinics. Eligibility criteria were: (i) residence within the catchment zone of our study clinics; (ii) signed maternal informed consent; and (iii) maternal willingness to undergo HIV testing. "Case" infants were those born to HIV-positive women and thus requiring prophylaxis per WHO guidelines. "Comparison" infants were infants born to HIV-negative women, unexposed to HIV and hence not requiring prophylaxis per WHO guidelines. Comparison infants were recruited contemporaneously 1:1 with case infants, and were age- and clinic-matched. Our sample size of 260 mother/infant pairs was powered to detect a 30% reduction in colonization and a twofold increase in co-trimoxazole resistance as a function of co-trimoxazole exposure, while accommodating up to 30% attrition.

As per WHO guidelines, case infants received daily prophylactic oral co-trimoxazole from 6 weeks until ≥ 12 months of age, dosed at 10 mg/kg daily for trimethoprim and 50 mg/kg daily for sulfamethoxazole. Case infants still on study at 12 months were tested for HIV infection. If positive at 12 months and again at 15 months, they were considered true positives and offered co-trimoxazole prophylaxis indefinitely. Case infants who tested negative at 12 or 15 months and had fully weaned were considered HIV-negative and co-trimoxazole was stopped.

All infants were enrolled at 6 weeks of age and followed according to a seven-visit, well-child care schedule at prespecified intervals (Fig. 1). At visit 1, no infants had started co-trimoxazole prophylaxis. From visits 2 to 5, all case infants received co-trimoxazole. After visit 5, most case infants tested negative for HIV and stopped co-trimoxazole. This schedule creates three distinct periods for comparison (Fig. 1): pre-co-trimoxazole (period 1), on co-trimoxazole (period 2), and post-co-trimoxazole (period 3).

The TZI project was jointly approved by the ethical review boards at Boston University and the Tropical Diseases Research Centre (TDRC) in Ndola. All mothers provided written informed consent.

Laboratory procedures

Mothers attending antenatal clinics underwent HIV voluntary counselling and testing according to local standards. HIV screening used the Determine® 1 + 2 test (Abbott Laboratories, Abbott Park, IL, United States of America) and confirmed using the Capillus® test (Cambridge Biotech Ltd, Galway, Ireland),[13] with the Bioline® test (Bionor AS, Skien, Norway) to resolve indeterminate/discrepant results. The sensitivity/specificity of this protocol exceeds 99%. This same protocol was used for case infants at 12 and 15 months.[14]

Table 1. **Baseline demographic and clinical characteristics of infants and mothers in longitudinal cohort study of the effect of co-trimoxazole prophylaxis on pneumococcal colonization rates, seroepidemiology and antibiotic resistance in Zambian infants**

Characteristics	Case group	Comparison group	*P*-value
Mothers			
No. single, of total (%)	13/129 (10.1)	14/128 (10.9)	NS
No. married, of total (%)	110/129 (85.3)	114/128 (89.1)	NS
No. divorced, of total (%)	2/129 (1.6)	0/128 (0.0)	NS
No. widowed, of total (%)	4/129 (3.1)	0/128 (0.0)	0.04
Mean age in years (SD)	25.9 (6.5)	24.2 (6.2)	NS
Mean weight in kg (SD)	56.2 (8.4)	56.5 (7.9)	NS
No. with primary language Bemba, of total (%)	111/132 (84.1)	109/128 (85.1)	NS
No. using insecticide-treated bednet, of total (%)	52/132 (39.4)	55/130 (42.3)	NS
Infants			
Mean birth weight in g (SD)	3059 (440)	3194 (622)	0.05
No. treated with co-trimoxazole since birth, of total (%)	6/132 (4.5)	5/128 (3.9)	NS
No. with infant health issues since birth			
No. having chronic cough, of total (%)	1/131 (0.8)	0/128 (0.0)	NS
No. having recurrent fevers, of total (%)	1/131 (0.8)	1/128 (0.8)	NS

NS, non-significant; SD, standard deviation.

HIV-positive mothers and their infants received peripartum nevirapine prophylaxis according to the HIVNET 012 protocol.[3] Antiretroviral drugs were virtually unavailable in Ndola during TZI.

At every visit, we screened for *S. pneumoniae* colonization using posterior nasopharyngeal samples obtained with sterile calcium-alginate-tipped aluminium swabs advanced into both nostrils until meeting resistance and then rotated 180°.[15] To maximize yields, swabs were plated immediately on to room temperature soy-trypticase agar plates with 5% sheep's blood/5% gentamicin (gent/BAP) and streaked later for optimal colony separation at the TDRC microbiology laboratory.[15–17] Gentamicin increases the yield for *S. pneumoniae* by approximately 40%.[16,17] Screening plates were incubated at 37 °C under 5% CO_2 atmosphere for 48 hours. Colonies were presumptively identified as *S. pneumoniae* by colony morphology (small grey "draftsman" mucoid α-haemolytic colonies), and typical diplococcoid morphology on Gram stain.[18] For confirmation, colonies from the screening gent/BAP plates were subcultured onto gentamicin-free BAP, and defined as *S. pneumoniae* on the basis of ≥ 14 mm optochin (ethylhydrocupreine, Difco, Detroit,. MI, USA) inhibition zones, or bile-solubility for isolates with < 14 mm optochin inhibition.[19]

Drug resistance was determined using the elipsometer method (Etest®, AB Biodisk, Solna, Sweden).[20,21] We measured the minimum inhibitory concentration (MIC) for each isolate against co-trimoxazole, penicillin, tetracycline, erythromycin, chloramphenicol and clindamycin. Subcultures of pure pneumococcal isolates were used to create a bacterial lawn on 150 mm Mueller–Hinton broth agar plates, each plate accommodating all Etests® simultaneously. Diameters of inhibition were measured at 24 hours of incubation. The point on the Etest® strip where inhibition was first noted indicated the MIC for that isolate and was read directly off the calibration guide printed on each strip. Classification of inhibition zones for isolates into sensitive, intermediately and highly resistant pneumococci was per the National Committee for Clinical Laboratory Standards guidelines.[18]

Subcultured isolates were characterized to the serogroup and serotype level using the Statens Serum Institute (Copenhagen, Denmark) latex slide agglutination system, with subsequent factor typing of the dominant serotypes.

Statistical analyses

Data were dual entered at TDRC and cleaned/reconciled at Boston University. We conducted univariate estimates of risk ratios (RRs) with 95% confidence intervals (CI), *t*-tests, and/or tests of proportions (χ^2 or Fisher's exact). Because MICs for antibiotic resistance operate on a logarithmic scale, we log transformed MICs before conducting *t*-tests on the means and back-transformed the result using the exponent of the difference in the log means. Note that this procedure yields the ratio of the two medians of the back-transformed MICs, not their means [exp(Ln mean1 - Ln mean2) = median1/median2].[22]

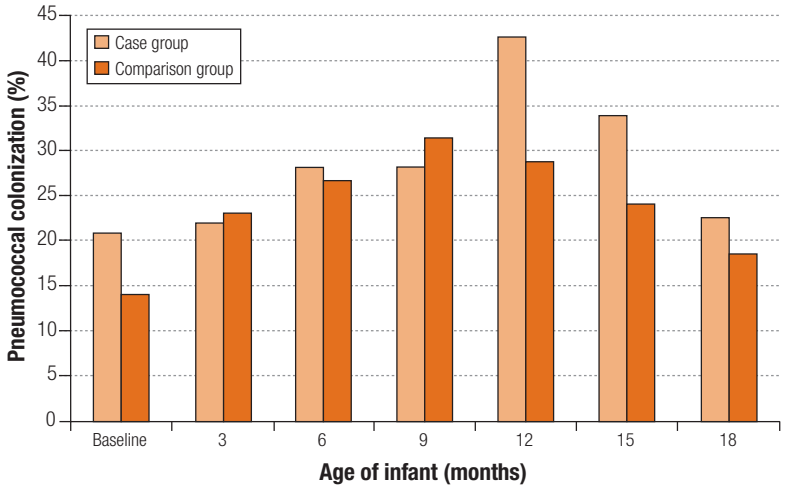
In univariate analyses, we assumed that case infants received co-trimoxazole during period 2 but not in periods 1 or 3 (adjusting for the handful who tested positive for HIV and remained on co-trimoxazole), and no comparison infants received co-trimoxazole. However, we also tested the effect on colonization and drug resistance of intercurrent short-term sulfonamide treatment among the comparisons, such as brief courses of co-trimoxazole for acute infections or malaria treatment with sulfadoxine-pyrimethamine, a pharmacologically similar drug to co-trimoxazole that has been linked to increased colonization with co-trimoxazole-resistant pneumococci.[23] Lacking a priori knowledge about what exposure level would be sufficient to induce resistance, we categorized any level of sulfonamide exposure during the preceding interval as 'exposed to co-trimoxazole' under this expanded definition. Conversely,

only those infants with no reported exposure to sulfonamides were categorized as unexposed to co-trimoxazole. Owing to the longitudinal structure of the data set with multiple observations on individuals, we recalculated the RRs using a log-linear model with robust standard errors to adjust for the clustering effect of repeated measures to see if our results changed significantly.[22] Our sample size was based on a projected 30% reduction in colonization between the two arms from a typical baseline of 60% colonized, while adjusting for predicted rates of attrition of up to 50% by study end.

Results

Between December 2003 and September 2004, we enrolled 132 HIV-exposed (case) and 128 HIV-unexposed (comparison) infants (260 total). We followed the mother/infant pairs for a total of 3096 person-months with the last patient visit in November 2005. Baseline characteristics were similar between the two groups (Table 1); 25 case versus 44 comparison infants were lost to follow-up ($P < 0.01$); 1 case versus 6 comparison infants withdrew ($P = 0.05$); 10 case versus 0 comparison infants died by study end ($P = 0.001$). Of these 10 case infants, only 2 had reached the 12-month visit and had undergone HIV testing; neither was HIV-positive. Fifteen of 105 HIV-exposed infants still on study by 12 months were HIV seropositive (14.3%, standard error: 3.0%). One tested positive at 12 months but negative at 15 months, presumably a false positive due to residual maternal antibody. Although 48 versus 42 unscheduled clinical illness visits occurred in the case and comparison arms respectively, the rates did not differ statistically after adjusting for person-time at risk. Intercurrent sulfonamide use occurred frequently among the comparison infants. By study end, 56.6% of the comparison infants had been treated at least once with a sulfonamide, occurring at 137 of 648 visits (21.1%).

Fig. 2. **Nasopharyngeal pneumococcal colonization among Zambian infants given co-trimoxazole prophylaxis and comparison group in longitudinal cohort study, by age**



TZI, co-Trimoxazole in Zambian Infants.

From 1394 nasopharyngeal swabs, 360 tested positive for *S. pneumoniae* (25.8%). Of the 360 isolates, 45 (12.5%) were from period 1; 231 (64.2%) from period 2; and 84 (23.3%) from period 3. The sample positivity rate did not vary by the time of year of sampling (data not shown). By contrast, nasopharyngeal colonization was strongly associated with the infants' age ($P < 0.001$), peaking in both groups at 12 months (Fig. 2).

Co-trimoxazole exposure modestly suppressed colonization among case infants. Colonization rates were 6.8% higher for cases than comparisons during period 1 (20.9% versus 14.1%, RR: 1.5; 95% CI: 0.9–2.6) and 7.1% higher in period 3 (28.7% versus 21.6%, RR: 1.3; 95% CI: 0.9–1.9). After combining the non-exposure periods (periods 1 and 3), case infants were significantly more likely to be colonized than comparisons (25.3% versus 18.1%, RR: 1.4; 95% CI: 1.0–1.9, $P = 0.04$). Adjusting for intercurrent sulfonamide treatments among the controls did not change this risk substantially (RR: 1.5; 95% CI: 1.2–1.9). By contrast, during co-trimoxazole exposure (period 2), colonization rates were similar between the two groups (29.8% case versus 27.2% comparison infants, RR: 1.1; 95% CI: 0.9–1.4, $P = 0.41$; Fig. 2).

Table 2. **Case:comparison ratios of median antibiotic MICs in pneumococci isolated from nasopharyngeal cultures taken from Zambian infants in longitudinal cohort study of co-trimoxazole prophylaxis, by study period[a]**

Antibiotic	Case:comparison ratio (95% CI)		
	Period 1	Period 2	Period 3
Co-trimoxazole	0.90 (0.30–2.70)	1.42* (0.98–2.08)	1.30 (0.58–2.85)
Penicillin	1.72 (0.86–3.47)	1.07 (0.76–1.51)	1.09 (0.66–1.81)
Chloramphenicol	0.95 (0.78–1.15)	0.94 (0.80–1.11)	1.11 (0.84–1.44)
Tetracycline	0.83 (0.23–3.00)	0.97 (0.52–1.83)	1.31 (0.41–4.17)
Erythromycin	1.17 (0.76–1.80)	0.92 (0.72–1.19)	1.01 (0.80–1.28)
Clindamycin	1.20 (0.77–1.87)	1.04 (0.84–1.28)	0.93 (0.73–1.19)

CI, confidence interval; MIC, minimum inhibitory concentration. *$P = 0.07$.
[a] Period 1: pre-co-trimoxazole; period 2, on co-trimoxazole; period 3, post-co-trimoxazole.

The onset of co-trimoxazole prophylaxis led to a rapid increase in co-trimoxazole-resistant pneumococci from the case infants. During period 1, the mean Ln MICs for co-trimoxazole were comparable between the two groups (difference in means: −0.11; 95% CI: −1.2 to 1.0, $P = 0.12$). In period 2, the mean Ln MICs for co-trimoxazole increased in the case arm but stayed constant in the comparison arm (difference: +0.35; 95% CI: −0.03 to 0.73, $P = 0.07$). During period 3, the difference in the mean Ln MICs for co-trimoxazole declined among case infants, though it remained elevated relative to the comparison infants (difference: +0.26; 95% CI: −0.54 to 1.05, $P = 0.52$). During period 2, the median co-trimoxazole MICs were 1.4 times higher for case than comparison infants ($P = 0.08$) but were similar during periods 1 or 3. The median MICs did not differ significantly for any of the other antibiotics tested during the three periods (Table 2).

When categorizing the MICs as sensitive (S), intermediately resistant (I) or resistant (R), the distributions were similar between cases and comparisons during period 1 but diverged during period 2 (Table 3). This was largely explained by rapid increases in co-trimoxazole resistance between visits 1 and 2 ($P = 0.04$). At baseline (visit 1), the distribution was virtually identical between the two arms (14.8% S, 25.9% I, 59.3% R in cases, versus 16.7% S, 22.2% I, 61.1% R in comparisons; $P = 0.96$). By visit 2, 6 weeks later, all isolates from case infants were intermediately or highly resistant to co-trimoxazole (case versus comparison infants, RR: 2.2; 95% CI: 1.6–2.9), whereas the comparisons showed only a modest shift towards more highly resistant pneumococci (0.0% S, 7.4% I, 93.6% R in cases, versus 25.0% S, 16.7% I, 76.5% R in comparisons; $P < 0.01$). Thereafter, resistance rates increased in both groups so that after visit 3, intermediately or highly resistant isolates occurred in similar proportions between the two groups (Fig. 3).

When combining both I and R categories together as non-susceptible, the relationship between co-trimoxazole prophylaxis and colonization with co-trimoxazole-resistant pneumococci became even stronger (RR: 3.2; 95% CI: 1.3–7.8, $P = 0.01$). Overall, approximately 10% of all co-trimoxazole non-susceptibility in this population was accounted for by co-trimoxazole prophylaxis (attributable risk: 12%; 95% CI: 6– 18). When also considering intercurrent non-prophylaxis exposure to sulfonamides among the controls, the risk of colonization with a co-trimoxazole non-susceptible pneumococcus increased further (RR: 4.4; 95% CI: 1.9–10.4).

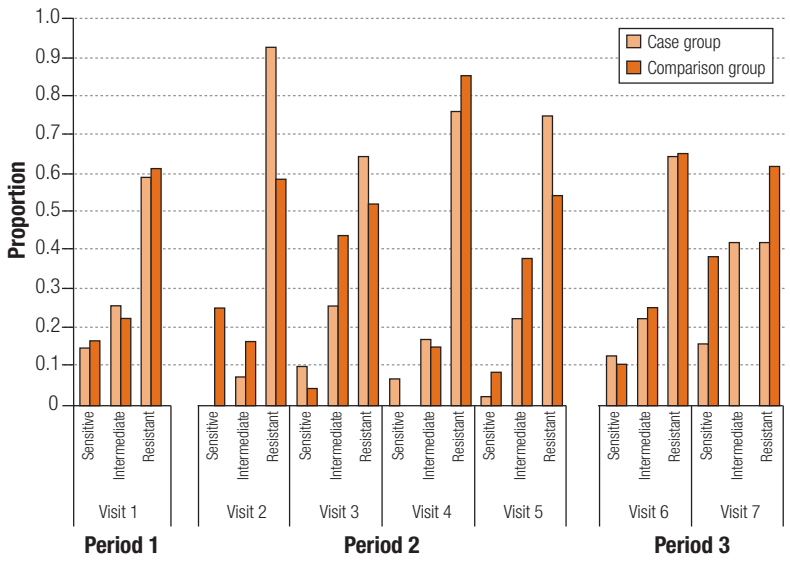
Repeating these analyses for the other antibiotics, co-trimoxazole prophylaxis led to a small but statistically significant increase of colonization with clindamycin non-susceptible pneumococci (RR: 1.6; 95% CI: 1.0–2.6, $P = 0.04$). Co-trimoxazole use did not increase the risk of non-susceptibility to

Table 3. **Degree of sensitivity to co-trimoxazole among pneumococci isolated from nasopharyngeal cultures taken from Zambian infants ($n = 359$)[a] given co-trimoxazole prophylaxis and comparison group, by study period[b]**

Period/ interpretation of MICs	Case group	Comparison group	Total
Period 1			
Sensitive	4 (14.8)[c]	3 (16.7)	7 (15.6)
Intermediate	7 (25.9)	4 (22.2)	11 (24.4)
Resistant	16 (59.3)	11 (61.1)	27 (60.0)
Subtotal	27	18	45 ($P = 0.96$)[d]
Period 2			
Sensitive	6 (4.6)	9 (9.0)	15 (6.5)
Intermediate	25 (19.1)	28 (28.0)	53 (22.9)
Resistant	100 (76.3)	63 (63.0)	163 (70.6)
Subtotal	131	100	231 ($P = 0.08$)[d]
Period 3			
Sensitive	7 (14.0)	7 (21.2)	14 (16.7)
Intermediate	15 (30.0)	5 (15.2)	20 (24.1)
Resistant	28 (56.0)	21 (63.6)	49 (59.3)
Subtotal	50	33	83 ($P = 0.27$)[d]

MIC, minimum inhibitory concentration.
[a] Antibiotic sensitivities were not measured for one isolate.
[b] Period 1: pre-co-trimoxazole; period 2, on co-trimoxazole; period 3, post-co-trimoxazole.
[c] Figures in parentheses are percentages.
[d] χ^2 of proportions within each exposure period.

Fig. 3. **Proportion of nasopharyngeal pneumococcal isolates classified as sensitive, intermediately resistant or resistant to co-trimoxazole, over time, in longitudinal cohort study of co-trimoxazole prophylaxis among Zambian infants**

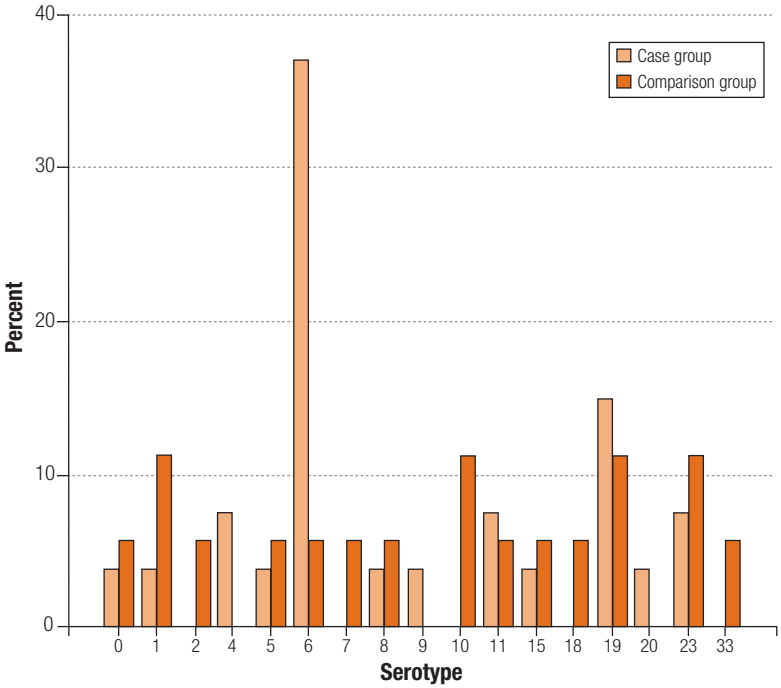


penicillin (RR: 1.1; 95% CI: 0.7–1.7), erythromycin (RR: 1.0; 95% CI: 0.6–1.7), tetracycline (RR: 0.9; 95% CI: 0.6–1.5) or chloramphenicol (RR: 0.8; 95% CI: 0.3–2.3).

In each of these analyses, the adjusted values after controlling for repeated measures and for baseline demographic variables were virtually identical to the results presented above (data not shown).

We had serotype data for 354/360 samples (98%), of which 44% were covered by the 7-valent conjugate vaccine. The probability that a given isolate would be a 7-valent vaccine strain was not altered by whether the infant was exposed or not to co-trimoxazole (RR: 1.0; 95% CI: 0.7–1.6). However, 7-valent vaccine strain isolates were more likely to be non-susceptible to co-trimoxazole than non-vaccine isolates (RR: 2.2; 95% CI: 1.0–4.8). The distribution of serotypes between the two groups across the three exposure periods is summarized in Fig. 4, Fig. 5 and Fig. 6. The five most common serotypes were: 19f (16.0%), 6b (9.9%), 23f (7.5%), 15 (7.0%) and 14 (6.4%), with 5.9% untypable. The most striking differences were a predominance of serotype 6 among the case infants (6b = 5, 6a = 3, 6 unfactorable = 3) during period 1 (Fig. 4), and an apparent loss of serotype diversity over time (Fig. 6): serotypes 2, 3, 5, 8, 12, 17, 18 and 33 were all found in period 1 and/or period 2, but were absent from both groups in period 3.

Fig. 4. **Frequency of nasopharyngeal pneumococcal serotypes in period 1 (pre-cotrimoxazole) of longitudinal cohort study of co-trimoxazole prophylaxis among Zambian infants (*n* = 45)**



Discussion

In this cohort of mostly HIV-negative Zambian infants followed from age 6 weeks through 18 months, pneumococcal colonization was common, peaked in incidence during the first year of life, and was dominated by serotypes represented by the conjugate 7-valent pneumococcal vaccine. These findings are all consistent with what is generally understood to be typical for colonization patterns of pneumococci in young infants[24] and thus increase our level of confidence in interpreting our subsequent findings.

Co-trimoxazole prophylaxis had the following effects on pneumococcal colonization dynamics. First, co-trimoxazole exposure significantly reduced colonization rates, though not below the rate in the comparison infants. While the magnitude of this effect was modest (ca 7% on an absolute scale), it may still be relevant at a population level, insofar as nasopharyngeal colonization is considered a precondition to invasive pneumococcal disease.[25,26]

Second, co-trimoxazole prophylaxis induced a rapid rise in intermediate and particularly high-level co-trimoxazole resistance. This was our most striking finding. On the one hand, co-trimoxazole-resistant pneumococci were exceedingly common in this population, cases and comparisons alike. While co-trimoxazole resistance in the colonizing pneumococci occurred rapidly with the onset of prophylaxis, resistance to co-trimoxazole increased over time in the comparison infants also, albeit somewhat later. Thus, in a setting of widespread sulfonamide exposure, one could argue that co-trimoxazole prophylaxis merely accelerated a process that was already under way. On the other hand, the induction of co-trimoxazole resistance clearly validates concerns about rising drug resistance from presumptive co-trimoxazole prophylaxis. Moreover, co-trimoxazole prophylaxis accounted for slightly over 10% of the total increase in co-trimoxazole resistance in this study, a surprisingly large fraction given the already high background prevalence of resistance and frequency of sulfonamide exposure. This necessarily prompts the question of whether co-trimoxazole prophylaxis might have a more profound effect in settings where co-trimoxazole resistance is uncommon and/or where other sulfonamide use is less widespread.

Third, co-trimoxazole exposure marginally increased the odds of non-susceptibility to clindamycin but did not appear to induce cross-resistance to other classes of antibiotics. Given that co-trimoxazole and clindamycin come from unrelated drug classes, this is unlikely to be explained by pharmacological cross-tolerance. An alternative explanation is co-selection of linked antibiotic-resistance genes. Supporting this hypothesis is the fact that multiple antibiotic-resistance genes among pneumococci have been demonstrated in clusters grouped together on transposons and that such transposons are linked with specific strains or clones of *S. pneumoniae*.[27–29] Our results contrast with those obtained by Abdel-Haq et al., who noted that co-trimoxazole prophylaxis selected for colonization with multidrug resistant pneumococci.[30] However, such selection presumably depends on whether such strains are

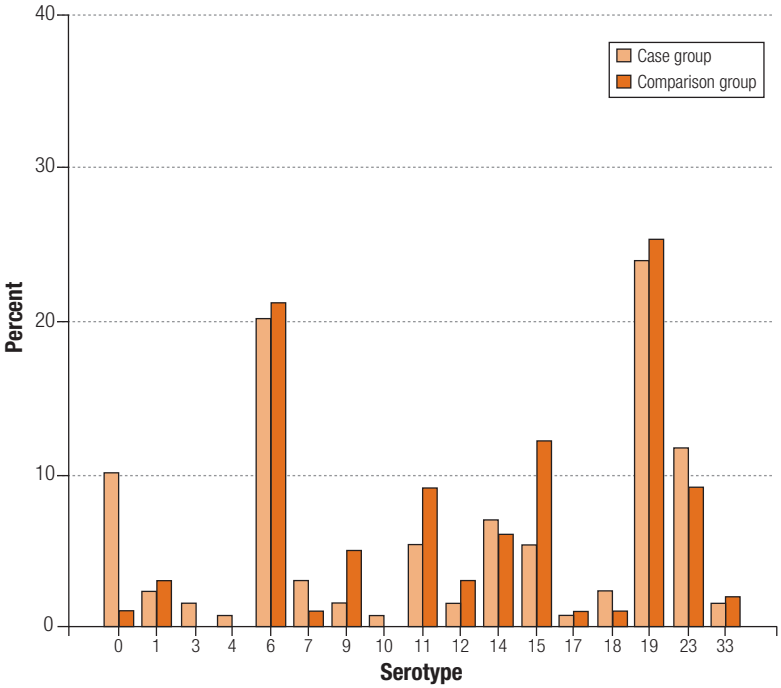
already circulating in a given community, so we do not feel that our results necessarily conflict with their findings. For the same reason, we would be cautious in inferring whether the pattern of cross-induction of clindamycin resistance observed in the context of this study and population should be extrapolated externally. In our view, a more generalizable interpretation is that our data provide additional evidence that co-trimoxazole exposure has consequences that may extend beyond induction of co-trimoxazole resistance alone but may include unrelated drug classes in patterns that are difficult to predict a priori.

Fourth, co-trimoxazole exposure was not associated with any notable shifts in the distribution of pneumococcal serotypes. Thus, our data provide no support for concerns that co-trimoxazole prophylaxis might reduce the future effectiveness of pneumococcal vaccines by shifting the distribution of prevailing serotypes away from vaccine strains.

The loss of serotype diversity that occurred in both study arms during period 3 was curious. This is unlikely to be explained by sample size, since there was a far greater diversity of serotypes during period 1, despite there being roughly half as many isolates as in period 3. More plausibly, this narrowed spectrum reflects the maturation of the infants' mucosal immunity, with infants becoming refractory to colonization by certain serotypes over time. In partial support of this theory, Simell et al. noted that nasopharyngeal pneumococcal colonization in infants was inversely correlated with development of strain-specific immunoglobulin A.[31]

Of concern, co-trimoxazole resistance was extremely common even at baseline, indicating a high community background prevalence of co-trimoxazole-resistant pneumococci – perhaps unsurprising given how frequent sulfonamide exposure was in this population. Though most of our infants were HIV negative, colonization rates were significantly higher among case infants even at baseline. Because this occurred so early in life and because so few of the infants proved to be infected with HIV, HIV-induced immunodeficiency is unlikely to be the explanation. More plausibly, it might reflect other aspects of these infants' home/environments that increase their pneumococcal exposure

Fig. 5. **Frequency of nasopharyngeal pneumococcal serotypes in period 2 (on co-trimoxazole) of longitudinal cohort study of co-trimoxazole prophylaxis among Zambian infants (*n* = 228)**



risk or susceptibility to colonization, or qualitative/quantitative differences in passive immunity from residual maternal antibody.

The TZI study was conducted to generate empirical population-level evidence about the possible microbiological effects of presumptive co-trimoxazole prophylaxis among HIV-exposed infants, but was not intended to study the efficacy of co-trimoxazole for reducing clinical disease. Clearly, we could have selected any number of pathogens to study, but we felt that the pneumococcus was a logical choice. Studying colonization dynamics in a relatively small cohort closely over an extended period of time reduced the risk that secular events (time of year, intercurrent outbreaks of pneumococcal disease in the community) would confound our results, and allowed us to evaluate the effect of starting and stopping co-trimoxazole prophylaxis, further strengthening inferences regarding cause and effect. However, an obvious limitation is that studying colonization dynamics is not equivalent to studying invasive pneumococcal disease. Another limitation is that the higher rates of study attrition in the comparison-arm infants could have introduced some degree of bias into our measurements. That said, given that pneumococcal colonization is generally asymptomatic, it seems unlikely that colonization and attrition would be confounded.

Conclusion

Co-trimoxazole prophylaxis modestly suppresses nasopharyngeal colonization with *S. pneumoniae*. Unfortunately, this comes at the price of accelerated acquisition of high-level resistance to co-trimoxazole and potentially other antibiotic classes as well (i.e. clindamycin). The clinical significance of the co-trimoxazole resistance is uncertain, particularly in settings where co-trimoxazole-resistant pneumococci are highly prevalent and sulfonamide exposure extremely common, as in this African community. The lack of effect of co-trimoxazole exposure on the distribution of pneumococcal serotypes is reassuring from the perspective of the future effectiveness of conjugate pneumococcal vaccines. Taken in the context of recent reports of co-trimoxazole's ancillary benefits in African populations,[32,33] on balance, our findings support WHO's current policy on presumptive co-trimoxazole prophylaxis. That said, our data reinforce the pressing need for a refined strategy

for early diagnosis of infant HIV infection, both to minimize unnecessary drug exposure and to optimize the use of precious resources. ■

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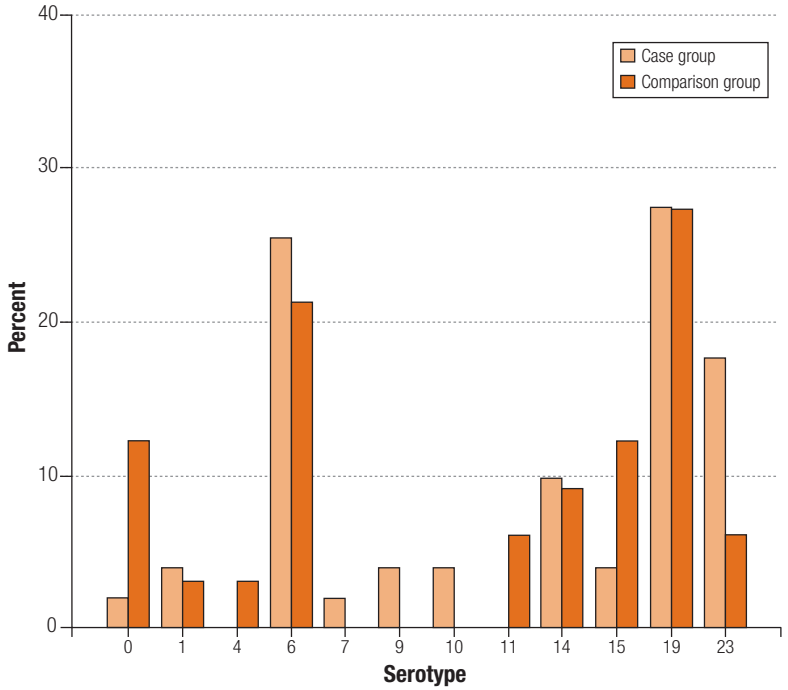
Competing interests: None declared.

Fig. 6. **Frequency of nasopharyngeal pneumococcal serotypes in period 3 (post-cotrimoxazole) of longitudinal cohort study of co-trimoxazole prophylaxis among Zambian infants (*n* = 84)**



Résumé

Effet d'un traitement prophylactique présomptif par le cotrimoxazole sur les taux de colonisation pneumococcique, la séro-épidémiologie et la résistance aux antibiotiques chez les nourrissons zambiens : étude de cohorte longitudinale

Objectif Evaluer les conséquences microbiologiques de la recommandation de l'OMS concernant le traitement présomptif par le cotrimoxazole des nourrissons exposés au VIH pendant la période périnatale.

Méthodes Dans le cadre d'une étude longitudinale de cohorte, nous avons suivi trois fois par mois des nourrissons exposés et non exposés au VIH sur une durée allant jusqu'à 18 mois par enfant. Les nourrissons exposés au VIH ont reçu un traitement prophylactique quotidien par le cotrimoxazole de l'âge de 6 semaines à celui de 12 mois au moins. En utilisant *Streptococcus pneumoniae* comme agent pathogène sentinelle, nous avons mesuré l'effet obtenu sur la colonisation nasopharyngée, la résistance aux antibiotiques et la distribution par sérotypes des pneumocoques, en fonction de l'exposition au cotrimoxazole.

Résultats Parmi 260 nourrissons suivis sur 3096 patients-mois, nous avons détecté des pneumocoques sur 360/1394 échantillons (25,8 %). Les nourrissons exposés au VIH étaient colonisés plus fréquemment que ceux non exposés à ce virus (rapport des risques, RR = 1,4 ; intervalle de confiance à 95 %, IC : 1,0-1,9, p = 0,04). La prophylaxie par le cotrimoxazole réduit la colonisation d'environ 7 %, mais accroît le risque de colonisation par des pneumocoques résistants à ce médicament dans les 6 semaines après le début du traitement (RR = 3,2 ; IC à 95 % = 1,3-7,8, p = 0,04). Ce traitement entraîne une augmentation faible, mais statistiquement significative de la colonisation nasopharyngée par des pneumocoques non sensibles à la clindamicyne (RR = 1,6 ; IC à 95 % = 1,0-2,6, p = 0,04), mais n'accroît pas le risque de non sensibilité à la pénicilline (RR = 1,1 ; IC à 95 % = 0,7-1,7), à l'érythromycine (RR = 1,0 , IC à 95 % = 0,6-1,7), à la tétracycline (RR = 0,9 ; IC à 95 % = 0,6-1,5) ou au chloramphénicol (RR = 0,8 ; IC à 95 % = 0,3-2,3). Le cotrimoxazole n'entraîne pas de divergence de la distribution sérotypique dominante des pneumocoques par rapport à celle visée par le vaccin antipneumococcique conjugé heptavalent (RR = 1,0 ; IC à 95 % = 0,7-1,6).

Conclusion Le traitement prophylactique par le cotrimoxazole élimine modérément la colonisation pneumococcique et accélère l'acquisition par les nourrissons de pneumocoques résistants au cotrimoxazole et à la clindamycine. Il semble peu probable qu'il compromette l'efficacité future des vaccins antipneumococciques conjugués.

Resumen

Efecto de la profilaxis de presunción con cotrimoxazol en las tasas de colonización, la seroepidemiología y la antibioticorresistencia neumocócicas en lactantes de Zambia: estudio longitudinal de cohortes

Objetivo Evaluar los efectos microbiológicos de la profilaxis de presunción con cotrimoxazol recomendada por la OMS para los lactantes con exposición perinatal al VIH.

Métodos Mediante un estudio longitudinal de cohortes, se siguió la evolución de lactantes expuestos al VIH y no expuestos al VIH con periodicidad trimestral por espacio de hasta 18 meses por lactante. Los expuestos al virus recibieron cotrimoxazol profiláctico desde las 6 semanas hasta como mínimo los 12 meses de edad. Usando *Streptococcus pneumoniae* como agente patógeno centinela, medimos los efectos del cotrimoxazol en la colonización nasofaríngea, la resistencia a los antibióticos y la distribución de los serotipos en función de la exposición al cotrimoxazol.

Resultados Entre los 260 lactantes sometidos a seguimiento durante 3096 meses-paciente, detectamos neumococos en 360/1394 (25,8%) muestras. Los lactantes expuestos al VIH presentaron colonización más a menudo que los no expuestos al virus (conciente de riesgos, RR: 1,4, intervalo de confianza (IC) del 95%: 1,0–1,9, $p = 0,04$). La profilaxis con cotrimoxazol redujo la colonización en un 7% aproximadamente pero aumentó el riesgo de colonización por neumococos resistentes al cotrimoxazol en las 6 semanas siguientes al comienzo de la profilaxis (RR: 3,2, IC95%: 1,3–7,8, $p = 0,04$). La profilaxis con contrimoxazol provocó un aumento ligero pero estadísticamente significativo de la colonización nasofaríngea por neumococos insensibles a la clindamicina (RR: 1,6, IC95%: 1,0–2,6, $p = 0,04$), pero no aumentó el riesgo de insensibilidad a la penicilina (RR: 1,1; IC95%: 0,7–1,7), eritromicina (RR: 1,0; IC95%: 0,6–1,7), tetraciclina (RR: 0,9; IC95%: 0,6–1,5) o cloranfenicol (RR: 0,8; IC95%: 0,3–2,3). La profilaxis con cotrimoxazol no alteró el perfil de serotipos neumocócicos en comparación con los establecidos como diana de la vacuna antineumocócica conjugada heptavalente (RR: 1,0, IC95%: 0,7–1,6).

Conclusión La profilaxis con cotrimoxazol suprime moderadamente la colonización por neumococos y acelera la adquisición de neumococos resistentes al cotrimoxazol y la clindamicina en los lactantes. Es improbable que esa profilaxis reste eficacia a las vacunas conjugadas en el futuro.

ملخص

تأثير الاتقاء الترجيحي بالكوتريموكسازول على معدّلات استعمار المكورات الرئوية، وعلى الوبائيات السيرولوجية، وعلى المقاومة للمضادات الحيوية لدى الرضّع في زامبيا: دراسة أترابية طولانية

الهدف: استهدفت هذه الدراسة الاستيقان من العواقب المكروبيولوجية لتوصية منظمة الصحة العالمية بإعطاء جرعة اتقائية ترجيحية من الكوتريموكسازول للرضّع الذين تعرَّضوا لفيروس الإيدز في الفترة المحيطة بالولادة.

الطريقة: استخدم الباحثون تصميماً أترابيا طولانياً لتتبع كل رضيع من الرضّع المتعرّضين لفيروس الإيدز وغير المتعرّضين له، وذلك كل 3 أشهر ولمدة 18 شهراً. وتلقّى الرضّع المتعرّضون لفيروس الإيدز جرعات اتقائية يومية بالكوتريموكسازول بداية من عمر 6 أسابيع إلى 12 شهراً فأكثر. كما قام الباحثون، على اعتبار أن العقدية الرئوية هي العامل الخافر المسبب للمرض، بقياس مدى تغيير الكوتريموكسازول للاستعمار الأنفي البلعومي، ومقاومة المكورات الرئوية للمضادات الحيوية، وتوزع النمط المصلي، باعتبار ذلك كله دالة للتعرض للكوتريموكسازول.

النتائج: بعد متابعة 260 رضيعاً لمدة 3096 مريض - شهر، اكتشف الباحثون وجود المكورات الرئوية لدى 360 من 1394 عينة (25.8%). ولوحظ تكرار المستعمرات لدى الرضّع المتعرضين لفيروس الإيدز أكثر مما كان لدى غير المتعرّضين للفيروس (نسبة الاختطار: 1.4، عند فاصلة ثقة 95%: إذ تراوحت من 1 إلى 1.9، عند نسبة احتمال 0.04). ولوحظ أن الجرعة الاتقائية بالكوتريموكسازول قللت الاستعمار بحوالي 7%، ولكنها رفعت خطر الاستعمار بالمكورات الرئوية المقاومة للكوتريموكسازول خلال 6 أسابيع من بدء إعطاء الجرعات الاتقائية (نسبة الاختطار 3.2، عند فاصلة ثقة 95%: إذ تراوحت من 1.3 إلى 7.8، عند نسبة احتمال 0.04). كما بيَّنت الدراسة أن الجرعة الاتقائية بالكوتريموكسازول أدت إلى زيادة بسيطة، ولكنها مهمة إحصائيا، في الاستعمار الأنفي البلعومي بالمكورات الرئوية العديمة التأثر بالكليندااميسين (نسبة الاختطار: 1.6 ، عند فاصلة ثقة 95%: إذ تراوحت من 1 إلى 2.6، ونسبة احتمال 0.04)، ولكن لم تؤد الجرعة الاتقائية إلى زيادة في خطر عدم التأثر بالبنسلين (نسبة الاختطار: 1.1 ، عند فاصلة ثقة 95% : إذ تراوحت من 0.7 إلى 1.7)، أو للإريثروميسين (نسبة الاختطار: 1 ؛ عند فاصلة ثقة 95%: إذ تراوحت من 0.6 إلى 1.7) ، أو للتتراسيكلين (نسبة الاختطار: 0.9 عند فاصلة ثقة 95%: إذ تراوحت من 0.6 إلى 1.5)، أو للكلورامفينيكول (نسبة الاختطار 0.8، عند فاصلة ثقة 95%: إذ تراوحت من 0.3 إلى 2.3). كما بينت الدراسة أن الجرعة الاتقائية بالكوتريموكسازول لم تؤد إلى اختلاف النمط المصلي السائد للمكورات الرئوية عن الأنماط المصلية المستهدفة بلقاح المكورات الرئوية المقترن السباعي التكافؤ (نسبة الاختطار: 1؛ عند فاصلة ثقة 95%: إذ تراوحت من 0.7 إلى 1.6).

الاستنتاج: تؤدي الجرعات الاتقائية من الكوتريموكسازول إلى كبت مستعمرات المكورات الرئوية بدرجة بسيطة، ولكنها أدت إلى تسريع إصابة الرضّع بالمكورات الرئوية المقاومة للكوتريموكسازول والكليندااميسين. ويبدو من غير المرجح أن تؤدي الجرعات الاتقائية بالكوتريموكسازول إلى تقليل نجاعة اللقاحات المتقارنة في المستقبل.

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