Am. J. Trop. Med. Hyg., 79(3), 2008, pp. 320–330

Does Cotrimoxazole Prophylaxis for the Prevention of HIV-Associated Opportunistic Infections Select for Resistant Pathogens in Kenyan Adults?

Mary J. Hamel,* Carolyn Greene, Tom Chiller, Peter Ouma, Christina Polyak, Kephas Otieno, John Williamson, Ya Ping Shi, Daniel R. Feikin, Barbara Marston, John T. Brooks, Amanda Poe, Zhiyong Zhou, Benjamin Ochieng, Eric Mintz, and Laurence Slutsker

Centers for Disease Control and Prevention, Atlanta, Georgia; Kenya Medical Research Institute, Centre for Global Health Research, Kisumu, Kenya; Atlanta Research and Education Foundation, Atlanta, Georgia

Abstract. We assessed the effect of daily cotrimoxazole, essential for HIV care, on development of antifolate-resistant *Plasmodium falciparum*, naso-pharyngeal *Streptococcus pneumoniae* (pneumococcus), and commensal *Escherichia coli*. HIV-positive subjects with CD4 cell count < 350 cells/μL (lower-CD4; $N = 692$) received cotrimoxazole; HIV-positive with CD4 cell count ≥ 350 cells/μL (higher-CD4; $N = 336$) and HIV-negative subjects ($N = 132$) received multivitamins. Specimens were collected at baseline, 2 weeks, monthly, and at sick visits during 6 months of follow-up to compare changes in resistance, with higher-CD4 as referent. *P. falciparum* parasitemia incidence density was 16 and 156/100 person-years in lower-CD4 and higher-CD4, respectively (adjusted rate ratio [ARR] = 0.11; 95% confidence interval [CI] = 0.06–0.15; $P < 0.001$) and 97/100 person-years in HIV-negative subjects (ARR = 0.62; 95% CI = 0.44–0.86; $P = 005$). Incidence density of triple and quintuple dihydrofolate-reductase/dihydropteroate-synthetase mutations was 90% reduced in lower-CD4 compared with referent. Overall, cotrimoxazole non-susceptibility was high among isolated pneumococcus (92%) and *E. coli* (76%) and increased significantly in lower-CD4 subjects by Week 2 ($P < 0.005$). Daily cotrimoxazole prevented malaria and reduced incidence of antifolate-resistant *P. falciparum* but contributed to increased pneumococcus and commensal *Escherichia coli* resistance.

INTRODUCTION

Globally, two thirds of the estimated 35 million HIV-infected persons live in sub-Saharan Africa, where the epidemic causes massive human suffering and economic loss.

Although recent international focus and funding have been directed toward increasing access to antiretrovirals in Africa, the vast majority of HIV-infected Africans still lack access to these life-saving drugs. Cotrimoxazole (CTX) or trimethoprim-sulfamethoxazole daily prophylaxis is a critical component of HIV care. Daily CTX is a low-cost regimen that has been shown to reduce morbidity and mortality in HIV-infected adults and children in sub-Saharan Africa.[1–3] CTX prophylaxis reduces the incidence of illness caused by bacterial and parasitic agents, including malaria, pneumonia, and diarrhea in areas with both low and high prevalence of CTX-resistant bacteria.[1–3] In addition, CTX prophylaxis slows HIV progression[3] and reduces morbidity and mortality among HIV-negative family members.[1,4] However, concerns remain about the consequences of widespread CTX prophylaxis, specifically whether such use would promote corresponding population-level resistance with resultant loss of the utility of CTX to treat life-threatening bacterial infections and the loss of sulfadoxine pyrimethamine (SP) to treat malaria caused by *Plasmodium falciparum*.[2,5–9]

Although many countries have changed their first-line antimalarial treatment to artemisinin-containing combination therapies, SP continues to be used for treatment of uncomplicated malaria, usually as a component of combination therapy or as monotherapy for intermittent preventive treatment of malaria in pregnancy (IPTp) and in infants (IPTi).[10] SP inhibits two critical enzymes in the *P. falciparum* folic acid biosynthetic pathway: pyrimethamine inhibits dihydrofolate reductase (DHFR), whereas sulfadoxine inhibits dihydropteroate synthetase (DHPS). Mutations in the *DHFR* and *DHPS* genes have been associated with SP resistance.[11–15] The presence of triple mutations in the *DHFR* gene have been correlated with a high level of clinical resistance to antifolate drugs in Africa. The addition of specific *DHPS* mutations, creating a quintuple mutation, increases resistance to SP and another antifolate antimalarial drug, chlorproguanil dapsone.[16]

We conducted a prospective study to assess whether the use of daily CTX resulted in significant changes in antifolate and CTX resistance among common organisms. We measured the change in prevalence of antifolate-resistant *P. falciparum* malaria by identifying mutations at the DHFR and DHPS alleles. We measured changes in the prevalence of CTX- and penicillin-resistant naso- or oro-pharyngeal *Streptococcus pneumoniae* (pneumococcus) as a surrogate for invasive disease.[17–21] Like other investigators in resource-poor settings, we measured the prevalence of CTX-resistant commensal *Escherichia coli* as a marker for CTX-resistance among other pathogenic *Enterobacteriaceae* (e.g., Shigella, *Salmonella*). Because of the rapid plasmid-mediated transfer of CTX resistance between fecal *E. coli* and other *Enterobacteriaceae*, the prevalence of fecal *E. coli* resistance to CTX could reflect similar resistance patterns in pathogenic Enterobacteriaceae.[22]

MATERIALS AND METHODS

This study was approved by the Institutional Review Boards of the Centers for Disease Control and Prevention (CDC) and the Kenya Medical Research Institute (KEMRI).

Study site. The study was conducted at the KEMRI/CDC Clinical Research Center at the Nyanza Provincial General Hospital in Kisumu city between May 2002 and December 2003. HIV prevalence is high in Kisumu; 20% of men and 30% of women 15–49 years of age are HIV infected.[23] At the initiation of this study, a single HIV voluntary counseling and testing site had recently opened in Kisumu, no centers had

* Address correspondence to Mary J. Hamel, Unit 64112, APO 09831. E-mail: mhamel@ke.cdc.gov

been established to provide medical care for HIV-infected persons and daily CTX for prophylaxis of opportunistic infections (OI) among HIV-infected persons was not yet recommended by the Kenyan Ministry of Health. Although antiretroviral access has increased in Kenya during the past 3 years, antiretrovirals were poorly accessible in Kenya throughout the study period.

Nyanza Province has perennial malaria transmission, with > 98% of infections caused by *P. falciparum*. Residents living in the periurban area of Kisumu receive up to 400 infectious bites per person per year.[24] At the time of this study, SP was the first-line therapy for uncomplicated malaria and for IPTp and was widely available in health facilities and in the community.

In addition to malaria, acute respiratory infections and diarrheal diseases are common illnesses and leading causes of childhood death in Nyanza Province.[25] CTX is the first-line antibiotic for empiric treatment of respiratory infections. Although not officially recommended by the Ministry of Health, CTX is also commonly prescribed for treatment of acute bloody or non-bloody diarrhea.[26]

Design. We conducted a prospective, open-label, nonrandomized cohort trial to compare the incidence density of antifolate-resistant *P. falciparum* and the prevalence of CTX-resistant nasopharyngeal *S. pneumoniae* and gastrointestinal *E. coli* among HIV-infected persons receiving daily CTX for OI prophylaxis with HIV-infected persons not receiving daily CTX. We aimed to enroll between 680 and 1,380 HIV-infected persons, depending on baseline prevalence of DHFR and DHPS mutations to measure a 3-fold difference in the incidence density of antifolate markers of resistance among HIV-infected subjects taking daily CTX and those not taking daily CTX with 80% power at the 95% confidence level. We aimed to enroll ~100 HIV-negative adults to reduce any stigma associated with enrollment in the study and to measure changes in the same resistance markers.

Eligible subjects 15 years of age and older, who agreed to HIV testing, were not severely ill nor in the first trimester of pregnancy, and who were not taking daily antibiotics for treatment of a chronic illness (excluding tuberculosis) were recruited into the study from local home-based care organizations. Home-based care organizations typically provided emotional support and nursing care to community members presumed to be HIV-infected, but no medications. Subjects were also recruited from the sole voluntary HIV counseling and testing site that had been established in Kisumu several months before the initiation of our study. At the initial screening visit, eligible subjects underwent HIV pre- and post-test counseling according to national guidelines and were tested for HIV infection, and their CD4 cell count was measured. All consenting eligible HIV-infected subjects and a systematic sample of eligible HIV-negative subjects were asked to return for enrollment. Subjects who began antibiotics or antimalarial drugs after screening were not enrolled until at least 3 full days after completing the antimicrobial course.

At the enrollment or baseline visit, demographic, socioeconomic, and risk factor data were collected. The study clinician performed a medical history and physical examination. A blood sample was taken to measure leukocyte count, hemoglobin, and malaria parasitemia and for genotyping at the *P. falciparum* DHFR and DHPS alleles. Naso- and oropharyngeal swabs, stool samples, and rectal swabs were collected.

Subjects were assigned to study arms according to HIV status and CD4 cell count at screening. HIV-infected subjects presenting with CD4 cell count < 350 cells/μL (lower-CD4) were provided daily CTX (800 mg sulfamethoxazole, 160 mg trimethoprim) as a single tablet per day. HIV-infected subjects with CD4 cell count ≥ 350 cells/μL (higher-CD4) and HIV-negative subjects (HIV-negative) were provided a daily multivitamin. The multivitamins provided contained no more than the recommended daily allowance of each vitamin and were provided so that clients in each arm received a study drug at scheduled visits. This was done to reduce identification by other participants of those who were HIV infected. Multivitamins were selected as the drug for the nonintervention arms before reports that higher doses of multivitamins reduce progression of HIV disease.[27]

The threshold of CD4 count < 350 cells/μL for initiation of CTX prophylaxis was selected after consultation with HIV experts including members of the Kenyan Ministry of Health and was based on findings from the randomized controlled trial of CTX prophylaxis conducted in Abidjan, which showed a mortality benefit for subjects with CD4 cell counts < 350 cells/μL[2].

All subjects were asked to return to the clinic for scheduled visits 2 weeks after beginning study drugs and every 4 weeks thereafter for a total of six monthly visits. Subjects were also asked to return for assessment and treatment any time they became ill between scheduled visits. To increase likelihood of adherence with this request, transport reimbursement was provided for all visits. At all visits, a blood sample was collected for malaria smears, hemoglobin, and parasite genotyping. At the initial, Week 2 and Months 2, 4, and 6 visits, naso- and oro-pharyngeal swabs were collected for pneumococcus isolation and antimicrobial sensitivity testing. A stool sample or rectal swab was collected for *E. coli* antimicrobial sensitivity testing at Months 2, 4, and 6 visits; a Week 2 sample collection was added in March 2003. Collection of routine stool specimens stopped in August 2003, when sufficient sample size was achieved. At every visit, subjects were examined by a study nurse or clinician and asked questions about recent illness, health facility visits, hospitalizations, antimicrobial use since the last visit, and adherence to study drug. Pill counts were conducted to verify reported adherence. Discrepancies in pill counts and reported adherence were probed and recorded adherence was adjusted accordingly. Study drug supplies were replenished, such that sufficient pills were provided to last to the next follow-up visit plus six additional pills. Subjects who presented late for follow-up visits and therefore ran out of study drug were considered to have missed doses of study drug.

At any visit, subjects with either *P. falciparum* infection with measured or reported fever or *P. falciparum* infection with parasite density ≥ 400 parasites/μL regardless of fever, were diagnosed with clinical malaria and provided SP for treatment of malaria according to national guidelines at the time of the study The cutoff of 400 parasites/μL was selected as a reasonable threshold based on earlier epidemiologic studies in children in the same area.[28]

Subjects treated with SP were asked to return to the clinic 7 and 14 days after treatment or any time if their condition worsened for a repeat malaria blood smear. Those who failed

SP were treated with artemether-lumefantrine or quinine. Daily CTX was not withheld when SP was provided.

Laboratory tests. *HIV testing.* HIV testing was done using Unigold (Abbott Laboratories, Tokyo, Japan) and Determine (Trinity Biotech, Bray, Ireland) rapid HIV tests, run in parallel. Discordant results were resolved using the Capillus (Cambridge Diagnostics, Wiclow, Ireland) test according to Kenyan national guidelines.

Malaria smears. Giemsa-stained thick and thin blood smears were prepared from finger stick blood samples. Parasite densities were estimated by counting the number of asexual parasites per 300 white blood cells. Measured leukocyte counts were used to calculate densities. Blood smears were examined independently by two microscopists; discordant results were resolved based on a reading by a third independent microscopist.

Plasmodium falciparum molecular analysis. All *P. falciparum* infections identified by light microscopic examination of thick and thin blood smears, regardless of parasite density, were genotyped for mutations at codons 50, 51, 59, and 108 in DHFR and at codons 437 and 540 in DHPS by restriction fragment length polymorphism (RFLP). When attempts to genotype by RFLP method failed, we used pyrosequencing to complete genotyping. All samples with DHFR Leu164 mutation by RFLP were confirmed using direct sequencing and/or pyrosequencing as previously described.[29,30]

We designated specimens with two mutations isolated at DHFR codons 108 and 51 or 59 a double mutation, those with mutations at codons 108, 51, and 59 a triple mutation, and a triple DHFR mutation with mutations at codons DHPS 437 and 540 a quintuple mutation.

Pneumococcus isolation and analysis. Naso- and oropharyngeal swabs were immediately placed in a culture media consisting of skim milk, glycerol, and glucose, and after transport, were frozen at −70°C. Swabs were plated onto defibrinated sheep's blood agar with 5% gentamicin and incubated at 37°C for 24–48 hours. Pneumococcal colonies were identified by morphology and optochin susceptibility. Subcultures of pneumococcal colonies were plated onto Mueller-Hinton sheep's blood agar and tested for susceptibility to CTX and penicillin using CTX and oxacillin disks. Isolates underwent minimum inhibitory concentration (MIC) testing using the broth microdilution technique for CTX and penicillin according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS).[31] Pneumococcus non-susceptibility to CTX and penicillin was defined as MIC of ≥ 1 μg/mL.

Enteric specimens. Swabs of fresh whole stool or rectal swabs were placed in Cary-Blair media, transported cold (4°C) to the CDC/KEMRI laboratory, and cultured for *E. coli* by plating on MacConkey agar and incubating for 24 hours at 37°C. One *E. coli* colony was selected from each plate and subcultured and then tested for susceptibility to CTX by the disc diffusion method per NCCLS guidelines.[31]

Other tests. All women of childbearing age not visibly pregnant had a rapid urine pregnancy test performed at enrollment. Hemoglobin was measured using Hemocue machines (Hemocue, Angelholm, Sweden).

Data analysis and statistical testing. Monthly visits were scheduled at 4-week intervals, giving a total follow-up time of 24 weeks. Those presenting late for scheduled visits were seen, and data were included in the analysis. In determining malaria incidence density, subjects who were treated with SP were not considered to be at risk for malaria for 28 days after SP treatment. Subjects with a positive malaria smear with low parasitemia, who did not receive treatment, were not considered to be at risk for malaria until after they had a subsequent blood smear negative for malaria parasitemia. All visits, both scheduled and sick visits, were used to calculate incidence density of *P. falciparum* parasitemia and antifolate resistance. Because baseline measurements differed between study arms, we compared the change in resistance from baseline to follow-up visits for each study arm for measurements of antifolate resistance, pneumococcus isolate non-susceptibility, carriage of non-susceptible pneumococcus, and *E. coli* resistance.

Categorical variables were compared with χ^2 or Fisher exact tests and continuous variables with rank-sum and *t*-tests. Kaplan-Meier survival plots were used to describe time-to-first-malaria-infection, right censored at the subject's last study visit. Study arm comparisons were made with the log-rank test.

We used Poisson regression to model changes in *P. falciparum* resistance from baseline through follow-up. Follow-up time was truncated at the last study date or first malaria infection date for the model of first or only malaria infection and at the last study date only for the model of multiple malaria infections. Follow-up time was decreased by 28 days for subjects who received SP. To model incidence of antifolate resistance markers, we used logistic regression models (generalized estimating equations with a logit link function; SAS 9.1; SAS Institute, Cary, NC) to account for correlated observations. Initially we adjusted for housing type, parasitemia at baseline, seasonality (high transmission versus low transmission), ITN use, prior antimalarial use, and hemoglobin at baseline. We removed seasonality, prior antimalarial use, and baseline hemoglobin from the model because they were non-significant. Although ITN use also was non-significant, we retained this variable in the model.

We modeled change in pneumococcus and *E. coli* resistance with logistic regression models using generalized estimating equations. We adjusted for housing type, seasonality (rainy versus dry), recent antibiotic use, and recent antifolate use for pneumococcus-specific data. Housing type and recent antibiotic use were significant, and seasonality approached significance. In the model for diarrhea-specific data, we adjusted for housing type, seasonality (rainy versus dry), recent antibiotic or antifolate use, and diarrhea during the prior 24 hours. Recent antifolate and antibiotic use was significant.

When analyzing *P. falciparum* resistance markers, we compared the incidence density at individual codons, triple mutations, and quintuple mutations. We calculated 95% confidence limits by adding a false record with small weight (0.05) to avoid the problem of zero counts in some cells. We classified mutations as triple and quintuple pure, indicating single clonal infections, and triple and quintuple mixed, indicating polyclonal infections, as described by Kublin and others.[16] Bonferroni correction was used when multiple comparisons were made.

RESULTS

We assigned 1,160 subjects to three study arms: 132 HIV-negative subjects (HIV-negative) and 336 HIV-positive subjects with CD4 cell count ≥ 350 (higher-CD4) received a daily

multivitamin, whereas 692 subjects with CD4 cell count < 350 (lower-CD4) received daily CTX. The study profile is shown in Figure 1.

Characteristics of subjects at baseline are shown in Table 1. Lower-CD4 subjects were older than higher-CD4 subjects (referent). HIV-negative subjects had higher median hemoglobin, whereas lower-CD4 subjects had lower median hemoglobin compared with the referent. Malaria parasitemia prevalence was lower in lower-CD4 than higher-CD4 subjects; this difference remained significant after controlling for hemoglobin, ITN use, prior antimalarial use, and housing type (adjusted rate ratio [RR] = 0.53; 95% confidence interval [CI] = 0.34 – 0.82; $P < 0.025$). Subjects in all study arms reported high recent antibiotic and antimalarial use, although HIV-negative subjects reported lower use than the referent. Lower-CD4 subjects had higher antibiotic and antimalarial use in the week before enrollment compared with the referent.

Median and mean follow-up time for all subjects was 24 and 20 weeks, respectively. HIV-negative and lower-CD4 subjects had less mean follow-up time (19 weeks in each arm) than higher-CD4 subjects (21 weeks; $P < 0.001$). Lower-CD4 subjects were nearly seven times more likely to die during follow-up (14%) than higher-CD4 subjects (2%; RR = 6.73; 95% CI = 3.16–14.33) and nine times more likely than HIV-negative subjects (2%; RR = 9.25; 95% CI = 2.31–37.06). The increase in deaths accounted fully for the decrease in follow-up time among lower-CD4 subjects. There was no significant difference in baseline characteristics within study arms between those who completed the study and those who did not (data not shown).

Eleven subjects discontinued the study because of a drug reaction: all in the lower-CD4 arm. Two subjects experienced a severe adverse reaction to CTX, both with blistering rash that resolved uneventfully after discontinuation of CTX; the other nine experienced mild rash. Other reasons for not completing 6 months of follow-up did not differ by study arm. Drug adherence was high; 85% of subjects in all study arms reported missing no doses of study drugs. A total of 7 subjects in the HIV-negative arm and 37 in the higher CD4 arm reported taking at least one course of CTX for treatment of illness during the follow-up period. There was no difference in number of sick visits by study arm, with an average of 2.8, 3.0, and 3.2 per person-year in the HIV-negative, higher-CD4, and lower-CD4 arms, respectively ($P > 0.05$ for HIV-negative and for lower-CD4 compared with reference).

CTX and parasitemia. Lower-CD4 subjects had an estimated 90% reduction in incidence density of first or only infection with *P. falciparum* parasitemia compared with higher-CD4 subjects (Figure 2; Table 2) and a similar reduction in incidence density for all episodes of *P. falciparum* parasitemia. This protective effect of 89–90% against *P. falciparum* infection in the CTX-administered group remained after adjusting for prior *P. falciparum* parasitemia, SP use, ITN use, and housing type in Poisson regression models ($P < 0.001$). HIV-negative subjects had a significant (38%) reduction in all episodes of *P. falciparum* parasitemia ($P = 0.018$ adjusted) compared with referent. Compared with lower-CD4 subjects who reported missing no study drug in the prior month, lower-CD4 subjects who missed any or all doses of CTX in the prior month were at a 3-fold (RR = 3.0, 95% CI = 1.20–7.06 adjusted for correlated data) and 11-fold (RR = 10.7, 95% CI = 3.46–33.17 adjusted for correlated data) increased risk of parasitemia during the subsequent follow-up visit, respectively.

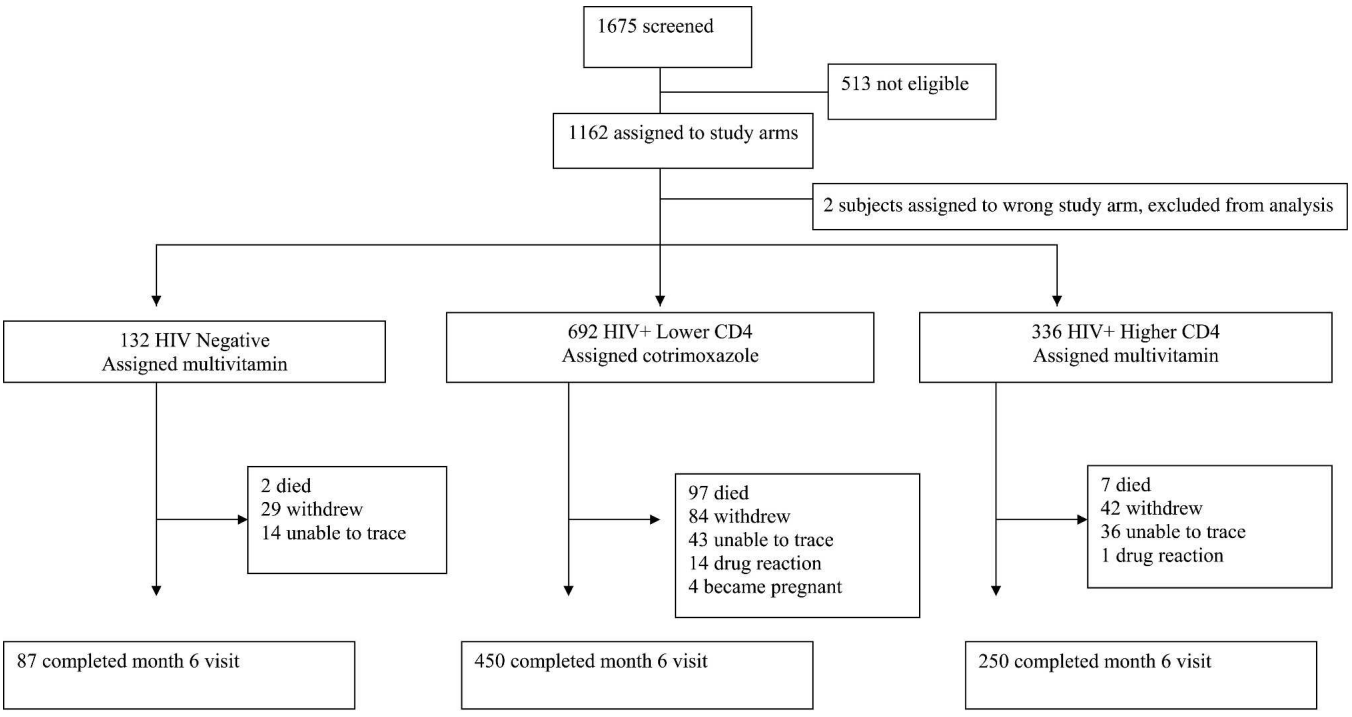

FIGURE 1. Trial profile: the effect of daily cotrimoxazole prophylaxis on the development of resistant pathogens, Kisumu, Kenya.

TABLE 1

Study population characteristics at enrollment among HIV-infected subjects taking CTX prophylaxis compared with those not taking CTX prophylaxis, Kisumu, Kenya

	HIV negative (N = 132)	HIV+ lower-CD4, daily CTX (N = 692)	HIV+ higher-CD4 (N = 336; Ref)
Median age in years (range)	29 (17–70)	34 (18–71)*	31 (16–74)
Female	57%	63%	59%
Completed primary school	55%	53%	52%
Household type			
Traditional mud hut	14%	20%	20%
Semi-permanent (corrugated iron roof)	66%	54%	57%
Permanent (concrete or stone walls)	20%	26%	23%
Slept under an ITN the prior night	12.9%	11.7%	13.7%
Median CD4 cell count in cells/μL (range)	NA	168 (0–349)†	561 (350–1,739)
Taking antiretrovirals	0%	2.4%	1.5%
Median hemoglobin (g/dL)	12.4*	9.9*	11.5
P. falciparum parasitemia prevalence	13.6%	7.4%*	13.1%
Geometric mean *P. falciparum* parasite density (per microliter) among those with parasites (N = 113)	346	776	399
Clinical malaria (*P. falciparum* parasitemia with fever)	0%	0.6%	0.6%
Antibiotic use in the past			
Week	8%†	27%*	16%
Month	49%*	70%	65%
Antimalarial use in the past			
Week	5%*	18%†	13%
Month	41%*	59%	58%
Either CTX or SP use in the past			
Week	3%*	14%	10%
Month	31%*	46%	44%

* $P < 0.025$ compared with HIV+ higher-CD4.
† $P < 0.05$ compared with HIV+ higher-CD4.
NA = not available.

DHFR and DHPS markers of antifolate resistance. *Plasmodium falciparum* parasitemia was present in 113 blood smear specimens at baseline (18 in HIV-negative, 44 in higher-CD4, and 51 in lower-CD4) and 300 specimens during follow-up (45 in HIV-negative, 209 in higher-CD4, 46 in lower-CD4). Baseline mutation prevalence ranged from 95% to 100%, 92% to 100%, and 56% to 82% at DHFR codons 108, 51, and 59, respectively at baseline. Baseline prevalence

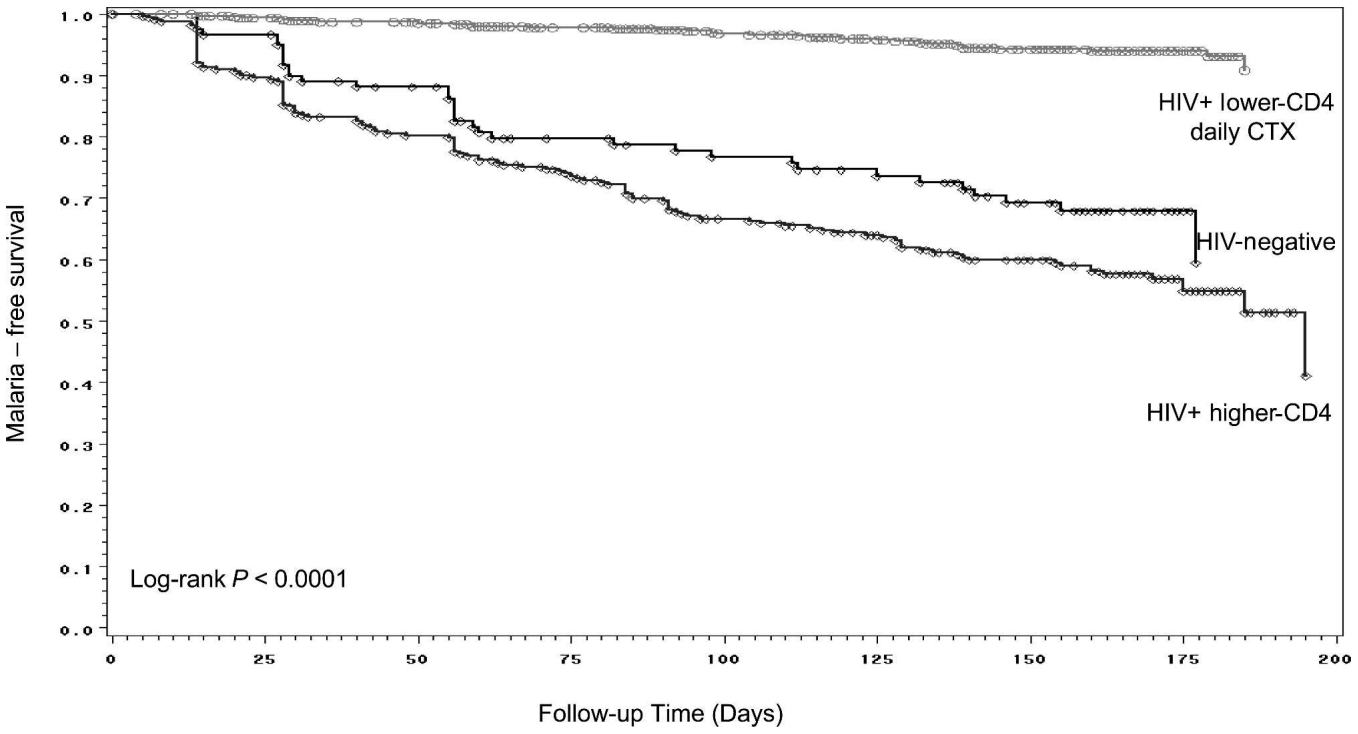

FIGURE 2. Probability of remaining free from malaria parasitemia among HIV-infected clients taking daily CTX and HIV-negative and HIV-infected clients not taking daily CTX, Kisumu, Kenya.

TABLE 2
Unadjusted and adjusted incidence density of first or only malaria episode and any malaria episode per 100 person-years among HIV-infected subjects taking CTX prophylaxis compared with those not taking CTX prophylaxis, Kisumu, Kenya*

	HIV-negative (N = 132)				HIV+ lower-CD4, daily CTX (N = 692_				HIV+ higher-CD4 (N = 336)		
Malaria	Events	Person-year at risk	Rate per 100 person-years (95% CI)	Rate ratio (95% CI) compared with higher-CD4	Events	Person-year at risk	Rate per 100 person-years (95% CI)	Rate ratio (95% CI) compared with higher-CD4	Events	Person-year at risk	Rate per 100 person-years (95% CI; reference)
First or only episode	36	38	95 (68–131)	0.69 (0.48–1.00)	34	241	14 (10–20)	0.10 (0.07–0.15)†	131	96	137 (115–162)
Adjusted				0.72 (0.49–1.13)				0.10 (0.06–0.15)†			
All episodes	43	44	97 (72–131)	0.62 (0.44–0.86)†	39	245	16 (12–22)	0.10 (0.07–0.14)†	186	119	156 (135–180)
Adjusted				0.77 (0.53–1.10)†				0.09 (0.06–0.14)†			

* Adjusted for housing type, baseline parasitemia, seasonality, and ITN use in a Poisson regression model. Malaria episode defined as any visit with *P. falciparum* parasitemia isolated, regardless of parasite density.
† $P < 0.025$.

of mutations at DHPS codons 437 and 540 was at least 89% in all study arms. During follow-up, prevalence of resistant mutations at these individual codons did not significantly increase in any study arm.

The prevalence of triple and quintuple mutations generally increased in all study arms, but there was no significant increase among lower-CD4 subjects compared with the higher-CD4 subjects (Table 3). Notably, because of the low parasite incidence density among lower-CD4 subjects, the incidence density of triple and quintuple mutant DHFR/DHPS genotypes in lower-CD4 subjects was significantly lower than in higher-CD4 subjects (Table 4).

SP effectiveness in subjects on CTX. We attempted to measure SP effectiveness among subjects presenting with clinical malaria before and after beginning daily CTX to determine whether those taking CTX were at increased risk for selecting resistant *P. falciparum* parasites; however, because of the low incidence of *P. falciparum* parasitemia, especially in lower-CD4 subjects, we did not have adequate statistical power to compare results. Among subjects who presented at baseline with clinical malaria (that is *P. falciparum* infection and reported fever during the prior 24 hours or axillary temperature > 37.5°C or *P. falciparum* infection with parasite density ≥ 400 parasites/μL regardless of fever), 0/8 (0%), 2/31 (6%), and 2/18 (11%) of subjects treated with SP in the HIV-negative, lower-CD4, and higher-CD4 arms, respectively, returned with *P. falciparum* parasitemia by Day 14. Among subjects who presented with clinical malaria during follow-up, after beginning study drug, 0/11 (0%) HIV-negative, 2/14 (14%) lower-CD4, and 2/67 (3%) higher-CD4 subjects had parasitemia detected by Day 14.

CTX non-susceptible pneumococcus. A total of 25/129 (19%), 241/687 (35%), and 112/334 (34%) of HIV-negative, lower-CD4, and higher-CD4 subjects, respectively, had pneumococcus isolated at baseline. The percentage of isolates that were CTX non-susceptible was high in all study arms (range: 76–93%) at baseline (Figure 3) and increased significantly in lower-CD4 subjects, with 223/241 (93%) isolates resistant at baseline and 196/196 (100%) isolates resistant 2 weeks later ($P = 0.005$). The prevalence of non-susceptible isolates remained high in lower-CD4 subjects at month 6, when 99% of 164 isolates were found to be non-susceptible to CTX.

The change in carriage of CTX non-susceptible pneumococcus isolates decreased significantly in higher-CD4 compared with lower-CD4 subjects at Month 6, after controlling for confounders ($P < 0.002$). This difference in non-susceptible pneumococcus carriage was caused by an overall decline in pneumococcus carriage in higher-CD4 subjects ($P = 0.006$) rather than an increase in non-susceptible pneumococcus carriage in lower-CD4 subjects.

At baseline, 56%, 85%, and 76% of pneumococcal isolates were non-susceptible to penicillin in HIV-negative, lower-CD4, and higher-CD4 subjects, respectively. The prevalence of penicillin non-susceptible isolates increased significantly in only HIV-negative subjects to 92% at 6 months ($P = 0.035$).

***Escherichia coli* resistance.** Commensal *E. coli* was isolated from 100% of samples taken at baseline and during follow-up. Prevalence of CTX-resistant *E. coli* was high in all study arms at baseline: 70% (92/132), 79% (542/688), and 74% (248/333) in HIV-negative, lower-CD4, and higher-CD4 subjects, respectively, but significantly increased only among lower-CD4 subjects, reaching 98% (172/176) by Week 2 ($P < 0.0001$).

TABLE 3

Prevalence of DHFR and DHPS mutant genotypes among parasitemic subjects at baseline and during follow-up among HIV-infected subjects taking daily CTX and HIV-negative and HIV-infected subjects not taking daily CTX, Kisumu, Kenya*‡§

	HIV-negative				HIV+ lower-CD4, daily CTX				HIV+ higher-CD4		
	Baseline mutant genotypes [*N* (%)]	Follow-up mutant genotypes [*N* (%)]	Change in prevalence, percentage points	Change in prevalence compared with higher-CD4 (*P*)	Baseline mutant genotypes [*N* (%)]	Follow-up mutant genotypes [*N* (%)]	Change in prevalence, percentage points	Change in prevalence compared with higher-CD4 (*P*)	Baseline mutant genotypes [*N* (%)]	Follow-up mutant genotypes [*N* (%)]	Change in prevalence, percentage points (reference)
Single	0/18 (0%)	0/45 (0%)	0	†	1/50 (2%)	0/41 (0%)	–2	†	0/43 (0%)	3/202 (1.5%)	1.5
Double	10/18 (56%)	16/45 (36%)	–20	0.75	17/50 (34%)	4/41 (10%)	–24	0.16	15/43 (35%)	46/202 (23%)	–12
Triple pure	3/18 (17%)	18/45 (40%)	23	0.31	22/51 (43%)	25/46 (54%)	11	0.93	20/44 (45%)	116/209 (56%)	11
Triple mixed	5/18 (28%)	10/45 (22%)	–6	0.48	10/51 (20%)	9/46 (21%)	1	0.70	6/44 (14%)	36/209 (17%)	3
Quintuple pure	2/18 (11%)	15/45 (33%)	22	0.26	20/51 (39%)	22/46 (48%)	9	0.93	18/44 (41%)	106/209 (51%)	10
Quintuple mixed	3/18 (17%)	9/45 (20%)	3	0.48	9/51 (18%)	6/46 (13%)	–5	0.13	3/44 (7%)	32/209 (15%)	8

* Mixed infections, with mutant and wild-type alleles found at a single codon, are considered to have mutant genotypes in this table. Denominators change because analysis failed at some codons.
† Statistical testing not done because of zero cells.
‡ Columns add to > 100% because quintuple mutations, by definition, contain triple mutations.
§ *P* values calculated from GEE model.

TABLE 4

Incidence density of triple and quintuple mutations per 100 person–years among HIV-infected subjects taking CTX prophylaxis compared with those not taking CTX prophylaxis, Kisumu, Kenya*

	HIV-negative (*N* = 132)				HIV+ lower-CD4, daily CTX (*N* = 692)					HIV+ higher-CD4 (*N* = 336)		
Malaria	Events	Person-year at risk	Rate per 100 person-years	Rate ratio (95% CI) compared with higher-CD4	Events	Person-year at risk	Rate per 100 person-years	Rate ratio (95% CI) compared with higher-CD4	Protective effect	Events	Person-year at risk	Rate per 100 person-years (reference)
Triple pure	18	44.56	40	0.46 (0.28–0.75)	19	245.73	8	0.09 (0.05–0.14)	91%	106	119.76	89
Triple mixed	12	44.93	27	0.86 (0.45–1.67)	8	245.94	3	0.11 (0.05–0.23)	89%	37	120.41	31
Quintuple pure	14	44.64	31	0.44 (0.25–0.77)	12	245.95	5	0.07 (0.04–0.12)	93%	86	119.90	72
Quintuple mixed	12	44.97	27	0.68 (0.36–1.29)	9	245.98	4	0.09 (0.05–0.19)	91%	47	120.19	39

* Results obtained from logistic regression model using GEE.

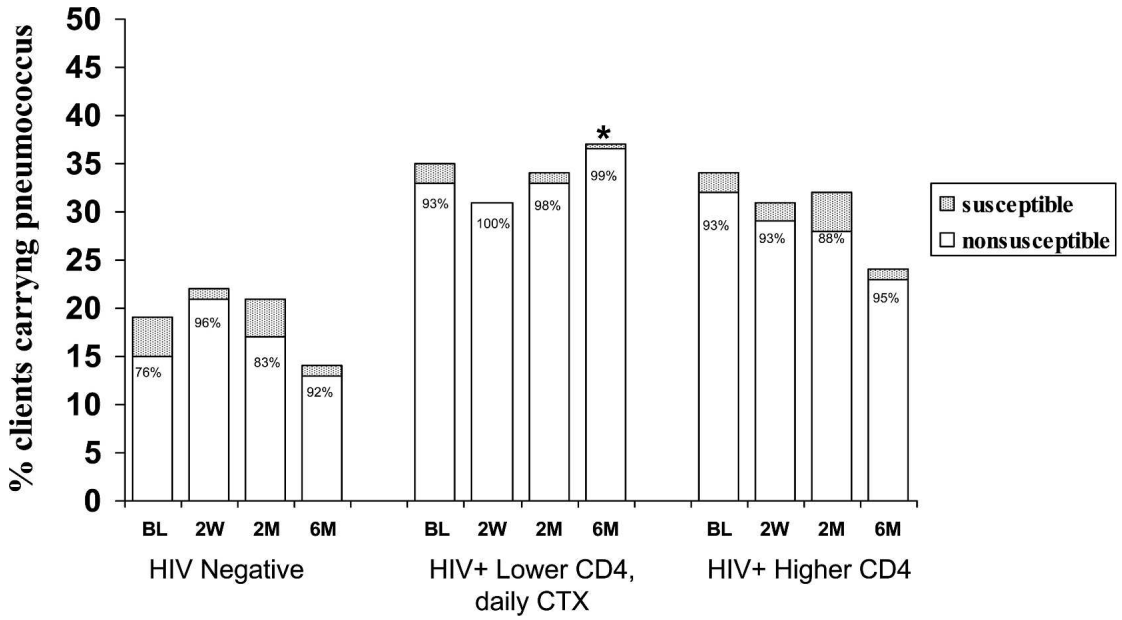

FIGURE 3. CTX non-susceptible and susceptible pneumococcus carriage among HIV-infected subjects taking daily CTX, and HIV-negative and HIV-infected subjects not taking daily CTX, Kisumu, Kenya. BL = baseline; 2W = two week visit; 2M = two month visit; 6M = six month visit. *Increased prevalence of non-susceptible pneumococcus carriage compared with HIV+ higher-CD4 arm from BL to 6M ($P = 0.004$) adjusted for housing type, seasonality, and recent antibiotic use.

CTX resistance remained high through Month 6 (96% [277/290] of isolates). The increase in CTX-resistant *E. coli* was significantly greater among lower-CD4 subjects compared with the referent ($P < 0.01$ adjusted).

DISCUSSION

We conducted a prospective study of the effects of daily CTX on the development of resistant pathogens among HIV-infected persons. In several sub-Saharan countries, daily CTX prophylaxis for HIV-infected persons has been adopted into national policy, and Ministries of Health require information on the potential effects of widespread CTX use to plan for the consequences.

In this malaria-endemic area, we found that daily CTX significantly reduced malaria incidence, with a protective efficacy of 89%. Because of the decreased incidence of malaria, daily CTX resulted in an almost 90% reduction in molecular markers of high-level antifolate resistance compared with HIV-infected subjects not taking daily CTX. Thus, in this setting, it seems unlikely that daily CTX use will contribute significantly to the loss of antifolate antimalarials for prevention or treatment of *P. falciparum* malaria. These findings support recommendations to provide CTX for HIV-infected persons in Africa.

Although SP monotherapy is no longer recommended as first-line treatment of malaria in most African countries, it still frequently is used as monotherapy,[32] as well as a component of combination therapy, remains the only recommended drug for IPTp and soon may be recommended for IPTi. Our finding that daily CTX use does not select for antifolate-resistant *P. falciparum* provides valuable reassurance to malaria control policy makers and managers who govern in areas where HIV and malaria endemicity overlap; SP use can continue in recommended capacities, even as daily CTX use by HIV-infected persons becomes widespread.

We were underpowered to measure the effectiveness of SP for treatment of clinical malaria in study subjects. There may have been a trend toward increased SP failure among lower-CD4 subjects; however, numbers were small and should be considered with caution. Nonetheless, effectiveness of SP remained > 85% in all study arms.

Importantly, taking daily CTX prevented malaria parasitemia despite high levels of triple and quintuple DHFR/DHPS mutations. This finding is particularly surprising when one considers that the subjects assigned to take daily CTX were the most immunosuppressed. Most likely, CTX adequately prevented malaria despite high prevalence of antifolate-resistant genotypes because of pre-existing levels of malaria-specific acquired immunity among this adult population living in an area of high malaria endemicity. Pre-existing acquired immunity likely also explains the low frequency of SP failure in these adults compared with children, in whom 26% younger than 5 years of age in this region fail SP treatment.[33] These findings underscore the complex nature of immunity and the limitations of *in vitro* measures of resistance, especially when extrapolating to the clinical effects of daily prophylaxis. Likewise, extrapolation of measures of pneumococcus and *E. coli* resistance to CTX prophylactic efficacy may not be straightforward. Prior studies in Uganda, Zambia, and Malawi have shown reduced morbidity and mortality among those taking daily CTX despite high resistance levels among pathogens targeted by the intervention.[3,9,34] In Zambia, *in vitro* CTX resistance among common pathogens was estimated between 60% and 80%, yet daily CTX decreased mortality in HIV-infected children.

We found that daily CTX resulted in relatively higher prevalence of non-susceptible pneumococcus carriage, with the unexpected finding that overall pneumococcus carriage declined among those in the higher-CD4 and HIV-negative arms while remaining stable in the lower-CD4 arm. We cannot definitively explain this decline; however, subjects in our study received regular medical care, including antibiotic prescriptions when needed, and those in the higher-CD4 and HIV-negative arms received a multivitamin. It may be that subjects with a more intact immune system responded to good health care and occasional antibiotic therapy by resolving pneumococcus carriage, whereas those who were immunosuppressed were unable to clear carriage.

The prevalence of penicillin non-susceptible pneumococcus isolates was also high at baseline and increased only among HIV-negative subjects, who had the lowest baseline prevalence of penicillin non-susceptible isolates. Notably, HIV-negative subjects had less recent antibiotic use at baseline compared with the other study arms, and this likely accounts for the lower proportion of non-susceptible isolates at baseline. Again, non-susceptible isolates reached the prevalence of the other two study arms during follow-up.

In Kenya, the rise in non-susceptible pneumococcus, similar to the rise observed in resistant *E. coli* carriage, may be statistically significant but not clinically significant. For both bacteria, the prevalence of *in vivo* CTX resistance was very high before the study drug was begun. Based on these findings, the Ministry of Health might consider alternatives to CTX, such as amoxicillin, for presumptive treatment of non-severe lower respiratory infections. They might also emphasize adherence to current guidelines that propose nalidixic acid, not CTX, for the treatment of bloody diarrhea and shigella.[35] However, the implication for countries with lower levels of CTX resistance merits serious consideration, especially considering the rapid rise in CTX resistance documented in our study.

Because daily CTX was shown to be an efficacious intervention before this study, we were unable to randomize subjects or blind treatment arms. We have been unable to explain the difference in malaria parasitemia among study arms at baseline, specifically the unexpected finding that *P. falciparum* prevalence was reduced among those with lower CD4 cell counts, even after controlling for prior antimalarial use and indicators of *P. falciparum* exposure, such as housing type and ITN use. The primary malaria-transmitting vectors in Nyanza Province, *Anopheles gambiae* and *Anopheles funestus*,[36] bite primarily late at night, when people are asleep indoors. Traditional and semi-permanent houses have open eaves for ventilation, and allow unobstructed movement of mosquitoes in and out of houses; thus, lack of movement out of doors at night among those in the lower-CD4 arm would not necessarily reduce exposure to anopheline mosquito bites. Additionally, there was no difference between study arms in ITN use to explain decreased exposure. We do find more antimalarial use in the week preceding enrollment among those in the lower-CD4 arm, which is not unexpected; in Kenya, reported fever is typically diagnosed and treated as malaria without blood smear confirmation. Subjects were informed at screening that enrollment would be deferred until at least 3 days after completion of any antimalarial drugs, and it may be that recent antimalarial use was therefore underreported.

Although subjects in the lower-CD4 arm had lower *P. falciparum* prevalence at baseline, they did not have lower geometric mean parasite density compared with the reference. This suggests that lower parasite prevalence is likely caused by unmeasured factors, such as recent antimalarial use not reported, and is not caused by an immunologic difference among those in the lower CD4 arm, which resulted in less malaria parasitemia. This assumption is further supported by the finding that missing doses of daily CTX significantly increased risk for subsequent malaria infection during the follow-up period, with an 11-fold increased risk if all CTX doses were missed in the prior month.

Our study had other limitations, including a relatively short study period, with ~24 weeks follow-up. As CTX use becomes widespread, the effectiveness in preventing malaria and preventing breakthrough parasitemia with highly resistant genomes may wane and should be monitored. We also experienced a moderately high loss to follow-up; 32% of subjects failed to make the Month 6 visits. This could have impacted our results; however, we are reassured that there were no differences in baseline characteristics within study arms between those who did and did not complete the Month 6 visit.

To study resistance in pneumococcus, we used nasopharyngeal pneumococcus as a surrogate for invasive disease. Although invasive disease is generally caused by strains that are carried in the nasopharynx, certain serotypes tend to be more or less invasive.[17–19] Because antibiotic resistance in pneumococcus is confined to a handful of serotypes, which tend to be the serotypes that predominate in carriage, it is possible that the evaluation of carried pneumococci for this study might slightly overestimate the impact of CTX prophylaxis in non-susceptibility among invasive pneumococci.

Finally, when looking at enteric organisms, we measured change in resistance in commensal *E. coli* and not enteric pathogens. Although it would have been more informative to have measured the change in diarrheagenic *E. coli* or other bacterial enteric pathogens, the relative rarity of these pathogens would have necessitated a much larger sample size. However, there is a high degree of rapid plasmid-mediated transfer of CTX resistance between fecal *E. coli* and *Salmonella,* as well as other *Enterobacteriaceae,* and thus, commensal *E. coli* can be used as a reasonable surrogate to measure the development of resistance in bacterial enteric pathogens.[37–39]

We have shown that daily CTX did not result in increased *P. falciparum* antifolate resistance; rather, daily CTX was associated with a reduced incidence of malaria and antifolate-resistant genotypes compared with HIV-infected individuals not taking daily CTX. In contrast, daily CTX increased carriage of non-susceptible pneumococci and CTX-resistant *E. coli* and may accelerate the development of CTX resistance among respiratory and diarrheal pathogens, especially in areas with lower baseline CTX resistance. Our findings add essential information and further support the widespread adoption of daily CTX prophylaxis for HIV-infected individuals in Africa. As CTX prophylaxis is adopted into national health programs across Africa, surveillance systems should be established to monitor for increasing incidence of breakthrough malaria infection and to look for increased CTX resistance among respiratory and diarrheal disease pathogens. Ministries of Health adopting daily CTX prophylaxis should introduce strategies to maintain good adherence to daily CTX

and should begin to identify alternative treatments other than CTX for respiratory and enteric diseases.

Received November 23, 2007. Accepted for publication May 30, 2008.

Acknowledgments: We thank the subjects who participated in this study, the CTX clinical staff who provided caring support to the subjects, including Monica Aluoch Ochieng, Mary Owino, Ida Ouma, Agnes Owiti, Grace Otom, and Mary Caesar, the scientific and laboratory team including Ananias Escalante and Venkatachalam Udhayakumar, who provided oversight and technical expertise for the molecular resistance laboratory work, and Cheryl Bopp, Richard R. Facklam, Mary Omwalo, Geoffrey Jagero, Dickens Olang, Lata Kumar, and Ira Goldman. We also thank Carolyn Wright and Kathleen Wannemuehler who contributed to data management and cleaning, Anne Schuchat for technical guidance, and John Vulule for support.

Financial support: Funding was provided for by the Opportunistic Infections Working Group, Centers for Disease Control and Prevention, and the Antimicrobial Resistance Working Group, Centers for Disease Control and Prevention. AAE was supported by NIH Grant R01 GM60740.

Disclosure: The authors do not have a commercial or other association that might pose a conflict of interest. The funding organizations had no role in the design and conduct of the study; in the collection, analysis and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Authors' addresses: Mary J. Hamel, KEMRI/CDC Research Station, Unit 64112, APO 09831, Tel: 254-722-772-616, Fax: 254-57-20-22981, E-mail: mhamel@ke.cdc.gov. Carolyn Greene, Bureau of Public Health Training, NYC Department of Health and Mental Hygiene, 2 Lafayette Street, New York, NY 10007. Tom Chiller, John Williamson, Barbara Marston, John T. Brooks, Amanda Poe, Zhiyong Zhou, Ya Ping Shi, Eric Mintz, and Laurence Slutsker, Center for Disease Control and Prevention, 1600 Clifton Road, NE, Atlanta, GA 30333. Peter Ouma, Kephas Otieno, and Benjamin Ochieng, Box 1678, Kisuma, Kenya. Christina Polyak, 1513 Belt Street, Baltimore, MD 21202.

REFERENCES

1. Anglaret X, Chene G, Attia A, Toure S, Lafont S, Combe P, Manlan K, N'Dri-Yoman T, Salamon R, 1999. Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1-infected adults in Abidjan, Cote d'Ivoire: a randomised trial. Cotrimo-CI Study Group. *Lancet 353:* 1463–1468.
2. Wiktor SZ, Sassan-Morokro M, Grant AD, Abouya L, Karon JM, Maurice C, Djomand G, Ackah A, Domoua K, Kadio A, Yapi A, Combe P, Tossou O, Roels TH, Lackritz EM, Coulibaly D, De Cock KM, Coulibaly IM, Greenberg AE, 1999. Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomised controlled trial. *Lancet 353:* 1469–1475.
3. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, Kaharuza F, Culver D, Kizito F, Bunnell R, Kigozi A, Nakanjako D, Wafula W, Quick R, 2004. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet 364:* 1428–1434.
4. Mermin J, Lule J, Ekwaru JP, Downing R, Hughes P, Bunnell R, Malamba S, Ransom R, Kaharuza F, Coutinho A, Kigozi A, Quick R, 2005. Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members. *AIDS 19:* 1035–1042.
5. Mwansa J, Mutela K, Zulu I, Amadi B, Kelly P, 2002. Antimicrobial sensitivity in enterobacteria from AIDS patients, Zambia. *Emerg Infect Dis 8:* 92–93.
6. Feikin DR, Dowell SF, Nwanyanwu OC, Klugman KP, Kazembe PN, Barat LM, Graf C, Bloland PB, Ziba C, Huebner RE, Schwartz B, 2000. Increased carriage of trimethoprim/sulfamethoxazole-resistant *Streptococcus pneumoniae* in Malawian children after treatment for malaria with sulfadoxine/pyrimethamine. *J Infect Dis 181:* 1501–1505.
7. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP, 2000. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. *Clin Infect Dis 31:* 170–176.
8. Iyer JK, Milhous WK, Cortese JF, Kublin JG, Plowe CV, 2001. *Plasmodium falciparum* cross-resistance between trimethoprim and pyrimethamine. *Lancet 358:* 1066–1067.
9. van Oosterhout JJ, Laufer MK, Graham SM, Thumba F, Perez MA, Chimbiya N, Wilson L, Chagomerana M, Molyneux ME, Zijlstra EE, Taylor TE, Plowe CV, 2005. A community-based study of the incidence of trimethoprim-sulfamethoxazole-preventable infections in Malawian adults living with HIV. *J Acquir Immune Defic Syndr 39:* 626–631.
10. WHO, 2006. *Facts on ACTs*. Geneva: WHO.
11. Bates SJ, Winstanley PA, Watkins WM, Alloueche A, Bwika J, Happi TC, Kremsner PG, Kublin JG, Premji Z, Sibley CH, 2004. Rare, highly pyrimethamine-resistant alleles of the *Plasmodium falciparum* dihydrofolate reductase gene from 5 African sites. *J Infect Dis 190:* 1783–1792.
12. Alker AP, Mwapasa V, Purfield A, Rogerson SJ, Molyneux ME, Kamwendo DD, Tadesse E, Chaluluka E, Meshnick SR, 2005. Mutations associated with sulfadoxine-pyrimethamine and chlorproguanil resistance in *Plasmodium falciparum* isolates from Blantyre, Malawi. *Antimicrob Agents Chemother 49:* 3919–3921.
13. Plowe CV, Cortese JF, Djimde A, Nwanyanwu OC, Watkins WM, Winstanley PA, Estrada-Franco JG, Mollinedo RE, Avila JC, Cespedes JL, Carter D, Doumbo OK, 1997. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *J Infect Dis 176:* 1590–1596.
14. Mberu EK, Nzila AM, Nduati E, Ross A, Monks SM, Kokwaro GO, Watkins WM, Hopkins Sibley C, 2002. *Plasmodium falciparum*: in vitro activity of sulfadoxine and dapsone in field isolates from Kenya: point mutations in dihydropteroate synthase may not be the only determinants in sulfa resistance. *Exp Parasitol 101:* 90–96.
15. Bwijo B, Kaneko A, Takechi M, Zungu IL, Moriyama Y, Lum JK, Tsukahara T, Mita T, Takahashi N, Bergqvist Y, Bjorkman A, Kobayakawa T, 2003. High prevalence of quintuple mutant dhps/dhfr genes in *Plasmodium falciparum* infections seven years after introduction of sulfadoxine and pyrimethamine as first line treatment in Malawi. *Acta Trop 85:* 363–373.
16. Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, Mukadam RA, Rogerson SJ, Lescano AG, Molyneux ME, Winstanley PA, Chimpeni P, Taylor TE, Plowe CV, 2002. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *J Infect Dis 185:* 380–388.
17. Lloyd-Evans N, O'Dempsey TJ, Baldeh I, Secka O, Demba E, Todd JE, McArdle TF, Banya WS, Greenwood BM, 1996. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. *Pediatr Infect Dis J 15:* 866–871.
18. Brueggemann AB, Griffiths DT, Meats E, Peto T, Crook DW, Spratt BG, 2003. Clonal relationships between invasive and carriage Streptococcus pneumoniae and serotype- and clone-specific differences in invasive disease potential. *J Infect Dis 187:* 1424–1432.
19. Hausdorff WP, Bryant J, Paradiso PR, Siber GR, 2000. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis 30:* 100–121.
20. Arason VA, Kristinsson KG, Sigurdsson JA, Stefansdottir G, Molstad S, Gudmundsson S, 1996. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. *BMJ 313:* 387–391.
21. Meynard JL, Barbut F, Blum L, Guiguet M, Chouaid C, Meyohas MC, Picard O, Petit JC, Frottier J, 1996. Risk factors for isolation of *Streptococcus pneumoniae* with decreased susceptibility to penicillin G from patients infected with human immunodeficiency virus. *Clin Infect Dis 22:* 437–440.
22. Zachariah R, Harries AD, Spielmann MP, Arendt V, Nchingula D, Mwenda R, Courtielle O, Kirpach P, Mwale B, Salaniponi FM, 2002. Changes in *Escherichia coli* resistance to co-trimoxazole in tuberculosis patients and in relation to co-

trimoxazole prophylaxis in Thyolo, Malawi. *Trans R Soc Trop Med Hyg 96:* 202–204.
23. Buve A, Carael M, Hayes RJ, Auvert B, Ferry B, Robinson NJ, Anagonou S, Kanhonou L, Laourou M, Abega S, Akam E, Zekeng L, Chege J, Kahindo M, Rutenberg N, Kaona F, Musonda R, Sukwa T, Morison L, Weiss HA, Laga M, 2001. Multicentre study on factors determining differences in rate of spread of HIV in sub-Saharan Africa: methods and prevalence of HIV infection. *AIDS 15 (Suppl 4):* S5–14.
24. Githeko AK, Service MW, Mbogo CM, Atieli FK, Juma FO, 1993. *Plasmodium falciparum* sporozoite and entomological inoculation rates at the Ahero rice irrigation scheme and the Miwani sugar-belt in western Kenya. *Ann Trop Med Parasitol 87:* 379–391.
25. Adazu K, Lindblade KA, Rosen DH, Odhiambo F, Ofware P, Kwach J, Van Eijk AM, Decock KM, Amornkul P, Karanja D, Vulule JM, Slutsker L, 2005. Health and demographic surveillance in rural western Kenya: a platform for evaluating interventions to reduce morbidity and mortality from infectious diseases. *Am J Trop Med Hyg 73:* 1151–1158.
26. Brooks JT, Ochieng JB, Kumar L, Okoth G, Shapiro RL, Wells JG, Bird M, Bopp C, Chege W, Beatty ME, Chiller T, Vulule JM, Mintz E, Slutsker L, 2006. Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya, 1997-2003. *Clin Infect Dis 43:* 393–401.
27. Fawzi WW, Msamanga GI, Spiegelman D, Wei R, Kapiga S, Villamor E, Mwakagile D, Mugusi F, Hertzmark E, Essex M, Hunter DJ, 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med 351:* 23–32.
28. Bloland PB, Boriga DA, Ruebush TK, McCormick JB, Roberts JM, Oloo AJ, Hawley W, Lal A, Nahlen B, Campbell CC, 1999. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children. *Am J Trop Med Hyg 60:* 641–648.
29. McCollum AM, Poe AC, Hamel M, Huber C, Zhou Z, Shi YP, Ouma P, Vulule J, Bloland P, Slutsker L, Barnwell JW, Udhayakumar V, Escalante AA, 2006. Antifolate resistance in *Plasmodium falciparum*: multiple origins and identification of novel dhfr alleles. *J Infect Dis 194:* 189–197.
30. Zhou Z, Poe AC, Limor J, Grady KK, Goldman I, McCollum AM, Escalante AA, Barnwell JW, Udhayakumar V, 2006. Pyrosequencing, a high-throughput method for detecting single nucleotide polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes of *Plasmodium falciparum. J Clin Microbiol 44:* 3900–3910.
31. NCCLS, 1998. *Performance Standards for Antimicrobial Susceptibility Testing: Eighth Informational Supplement.* Wayne, PA: National Committee for Clinical Laboratory Standards.
32. Zurovac D, Midia B, Ochola SA, English M, Snow RW, 2006. Microscopy and outpatient malaria case management among older children and adults in Kenya. *Trop Med Int Health 11:* 432–440.
33. Obonyo CO, Ochieng F, Taylor WR, Ochola SA, Mugitu K, Olliaro P, ter Kuile F, Oloo AJ, 2003. Artesunate plus sulfadoxine-pyrimethamine for uncomplicated malaria in Kenyan children: a randomized, double-blind, placebo-controlled trial. *Trans R Soc Trop Med Hyg 97:* 585–591.
34. Chintu C, Bhat GJ, Walker AS, Mulenga V, Sinyinza F, Lishimpi K, Farrelly L, Kaganson N, Zumla A, Gillespie SH, Nunn AJ, Gibb DM, 2004. Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial. *Lancet 364:* 1865–1871.
35. Ministry of Health Kenya, 2005. *Integrated Management of Childhood Illness Guidelines.* Nairobi: Ministry of Health Kenya.
36. Phillips-Howard PA, Nahlen BL, Alaii JA, ter Kuile FO, Gimnig JE, Terlouw DJ, Kachur SP, Hightower AW, Lal AA, Schoute E, Oloo AJ, Hawley WA, 2003. The efficacy of permethrin-treated bed nets on child mortality and morbidity in western Kenya I. Development of infrastructure and description of study site. *Am J Trop Med Hyg 68:* 3–9.
37. Marsik FJ, Parisi JT, Blenden DC, 1975. Transmissible drug resistance of *Escherichia coli* and *Salmonella* from humans, animals, and their rural environments. *J Infect Dis 132:* 296–302.
38. Murray BE, Rensimer ER, 1983. Transfer of trimethoprim resistance from fecal *Escherichia coli* isolated during a prophylaxis study in Mexico. *J Infect Dis 147:* 724–728.
39. Balis E, Vatopoulos AC, Kanelopoulou M, Mainas E, Hatzoudis G, Kontogianni V, Malamou-Lada H, Kitsou-Kiriakopoulou S, Kalapothaki V, 1996. Indications of *in vivo* transfer of an epidemic R plasmid from *Salmonella enteritidis* to *Escherichia coli* of the normal human gut flora. *J Clin Microbiol 34:* 977–979.