

Differential Effects of Azithromycin, Doxycycline, and Cotrimoxazole in Ingested Blood on the Vectorial Capacity of Malaria Mosquitoes

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Background. The gut microbiota of malaria vector mosquitoes grows after a blood meal and limits *Plasmodium* infection. We previously showed that penicillin and streptomycin in the ingested blood affect bacterial growth and positively impact mosquito survival and permissiveness to *Plasmodium*. In this study, we examine the effects of doxycycline, azithromycin, and co-trimoxazole. All 3 antibiotics are used in mass drug administration programs and have antimicrobial activities against bacteria and various stages of malaria parasites.

Methods. The effects of blood meal supplementation with antibiotics on the mosquito microbiota, lifespan, and permissiveness to *Plasmodium falciparum* were assessed.

Results. Ingestion of any of the 3 antibiotics significantly affected the mosquito microbiota. Azithromycin decreased *P falciparum* infection load and mosquito lifespan, whereas at high concentrations, doxycycline increased *P falciparum* infection load. Co-trimoxazole negatively impacted infection intensity but had no reproducible effect on mosquito lifespan.

Conclusions. Our data suggest that the overall effect of antibiotic treatment on parameters critical for mosquito vectorial capacity is drug specific. The negative effect of azithromycin on malaria transmission is consistent with current efforts for disease elimination, whereas additional, larger scale investigations are required before conclusions can be drawn about doxycycline.

Keywords. *Anopheles*; antibiotics; azithromycin; microbiota; *Plasmodium falciparum*.

Anopheles mosquitoes are vectors of malaria, which causes 200 million disease incidences per year [1]. Mosquitoes become infected after ingesting a blood meal containing *Plasmodium* gametocytes, the mosquito infectious parasite stages. Parasites reproduce sexually in the mosquito midgut lumen, and the motile ookinetes mature into replicative oocysts after they traverse the midgut epithelium [2]. After approximately 1 week of mitotic divisions, the oocysts rupture and release thousands of sporozoites that infect the salivary glands and, subsequently, can infect humans. The mosquito midgut stages represent a major bottleneck of parasite development in the mosquito and are critical in disease transmission. The drop in parasite numbers that occurs before the oocyst development is partly explained by the activity of the mosquito immune system, largely a complement-like reaction that occurs during the ookinete-to-oocyst transition, as soon

as ookinetes come into contact with the hemolymph [3], but also by colonization resistance due to the mosquito microbiota, known to multiply after a blood meal [4–7]. The proliferating gut bacteria induce the nuclear factor- κ B immune-deficiency pathway in the gut epithelium [6, 8] and directly affect parasites by producing reactive oxygen species [9].

In a recent study, we investigated how a broad-spectrum antibiotic cocktail of penicillin-streptomycin present in ingested human blood at pharmaceutical concentrations affect the mosquito microbiota and, indirectly, the mosquito vectorial capacity [10]. We showed that the antibiotic cocktail reduced the bacterial growth by 70% after the blood meal and resulted in an increase in mosquito permissiveness to the parasite, lifespan, and fecundity. These data suggested that the presence of antibiotics in the blood meal might increase the risk of malaria transmission, which would be of particular importance when implementing mass drug administration programs, as the chance of a mosquito feeding on an antibiotic treated person is increased.

Besides their antibacterial activity, some antibiotics also affect parasites [11, 12]. In these cases, we hypothesize that the resulting impact of an antibiotic treatment on malaria transmission will depend on the balance between the microbiota-dependent and parasite-dependent effects. Among these, doxycycline, azithromycin, and co-trimoxazole are known to have a negative impact on the asexual stages of parasite multiplication in the blood [11, 12] but not on gametocytemia [13–15]. Considering

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mosquito stages of parasite development, *in vitro* assays have shown that azithromycin and doxycycline reduce *Plasmodium falciparum* gametocyte differentiation into male gametes and *Plasmodium berghei* ookinete formation, respectively [12]. Co-trimoxazole negatively impacts on the intensity of infection in *Anopheles stephensi* mosquitoes [15].

In this study, we investigate the effects of doxycycline, azithromycin, and co-trimoxazole on the vectorial capacity of *Anopheles coluzzii* mosquitoes (formerly known as *Anopheles gambiae* M form) when added to the blood meal. All 3 antibiotics are used in large-scale administration programs or trials, including World Health Organization (WHO)-led programs towards the elimination of neglected tropical diseases using azithromycin [16, 17], doxycycline-based trials to fight against onchocerciasis and lymphatic filariasis [18, 19], and co-trimoxazole prophylaxis for human immunodeficiency virus (HIV)-infected or HIV-exposed people [20].

METHODS

Ethics Statement

The protocols for human blood collection and for mosquito maintenance were approved by the Centre Muraz Institutional Ethics Committee under the ethical clearance number 003-2009/cE-cM. Before enrollment, written informed consent was taken from each human volunteer and/or their legal guardian.

Mosquito Colony and Maintenance

Most experiments were performed with female mosquitoes from an *A. coluzzii* Ngouso colony, established from field mosquitoes collected in Cameroon in 2006, maintained on human blood, and fed as adults with 5% fructose. Field parasite infections were performed with an *A. coluzzii* colony established from field mosquitoes collected in Burkina Faso in 2008, maintained on rabbit blood, and fed as adults with 5% glucose. Larvae were fed TetraMin fish food (Tetra). Insectary temperature was maintained at 28°C ($\pm 1^\circ\text{C}$) with 70%–80% humidity on a 12-hour light/dark cycle.

Human Blood Feeding and *Plasmodium* Infections

Plasmodium falciparum NF54 gametocytes were cultured in RPMI medium (GIBCO) including 300 mg L-1 L-glutamine supplemented with 50 mg/L hypoxanthine, 25 mM HEPES plus 10% heat-inactivated human serum without antibiotics. Two 25-mL cultures were started 17 and 14 days before the infection at 0.5% parasitemia in 6% v/v washed O+ red blood cells (RBCs). Media was changed daily. Before mosquito infection, centrifuged RBCs were pooled and supplemented with 20% of fresh washed RBCs and human serum (2:3 v/v ratio between RBCs and serum). For experiments carried out from field parasites, gametocyte carriers were screened among 5- to 14-year-old school children in Burkina Faso. Vein blood was sampled into a lithium heparin-coated tube. After centrifugation,

serum was replaced by European AB serum to limit the chance of transmission-blocking immunity. Mosquitoes were offered a blood meal from a membrane-feeding device covered with Parafilm and kept at 37°C. Stock solutions of doxycycline, co-trimoxazole (trimethoprim-sulfamethoxazole), and azithromycin were prepared from powders (Sigma-Aldrich) in water, dimethyl sulfoxide (DMSO), and DMSO, respectively, so that therapeutic concentrations for each antibiotic is reached when 1.2 μL of the stock is added to 1 mL blood: 5 $\mu\text{g}/\text{mL}$ for doxycycline, 1 $\mu\text{g}/\text{mL}$ for azithromycin, 2.5 $\mu\text{g}/\text{mL}$ for trimethoprim, and 60 $\mu\text{g}/\text{mL}$ for sulfamethoxazole. For experiments performed on *A. gambiae*, the co-trimoxazole solution was prepared in water, to reach therapeutic concentrations when adding 60 μL of the stock to 1 mL blood. These antibiotics do not require metabolic activation, and they circulate in the blood until elimination, mostly in an unmetabolized form [21–24]. Control blood was supplemented with the corresponding amount of solvent. For each *Plasmodium* infection, at least 100 mosquitoes per condition were offered a blood meal and nonengorged mosquitoes were removed. All (or at least 50) mosquitoes that blood fed and survived until the end of the experiment were dissected for oocyst counts using mercurochrome staining.

Microbiota Analysis by Quantitative Polymerase Chain Reaction

Nonengorged mosquitoes were discarded after the blood meal. Before dissection, mosquitoes were immersed in 70% ethanol for 5 minutes in order to kill mosquitoes and to fix surface bacteria to the cuticle, then rinsed 3 times in sterile phosphate-buffered saline (PBS) to wash out nonattached bacteria, thus minimizing sample contamination with cuticle bacteria during dissection. Midguts were removed and frozen immediately on dry ice in 3 groups of at least 5 mosquitoes per condition and stored at -20°C until processing. Midguts were only excluded from analysis if they burst and a substantial amount of the gut content was lost. Samples were homogenized in phenol-chloroform in a Precellys 24 homogenizer (Bertin) using 0.5 mm-wide glass beads (Bertin) for 30 seconds at 6800 rpm and deoxyribonucleic acid (DNA) was extracted with phenol-chloroform. The 16S ribosomal DNA (rDNA) (Figure 1A) or complementary DNA of the 16S ribosomal ribonucleic acid (rRNA) (Figure 1C) was used for bacterial quantification and is shown as a ratio of the *Anopheles* housekeeping gene 40S ribosomal protein S7 (Vector-Base gene ID AGAP010592), referred to as S7. Primer sequences are listed in (Supplementary Table 1). Quantitative polymerase chain reaction (qPCR) was performed on a 7500 Fast Real-Time thermocycler (Applied Biosystems) using the SYBR *Premix Ex Taq* kit (Takara), following the manufacturer's instructions.

Microbiota Analysis by 454 Pyrosequencing

The microbiota was analyzed in parallel with our previous analysis of penicillin-streptomycin treatment, so the control results are the same [10]. Mosquitoes and midguts samples were

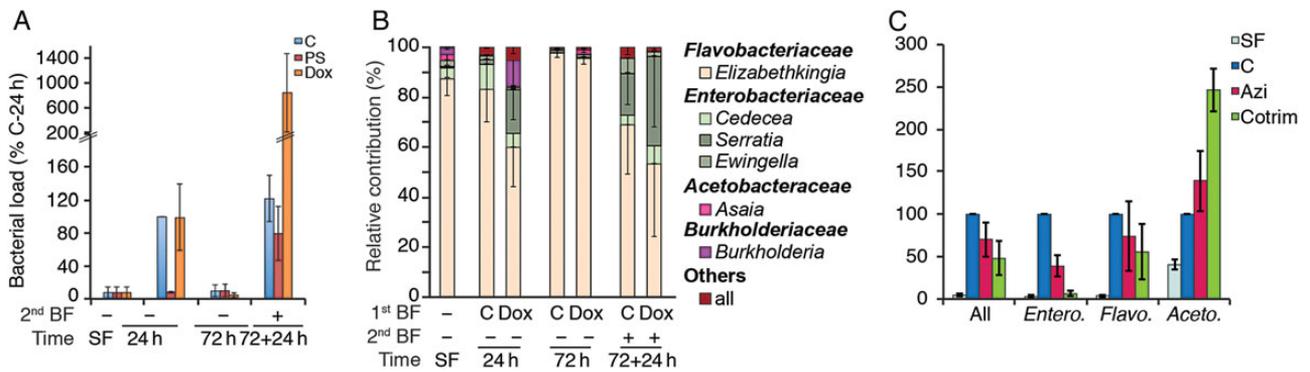


Figure 1. Doxycycline, azithromycin, and co-trimoxazole affect the mosquito microbiota. *16S* quantitative polymerase chain reaction (qPCR) (A) and 454-pyrosequencing (B) analysis of the bacterial load in dissected midguts during a course of 2 blood meals, the first one being supplemented with doxycycline (Dox), penicillin-streptomycin (PS), or water as a control (C). (C) *16S* qPCR analysis in sugar-fed mosquitoes (SF) or 24 hours after a blood meal using generic (All) or family-specific primers (*Entero.*, *Enterobacteriaceae*; *Flavo*, *Flavobacteriaceae*; *Aceto.*, *Acetobacteraceae*). The blood meal was supplemented with azithromycin (Azi), co-trimoxazole (Cotrim), or dimethyl sulfoxide as a control (C). Results show average (\pm standard error of the mean) from 3 independent experiments. *, $P < .05$, analysis of variance χ^2 test on a common linear regression (lmer).

handled as described above for qPCR analysis, with added precaution to reduce environmental contamination: dissecting slides and forceps were washed with detergent, rinsed with water and then with ethanol between each sample, and sample-containing tubes were pretreated with ultraviolet radiation. For each sample, at least 15 mosquitoes were dissected. To ensure contamination control, drops of PBS were transferred with forceps from a dissecting slide into a sampling tube in 10 steps to mimic dissection routine. Deoxyribonucleic acid extraction conditions are adapted from method 2 of Yuan et al [25]. Samples were first homogenized for 15 seconds at 6800 rpm in a Precellys 24 homogenizer (Bertin) in a lysis buffer (20 mM Tris pH 8.0, 2 mM EDTA, 1.2% Triton, 20 mg/mL lysozyme, 250 U/mL mutanolysin, 20 U/mL lysostaphin [Sigma-Aldrich]), then incubated for 30 minutes at 37°C, and homogenized again for 15 seconds. Deoxyribonucleic acid was extracted under sterile conditions using a DNeasy blood and tissue kit (QIAGEN). The regions V2–V3 of the 16S rDNA were amplified with Phusion polymerase ([New England Biolabs] 27 cycles: 5 seconds at 98°C, 20 seconds at 63°C, 20 seconds at 72°C, followed by 5 minutes at 72°C) using the following primers: 16S-0027F (AGAGTTTGATCCTGGCTCAG) and 16S-0533R (TTACCGCGGCTGCTGGCAC) attached in 5' to Roche MID adapters (Supplementary Table 2). After Picogreen (Life Technologies) quantification, a pool of 48 samples (including the 24 samples reported here), each of 50 ng DNA, was 454-pyrosequenced in one-half run of a 454 GS FLX sequencer (Roche) by Beckman Coulter Genomics. The data set was deposited on the European Nucleotide Archive [26]. Sequencing resulted in 544 038 sequences processed with Qiime (version 1.8.0). Of these, 181 967 reads of 477-base pair average length after primer truncation were assigned to the 24 samples (no primer mismatch allowed), and 180 074 of them were identified as nonchimeric using usearch61 [27] (see Supplementary Table 2). Operational

taxonomic units (OTUs) were picked using UCLUST software with a 97% identity threshold [27]. Among contamination controls, 1 replicate produced only 12 reads after filtering; the other 2 were significantly different from mosquito midgut samples (ANOSIM test output $P = .024$ and $P = .004$ on weighted and unweighted Unifrac distances [28], respectively) and were thus excluded from subsequent analyses. Alpha diversity was monitored using the number of observed species, the Chao1 species richness estimation [29, 30], the Shannon index, and the phylogenetic diversity whole tree index [31] (Supplementary Table 3). The OTUs were assigned to taxonomy against the Silva reference dataset (Release 119) [32].

Survival

Age-matched, 2- to 3-day-old mosquitoes were offered a first control or experimental blood meal, and nonengorged mosquitoes were discarded. Mosquitoes were then offered the same type of blood meal twice a week until the death of the last mosquito. Between blood meals, mosquitoes were provided with a cotton pad moistened with distilled water for oviposition. Unfed mosquitoes were not removed after the second and later blood meals. Deaths were counted and carcasses were removed on a daily basis. At least 50 mosquitoes per condition were used for each of 3 independent replicates.

Statistical Analyses

Statistical analyses were performed by generalized linear mixed models (GLMM) in R (version 2.15.3). Prevalence data were analyzed by analysis of variance (ANOVA) χ^2 test on a logistic regression (glmer). Oocyst counts were analyzed by Wald Z-test on a zero-inflated negative binomial regression (glmmADMB). *16S* data (qPCR, family proportions from pyrosequencing) were analyzed by ANOVA χ^2 test on a common linear regression (lmer). Survival data were analyzed using an ANOVA χ^2 test on a mixed effect Cox model (coxme), using replicates as

random effects. Mixed model analyses decompose model effects into the contribution of a fixed component (the covariates in question, the treatments in this study) and a random component (the experiments in this study). The fixed effect estimates are the regression coefficients, and the odds ratios are their exponential value.

Supporting Data

The data set supporting the results of this article is available in the European Nucleotide Archive repository, under accession code PRJEB7708 (control) and XX (doxycycline-treated) (<https://www.ebi.ac.uk/ena/data/view/XX>).

RESULTS

Effect of Antibiotics on the Microbiota

We investigated the outcome of the presence of antibiotics in the blood on the mosquito midgut microbiota. We supplemented human blood with doxycycline at a therapeutic concentration of 5 µg/mL [33] and offered it to mosquitoes as a blood meal through standard membrane feeding. Blood supplemented with water (the solvent of doxycycline) was used as a control. Treating the blood with doxycycline did not impact the bacterial load of dissected mosquito midguts 24 hours after the blood meal ($P = .85$) (Figure 1A). This could mean that either the concentration of doxycycline was too low to affect the mosquito microbiota or that the reduced growth of some bacteria was compensated by the expansion of others. To address this question, we followed the composition of the microbiota by 454 pyrosequencing of the *16S rDNA* from dissected midguts (Figure 1B). We observed that the relative contribution of *Flavobacteriaceae* significantly decreased ($P = .037$), corresponding to a marginally significant increase in the proportion of *Enterobacteriaceae* ($P = .058$).

Next, we investigated whether this antibiotic treatment had any persistent consequences on the microbiota after a subsequent untreated blood meal. Twenty-four hours after the second blood meal, we observed higher bacterial growth in doxycycline pre-exposed mosquitoes ($P = .023$) (Figure 1A). A microbiota composition analysis showed that this expansion could not be explained by the growth of 1 bacterial genus alone, which may have been selected for its resistance to doxycycline (Figure 1B). On the contrary, we observed that the species diversity increased in the midgut upon exposure to doxycycline (Supplementary Table 3). These results were in contrast to the results obtained previously following blood supplementation with penicillin-streptomycin. This cocktail reduced bacterial growth after a first blood meal, almost clearing the *Enterobacteriaceae* and reducing the species diversity, and did not affect the microbiota after a subsequent nontreated blood meal [10].

We examined the effects of azithromycin or co-trimoxazole by dissolving them in DMSO and adding them to human blood also at the therapeutic concentrations of 1 µg/mL for azithromycin and 2.5 µg/mL trimethoprim and 60 µg/mL sulfamethoxazole

for co-trimoxazole [33, 34]. Blood supplemented with DMSO was used as a control. We observed that azithromycin and co-trimoxazole led to a 30% marginally significant reduction ($P = .060$) and to a 52% reduction ($P = .0085$) of the bacterial load 24 hours after the blood meal, respectively (Figure 1C). Using bacterial family-specific qPCR on *16S rRNA*, we observed that the reduction was stronger on *Enterobacteriaceae* than on *Flavobacteriaceae* ($-61%$, $P = .0049$ vs $-26%$, $P = .072$ for azithromycin; $-94%$, $P = .00015$ vs $-44%$, $P = .020$ for cotrimoxazole), whereas *Acetobacteraceae* grew better in the presence of both antibiotics ($+39%$, $P = .037$ for azithromycin; $+150%$, $P = 7.5e-5$ for co-trimoxazole). The results show that these antibiotics, especially co-trimoxazole, have similar effects on the mosquito microbiota as penicillin-streptomycin [10].

Effect of Antibiotics on *Plasmodium* Infections

In addition to their effect on bacteria, the 3 antibiotics tested in this study are also known to exert an antiparasitic activity [12, 15]. This suggests that each of these antibiotics may have 2 opposing actions on the mosquito vectorial capacity, by clearing parasites and by reducing bacteria-dependent colonization resistance. We directly investigated the outcome of these antibiotics on mosquito permissiveness to *P falciparum* (ie, vector competence) by adding the antibiotics at therapeutic concentrations to an infectious blood meal minutes before offering it to mosquitoes and monitoring infection at the oocyst stage. We first infected mosquitoes with cultured *P falciparum* gametocytes and observed that doxycycline had a nonsignificant effect, but a positive trend, on the infection intensity ($+59%$ mean oocyst load, $P = .34$) (Figure 2A and 2C). To investigate whether doxycycline was able to affect parasites at higher concentrations, we repeated this experiment by doubling the concentration (10 µg/mL) and observed a significant augmentation in parasite infection intensity ($+39%$ mean oocyst load, $P = .027$) (Figure 2B and 2C). The effects on infection prevalence were not significant ($+5.7%$) (Figure 2A and 2B).

We investigated the effect of doxycycline in a setting more reflective of field conditions, by infecting laboratory-reared mosquitoes with blood drawn from *P falciparum* gametocyte carriers in Burkina Faso and supplemented with doxycycline (at 5 µg/mL) or water just before mosquito feeding. The results revealed a positive but statistically not significant effect of doxycycline on the infection prevalence ($+7.3%$) (Figure 2A and 2D).

In the same analysis with azithromycin, we observed a clear negative impact on *P falciparum* infection intensity, whether infecting with cultured gametocytes or with blood from gametocyte carriers ($-42%$ in mean oocyst intensity, $P = 5e-7$) (Figure 3A and 3B). No significant effect on infection prevalence was observed ($-8.3%$, $P = .25$). This indicates that the antiparasitic activity of azithromycin has a stronger impact on parasite infection than the reduction in bacteria-dependent antiparasitic activity.

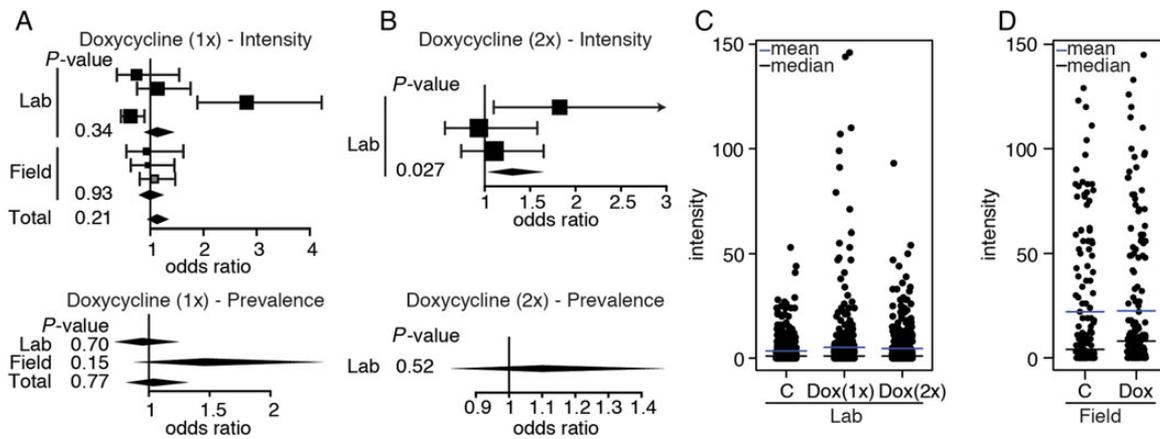


Figure 2. Impact of doxycycline (Dox) on mosquito permissiveness to *Plasmodium falciparum*. (A and B) Forest plots showing the effects of Dox supplementation on the intensity and prevalence of infection with *P. falciparum* measured as oocyst load on the mosquito midgut. The therapeutic concentration is 1x. Mosquitoes were infected using laboratory-cultured *P. falciparum* gametocytes (laboratory) or blood from gametocyte carriers (field). Each line shows odds ratio (\pm confidence interval) of independent replicates, and diamonds report summaries. Results shown in gray correspond to mosquitoes raised in water sampled from an *Anopheles coluzzii*-larvae-containing pool. Data were analyzed by Wald Z-test on a zero-inflated negative binomial regression (glmmADMB). Strip chart showing the oocyst counts in each individual mosquito infected with naturally infected blood (C) or laboratory-cultured parasites (D). For C and D, data from all experiments shown in A and B were pooled.

We also infected *A. coluzzii* mosquitoes with blood from gametocyte carriers to monitor the effect of co-trimoxazole. As observed with azithromycin, co-trimoxazole significantly reduced the parasite intensity (-27% mean oocyst load, $P = .026$) at the oocyst stage (Figure 3C and 3D). However, it increased the infection prevalence ($+18\%$, $P = .039$). To explore further the observed dysjunction between infection prevalence and intensity, we replaced *A. coluzzii* with its sibling *A. gambiae* mosquito species, previously known as *A. gambiae* S form. We observed that not only the intensity but also the prevalence significantly decreased upon co-trimoxazole treatment (-12% prevalence, $P = .031$; -34% mean oocyst load, $P = .00074$) (Figure 3C and 3D). These data confirm the negative effect of co-trimoxazole on intensity and suggest that the previously observed effect on prevalence was likely not biologically relevant.

To control for potential differences in the microbiota composition between our laboratory-reared and field mosquitoes, 1 experiment for each antibiotic was performed using mosquitoes that were reared in the laboratory but emerged in water sampled from a pool containing *A. coluzzii* larvae. It has been previously shown that the microbiota composition is highly dependent on the larval breeding site [35]. The results revealed that the effects of antibiotics on *P. falciparum* infection in mosquitoes raised in water collected from natural larval habitats were similar to those performed using conventionally reared mosquitoes (Figures 2A, 3A, and 3C, gray color).

Effect of Antibiotics on the Mosquito Lifespan

Because the parasite extrinsic incubation period spans approximately half of the mosquito lifespan, the latter is a major determinant of the mosquito vectorial capacity. Thus, we analyzed the effect of antibiotics on the lifespan of *A. coluzzii*. Mosquitoes

that ingested an antibiotic-treated or control blood meals were offered a similar blood meal twice a week and their survival was monitored daily. Doxycycline increased the lifespan by 2 days until day 23 and had no significant effect later ($P = .030$ until day 23, $P = .53$ until day 41) (Figure 4A), whereas azithromycin decreased the lifespan by 1 to 5 days ($P = .037$) (Figure 4B).

Co-trimoxazole had no consistent effect on mosquito survival ($P = .56$) (Figure 4C). Indeed, independent experiments showed a significant increase ($P = .03$ on the 1st replicate) or decrease ($P = .02$ on the 3rd replicate) in the lifespan of co-trimoxazole-exposed mosquitoes. Again, this may be explained by a difference in the initial microbiota that may be or not sensitive to co-trimoxazole.

DISCUSSION

In this study, we show that treatments with different antibiotics result in contrasting effects on the microbiota, the vector competence to *P. falciparum*, and the lifespan of mosquitoes. Azithromycin and co-trimoxazole reduce the load of the mosquito microbiota and modify its composition in a similar fashion to what we previously reported for penicillin-streptomycin, characterized by a decrease in *Enterobacteriaceae* [10]. Doxycycline shows a different effect by decreasing the proportion of *Flavobacteriaceae* and concomitantly increasing the microbiota species diversity. This increase may be explained by the reduction in *Elizabethkingia*, shown in vitro to have broad antimicrobial activity, against both Gram-positive and Gram-negative bacteria [36].

We examined mosquito vectorial capacity and observed that azithromycin treatment significantly decreased infection by *P. falciparum* and the lifespan of *A. coluzzii* mosquitoes, whereas

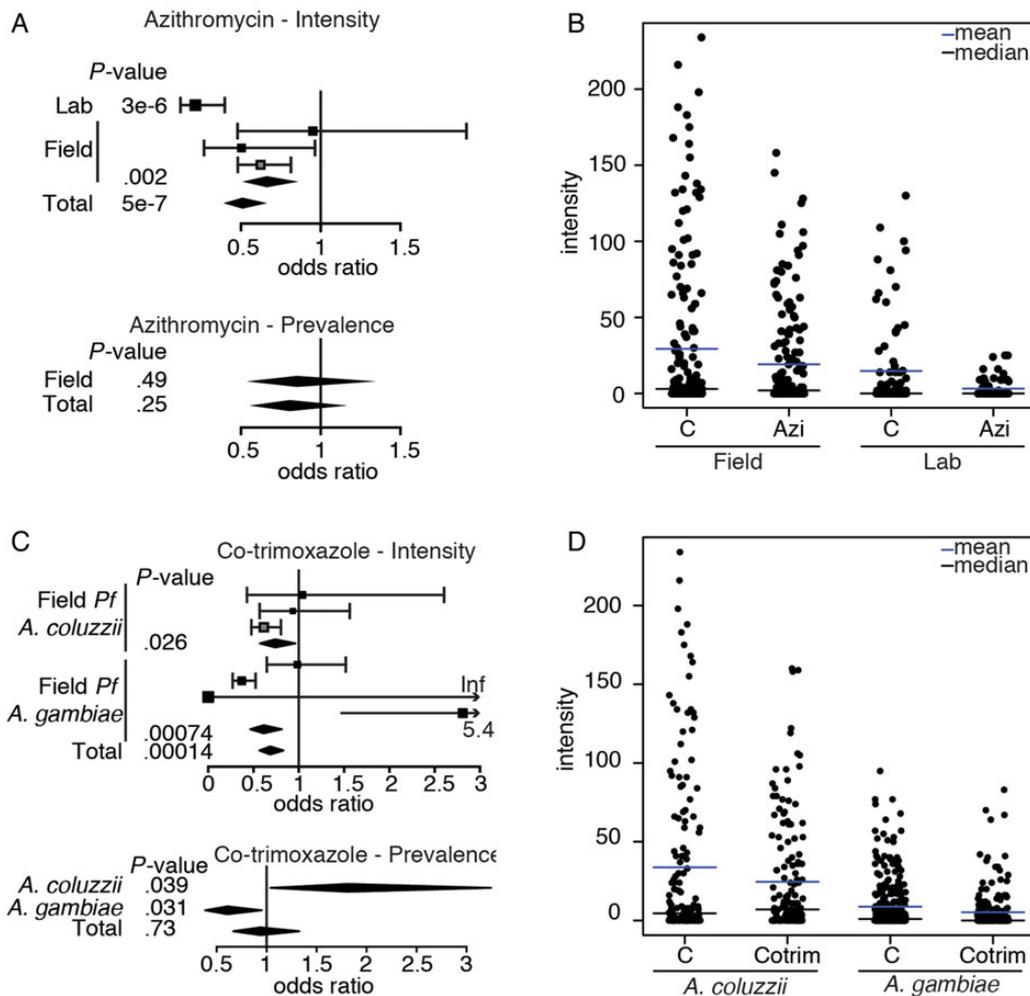


Figure 3. Impact of azithromycin and co-trimoxazole on mosquito permissiveness to *Plasmodium falciparum*. Forest plots showing the effects of azithromycin (A) or co-trimoxazole (C) supplementation on the intensity and prevalence of infection with *P. falciparum* at the oocyst stage. Mosquitoes were infected using laboratory-cultured *P. falciparum* gametocytes (laboratory) or blood from gametocyte carriers (field). Each line shows odds ratio (\pm confidence interval) from an independent replicate, and diamonds report summaries. Results shown in gray correspond to mosquitoes raised in water sampled from an *Anopheles coluzzii*-larvae-containing pool. Co-trimoxazole experiments were performed using *A. coluzzii* and *Anopheles gambiae*, whereas all azithromycin experiments were performed using *A. coluzzii*. Data were analyzed by Wald Z-test on a zero-inflated negative binomial regression (glmmADMB). Strip chart showing the oocyst counts in each individual mosquito upon azithromycin (B) or cotrimoxazole (D) treatment. For C and D, data from all experiments shown in A and B were pooled.

doxycycline had no significant effect on these same parameters. This result suggests that the balance between the antiparasitic effect and the microbiota-mediated “proparasitic” effect is highly drug-specific. Aside from mosquito permissiveness to parasites and lifespan, malaria transmission success has been modeled to depend on the biting rate, the number of mosquitoes per human, the infectivity of parasites to humans, and the time of parasite development in the mosquito [37]. Further studies deciphering the effect of antibiotics on these parameters may highlight additional impacts on the mosquito vectorial capacity.

The therapeutic concentrations considered here corresponded to the peak concentrations reported in the patient blood during the course of a treatment [34, 38] to assess the maximum possible effect of these antibiotics on malaria transmission. Blood antibiotic concentrations decrease after the end of a treatment. In

particular, azithromycin and doxycycline concentrations decrease to approximately 40%–20% of their peak concentrations soon after the end of the treatment and remain almost constant for 40 hours and 16 hours thereafter, respectively. The effects of these antibiotics at such lower concentrations must be further investigated.

Mass drug administration with azithromycin is currently recommended by WHO to fight neglected tropical diseases including trachoma and yaws, 2 bacterial diseases that cause blindness and affect the skin and bones, respectively [16, 17]. Trials are also being performed to analyze the effect of this antibiotic to fight other infectious diseases in sub-Saharan Africa and have notably evidenced a reduction in child mortality [39]. Our data are indicative of a reduction in malaria transmission as a result of a 42% reduction in mosquito infection intensity and

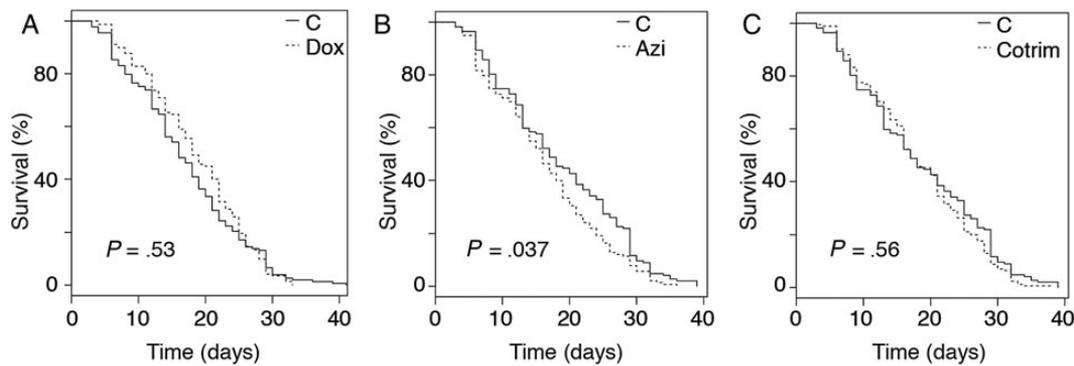


Figure 4. Effect of doxycycline (Dox), azithromycin and co-trimoxazole on mosquito lifespan. Kaplan-Meier survival plots of mosquitoes fed every 3–4 days with blood supplemented with Dox (A), azithromycin (Azi; B), co-trimoxazole (Cotrim) or their solvents as controls ([C] water in A, and dimethyl sulfoxide in B and C). Results from 3 independent experiments were analyzed using an analysis of variance χ^2 test on a mixed effect Cox model.

a nonsignificant 8% reduction in infection prevalence. These data fit nicely with a previously published model on transmission-blocking efficacy, given the mean infection intensity of 24 oocysts/mosquito observed in our experiments [40]. At infection intensities of 1–5 oocysts/mosquito that are observed in the field [41], this model can predict an effect on prevalence between 15% and 30% [40]. Such an effect may have a substantial impact on malaria transmission during azithromycin large-scale distribution programs, which commonly cover 90% of the population [42]. Therefore, our data suggest that a reduction in malaria transmission may contribute to the observed general reduction in infectious diseases upon large-scale treatment with azithromycin [39].

Our results of the impact of doxycycline exposure on the mosquito lifespan and permissiveness to *P. falciparum* are nonsignificant, suggesting that one-off treatments may not affect malaria epidemiology. Nonetheless, the observed positive trend in both parameters suggests that mass drug administration of doxycycline, suggested to fight lymphatic filariasis and onchocerciasis [18, 19], may benefit from testing its impact on malaria transmission at a larger scale. Both diseases are also vector borne, and microbiota are thought to interfere with nematode infection [7, 43], so this antibiotic may also affect the transmission of these diseases.

A high proportion of *Enterobacteriaceae* in the microbiota has been previously shown to correlate with successful *P. falciparum* infections in *Anopheles* [44]. In that case, the microbiota analysis was performed 8 days after *P. falciparum* infection, so it is unclear whether a higher contribution of *Enterobacteriaceae* supports *P. falciparum* infection or whether the parasite promotes *Enterobacteriaceae*. In this study, we observed that doxycycline treatment increases the proportion of *Enterobacteriaceae* in uninfected mosquitoes and tends to increase the permissiveness to *P. falciparum*. Together, these studies suggest that a higher proportion in *Enterobacteriaceae* promotes the development of *P. falciparum* in the mosquito gut. However, such

correlations at the family level must be considered with care, because the effect of bacteria on *Plasmodium* is at least partially strain-specific [9, 45].

Finally, the results on co-trimoxazole, with high variations between experiments, highlight the conflicting effects of the antibacterial and antiparasitic activities of this antibiotic. This may be linked to the fact that co-trimoxazole has a strong effect on the mosquito microbiota. The positive effect on prevalence versus negative impact on intensity of *A. coluzzii* infections suggests that a positive impact due to the effect on the microbiota only happens in certain mosquitoes, depending on the microbiota composition, whereas a negative direct effect inhibits all the parasites.

CONCLUSIONS

Taken together, our results indicate that doxycycline, azithromycin, and co-trimoxazole have diverse actions on the midgut microbiota, the permissiveness to malaria parasites, and the lifespan of malaria vector mosquitoes. The clearest effect is seen with azithromycin, which, despite reducing the bacterial load in the mosquito midgut, has a negative impact on its permissiveness to malaria infection. Because it also negatively affects the mosquito lifespan, azithromycin treatment may reduce malaria transmission.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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Authors' contributions. M. G. and G. K. C. designed the experiments. M. G. performed the experiments. R. S. Y., J. B. O., T. L., and A. C. provided infrastructure and organized the recruitment of gametocyte-carriers in

Burkina Faso. M. G. and G. K. C. wrote the manuscript. All the authors revised the manuscript and approved the final version.

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