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Long-term effect of mass azithromycin distributions for childhood mortality on antibiotic resistance

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Biological Sciences - Article

Keywords:

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10021703/v1

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Additional Declarations: Yes there is potential Competing Interest. Gates Foundation Funding. Grant number OP1032340

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Title: 88 characters
Authors: 17
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Abstract: 208 words
Main Text: 2402 words
Tables: 2
Figures: 3
References and Notes: 56
Acknowledgements: MORDOR, REVENIR: R01AI175250
List of Supplementary Materials:

Abstract

Biannual mass azithromycin distributions reduce childhood mortality in Sub-Saharan Africa.[1-4] However, stakeholders have struggled to balance this short-term benefit against the potential long-term risk of antibiotic resistance.[5-19] Here, we monitor resistance in 286 community surveys in Niger over 8 years of biannual azithromycin distributions to pre-school children. Communities were randomized to either azithromycin or placebo at 3 timepoints, allowing assessment of the effects of recent treatment, cumulative treatment, and secular trend. Macrolide resistance genes found in the stool of pre-school children increased during the first 3 years of intervention (the first 6 biannual distributions). Assessment at 4½, 6, and 8 years (after 9, 11, and 15 biannual distributions, respectively) failed to detect any further incremental increase. When distributions were discontinued, macrolide resistance decreased to pre-treatment levels. Beta-lactam resistance increased significantly over the 8 years. This increase was not associated with the mass azithromycin distributions and may represent a secular trend in the region. Mass azithromycin distribution for childhood mortality clearly selects for macrolide-resistant bacteria. This resistance apparently plateaus after 3 years of intervention, remains limited to macrolide antibiotics, and returns to baseline after distributions are discontinued. Monitoring can inform whether the benefit of improved childhood survival with azithromycin distributions continues to outweigh any long-term harm from antibiotic resistance.

Text

A single oral dose of the antibiotic azithromycin is effective in clearing the ocular strains of chlamydia from an individual with trachoma.[20-22] Mass distribution of azithromycin dramatically reduces the prevalence of trachoma in a community.[23-26] Repeated distributions progressively reduce infection, offering indirect protection even to those who themselves did not receive treatment.[27-29] Over time, these distributions can eliminate infection from even the most severely affected communities.[30-32] In the past two decades, trachoma control programs have distributed over one billion doses of azithromycin, bringing the blinding disease to the brink of elimination.[33]

Azithromycin has efficacy against a broad-spectrum of pathogens other than chlamydia, and against apicomplexa such as malaria.[34-36] All-cause childhood mortality was found to be significantly lower in trachoma-endemic communities randomized to receive azithromycin.[1] Several larger community-randomized trials have since confirmed lower childhood mortality in areas without trachoma, even when biannual treatments were targeted to just pre-school children aged 1-59 months.[2,4,37] The mechanism is presumably the reduction of a range of pathogens. In communities where children were randomized to receive azithromycin, fewer deaths were attributed to malaria, diarrhea, and respiratory disease.[38] In addition, fewer total infectious disease clinic visits were observed.[38]

Azithromycin distributions also have collateral risk. Treatments clearly select for antibiotic-resistant bacteria.[36] After 6 community-wide trachoma distributions, 78% of *Streptococcus*

pneumoniae isolates were found to be resistant to macrolides.[1,5] When azithromycin is distributed for childhood mortality, the intervention targets a narrower age group than when distributed for trachoma control. The pre-school children included in childhood mortality programs make up only about 1/6th of the total population included in community-wide trachoma treatments. But these more limited distributions for childhood mortality are still associated with significantly increased macrolide resistance in both the nasopharynx and the gut.[6,15] Co-selection of resistance to other classes of antibiotics is also theoretically possible.[9,39] A significant increase in resistance to beta-lactam antibiotics (penicillins, cephalosporins, and carbapenems) would likely raise more concern about the intervention.

Childhood mortality programs have now provided over 8 years of azithromycin distributions. The MORDOR clinical trials (*Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance*) have distributed up to 15 biannual azithromycin distributions in Niger.[2,40] Communities were periodically randomized to receive azithromycin or placebo, allowing assessment of the impact of starting and stopping antibiotics. Treatment, sampling, and specimen collection protocols have remained similar over the 15 distributions, allowing for comparison between communities randomized to MDA or no MDA, and also between communities over time. Here, we performed metagenomic deep sequencing (DNA-seq) on rectal samples collected in the trials to assess antimicrobial resistance (AMR). We analyzed whether recent MDA selected for increased antibiotic resistance genes, whether this resistance continued to increase with additional

distributions, and whether resistance returned to baseline levels after MDA was discontinued. We estimated whether resistance increased over time independent of the MDAs, as a secular trend. Co-selection of resistance to non-macrolide antibiotics is also theoretically possible, so we also monitored resistance in other classes of antibiotics such as beta-lactams.[39]

Rectal swabs were taken from 9,932 children from 286 community surveys at 5 time points: 0, 3, 4½, 6, and 8 years (Table 1). The time between collections varied between 18 and 36 months. After the baseline (year 0) collection, communities were randomized to receive either azithromycin or placebo. At both 3 and 6 years, communities were re-randomized to azithromycin or placebo, offering ten different treatment histories. The same communities were not monitored longitudinally over all the time points. Instead, each set of surveys was a simple random sample of communities drawn from the same pool. This allowed a statistical comparison between different treatment histories at the same timepoint, as well as a comparison between timepoints. The collections were all performed by the same field program. The first 3 time points have been previously reported, and samples were maintained at -80 degrees, allowing for re-processing.[3,6,41] The last 2 time points (6 and 8 years) have not previously been reported. All samples were processed together in a randomized order with masked laboratory personnel, to prevent any bias between sequencing runs. DNA-seq allowed identification of all known genetic resistance determinants, as previously described.[7]

Macrolide resistance increased, but plateaued after 3 years of azithromycin MDA. Recent azithromycin distributions clearly selected for macrolide antibiotic resistance genes (ARGs) (Figure 1a).[41] When azithromycin, and not placebo, had been distributed in the previous phase, the mean community load of macrolide ARGs was 25% higher (95% CI 19% to 30% higher, $P<0.001$, Table 1). Previous studies found an increase in genetic determinants of macrolide resistance in the gut, as well as an increase in the prevalence of macrolide resistance in nasopharyngeal *Streptococcus pneumoniae*.[15,41] Thus, selection of macrolide resistance has been observed whether azithromycin was distributed to the entire community as in trachoma control, or limited to pre-school children as in childhood survival programs. Here, we found that this increase in resistance in the gut did not increase further after 36 months (Figure 1a). While recent azithromycin MDA in the previous phase was significantly associated with ARG load, the total number of doses prior to that last phase was not (1.0% increased with each additional MDA, 95% CI -0.4% to +2.0%, $P=0.201$, Table 1). This suggests that with biannual MDA, macrolide-resistant strains may not completely take over for the susceptible strains, even in the presence of biannual mass childhood distributions. We found no evidence for a secular trend of macrolide resistance increasing independent of the MDAs, outside of the program (-1.2%/year, 95% CI -3.1%/year to +1.0%/year, $P=0.204$).

Macrolide resistance decreased after distributions were discontinued. When communities treated with azithromycin for the first 6 years were randomized to discontinue azithromycin MDA and switch to placebo, the mean load of macrolide-resistant

determinants decreased 14.6% (95% CI 5.4% to 22.9% decrease, P=0.003, Table S1). In fact, levels returned to those seen at baseline, pre-treatment (0.7% lower than baseline*, 95% CI 11.1% to +11.0%*, *P*=0.90) despite receiving 11 previous distributions of MDA. A similar decline in resistance post-MDA had been observed earlier in trachoma programs.[42,43] Phenotypic resistance in nasopharyngeal *S. pneumoniae* decreased from 76% to 25% in the two years after trachoma distributions had been discontinued.[43] That macrolide-resistance dramatically decreases in the absence of continued antibiotic pressure supports the hypothesis that resistant strains are less fit than susceptible strains.[44]

Co-selection of antibiotic resistance to other classes of antibiotics was not observed. We combined known genetic determinants into 110 categories that confer resistance to 13 antibiotic classes. We were unable to show a significant increase in resistance due to recent azithromycin treatment to any class other than macrolides (Figure 3). In particular, beta-lactam resistance was not associated with azithromycin treatment in the previous phase, or with cumulative number of azithromycin distributions prior to the last phase (Table 1). We did observe a significant increase in resistance to beta-lactam antibiotics over time (Figure 1b, Table 1), but this increase was unrelated to the azithromycin distributions themselves.

Individual macrolide antimicrobial resistance genes (ARGs) increased with MDA. In the above analyses, macrolide resistance genetic determinants were combined into a single

group. We also compared individual ARGs. *MLS23S* was the most frequent macrolide ARG found, followed by *ermF*, *ermA*, and *mphB* (Supplemental Material, Figure S2). *ermF* increased significantly with recent azithromycin distribution (119% higher, 95% CI 97% to 142%). A similar increase was noted in *ermA* with recent azithromycin distribution (170% higher, 95% CI 73% to 321%). Both *ermA* and *ermF* decreased when distributions were discontinued (*ermF* 47.3% lower, 95% CI 36.3% to 56.4% lower; *ermA* 83.8% lower, 95% CI -98.3% to +54%, Table S1).

Antibiotic resistance genes can be correlated for a number of epidemiological and physiological reasons. For example, ARGs may be on the same plasmid or closely linked on the bacterial chromosome. Epidemiologically, the same child exposed to one class of antibiotics may be exposed to other classes as well.[39] The community mean load of genes conferring resistance was clearly correlated between the different classes, although more so at baseline and after placebo MDA than after azithromycin MDA (Figure S1). For example, macrolide and beta-lactam resistance were correlated before any communities had received MDA (r=0.29, 95% CI -0.13 to 0.64). A similar correlation was found in communities which had recently received placebo (*r=0.59, 95% CI 0.43 to 0.71*). The correlation was lower in those communities recently treated with azithromycin (r=0.23, 95% CI 0.05 to 0.39). Although correlation is a risk factor for co-selection, we were unable to show that the correlation found at baseline contributed to any co-selection.[39] Similarly, antibiotic resistance genes (not divided into antibiotic class) were significantly correlated (Supplementary Table S4). The highest correlations with macrolide AMR were found with

aminoglycoside and tetracycline ARGs. The highest correlations with *ermF* were the genes *ant6*, *ermA*, *cfx*, and*tetQ, and tet32*.

While short-read metagenomic deep sequencing typically does not reveal which organism harbors an antibiotic resistance gene, read numbers for the resistance genes correlate with those for specific bacterial genera. For example, *ermF* correlated significantly with *Prevotella species*, which make up a large portion of the gut microbiota in these children. *Prevotella species* are well recognized as hosts of the *ermF* gene (Table S5).

Limitations. Monitoring genetic resistance determinants may be a relatively sensitive way to detect changes in resistance between interventions or over time. But the clinical significance can be difficult to assess. Resistance would presumably not affect illness unless present in pathogenic bacteria. Resistance to macrolides (and in the case of *erm*, lincosamides and streptogramins) would not itself affect susceptibility to more widely prescribed antibiotics such as beta-lactams. The presence of genetic resistance to a class of antibiotics does not necessarily translate to clinical resistance to that class. Although it should be noted that phenotypic resistance (decreased growth in the presence of antibiotic) also does not completely correlate with clinical resistance.[45] The relatively short DNA fragments identified with DNA-seq do not directly identify the organism that harbors the resistance, and a statistical correlation between a gene and a specific genus does not guarantee that genus harbors the gene. Isolation of individual colonies or longer-read sequencing may allow better identification of the organisms which are resistant, although

results would be limited to the few colonies typically selected.[19] In this study, communities were all sampled randomly from the same pool with identical collection protocols, and azithromycin (vs placebo) was randomized at 3 time points. However, not all treatment histories were represented. For example, due to ethical considerations, no communities received placebo for the entire 8 years. The 6-year treatment was technically after the last collection in the MORDOR I-II-III research studies and was provided for purely ethical reasons. While our analyses assumed the official treatment records were correct and communities were treated with the same agent as the previous round, the communities may actually have all received azithromycin. That might explain why year 6 had the highest load found in communities thought to have been recently treated with placebo. Inclusion or exclusion of this time point does not qualitatively affect the results.

Discussion

The benefits of mass azithromycin distributions on child survival need to be balanced against any risks caused by long-term selection of antibiotic resistance.[46] Distributions clearly select for macrolide-resistant bacteria in the gut in the short term.[6,9,15,19] In this analysis, the mean load of genetic determinants conferring resistance to macrolides increased by 25% in the first 3 years of distribution.[6] But we found no evidence of escalation beyond that point. In fact, macrolide resistance plateaued, with no observed increase from 3 to 8 years of MDA. When distributions were discontinued, levels of macrolide resistance returned to pre-intervention levels. The increased macrolide resistance from trachoma distributions, which treat the entire community rather than only

1-59 month olds, has not been observed to cause increased disease. In fact, fewer childhood clinic visits have been observed in households and communities randomized to receive azithromycin in childhood mortality interventions.[47,48]

Co-selection of beta-lactam resistance could cause considerable concern, as penicillins are by far the most commonly prescribed antibiotics in rural Niger and similar settings. We did find that beta-lactam resistance had increased significantly over time in the study area. The randomization of communities to azithromycin or placebo allowed an assessment of the cause of this increase. We were unable to find any evidence that the increase was related to the azithromycin distributions themselves. The increased beta-lactam resistance likely reflects a secular trend independent of azithromycin distributions in the childhood mortality program.

Macrolide resistance was correlated with resistance to other classes of antibiotics across the different surveys. In communities where macrolide resistance was elevated, aminoglycoside and tetracycline tended to be as well. Similar correlations were found at the level of the gene. When *ermF* or *ermA* were elevated, certain genes conferring resistance to other classes were also elevated. This was not unexpected, as resistance genes may be found on the same plasmid or integrative and conjugative elements, or nearby on the bacterial genome itself.[19] Children who live closer to health care facilities may be more likely to be exposed to antibiotic pressure from several classes of antibiotics. Potentially, this could lead to co-selection of other classes of antibiotic.[39] However, we

were unable to demonstrate that azithromycin MDA selected for AMR in any other class of antibiotic. This may be because the design and size of this study lacked the power to detect a small effect. Additionally, the observed correlation between resistance to different classes does not in itself guarantee co-selection.[39] For example, the majority of resistance may not be linked genetically. Each additional AMR gene may have an associated fitness cost, in which case resistance to multiple drugs may not be a particularly effective strategy for any given bacterial strain.[44]

The benefits of mass azithromycin distributions are likely due to effects on a number of different organisms. Distributions dramatically reduce the prevalence of the ocular strains of chlamydia that cause blinding trachoma.[26,27] Respiratory, gastrointestinal, and skin diseases have been observed to decrease with azithromycin treatments.[35,36] Clinic visits have decreased, as has childhood mortality due to pneumonia, diarrhea and dysentery, and malaria.[34,38,49] But this same broad-spectrum activity also puts communities at risk for selection of resistant bacteria. When deciding whether to implement mass azithromycin distributions in areas without trachoma, the benefit of decreased mortality will need to be balanced against the potential long-term harm of increased antibiotic resistance. Here, we did not observe an escalating threat past the first 3 years of intervention. In fact, changes were reversible after the intervention was discontinued. Longer-term monitoring can continue to inform stakeholder decisions.

Methods

Population-based sampling of children from enrolled communities in the MORDOR trials was performed as previously described. Field procedures for sample collection were consistent across the timepoints. Briefly, a door-to-door census was performed and 25-40 children aged 1-59 months were randomly chosen from each community for AMR monitoring.

Study description and participants

MORDOR I, II, and III and AVENIR I were cluster-randomized trials assessing the impact of biannual azithromycin distribution to 1–59-month-old children on childhood mortality. The samples used in this analysis were collected from the Loga, Boboye, and Falmey *departements* of the Dosso region. MORDOR I included sites in Tanzania and Malawi, but only the Niger communities were included in subsequent studies, so this study was limited to Niger samples.[50] Sentinel communities (grappes), where AMR-monitoring was conducted, were randomly selected from the same pool of 594 Nigerien communities monitored for mortality. At baseline, communities were randomized in a 1:1 ratio to biannual mass distribution of either azithromycin or placebo (both Pfizer Inc). Communities were re-randomized at 36 and 72 months as displayed in Figure 2. Rectal swab samples were collected and stored in DNA/RNA Shield (Zymo Research, placed at 4°C in the field, stored in a −20 °C freezer in Niger, then shipped on ice to the University of California, San Francisco, where they were stored at −80 °C. Samples from 0, 36, and 54 months have previously been analyzed and reported, while those from 72 and 96 months

have not. For this study, all samples were reanalyzed from the original specimen in a randomized manner, allowing comparison between the time points.

Ethical approval

We obtained ethical approval for the collections from the Committee for Human Research at the University of California, San Francisco and the Ethical Committee of the Niger Ministry of Public Health (institutional review board number 10-01036). The study was undertaken in accordance with the Declaration of Helsinki (6th version). Given the low literacy rate in Niger, oral consent was obtained from guardians of children before treatment and rectal swab collection. No incentives were offered for participation, and all communities received azithromycin at some time point.

DNA-sequencing and bioinformatics

Researchers performing the assays were masked. Ten samples from each village were randomly chosen for sequencing and analysis. DNA sequencing was performed as previously described.[9] DNA was extracted from pooled samples using the Maxwell RSC Enviro TNA Kit (Promega, Madison, WI, United States) according to the manufacturer's instructions. DNA sequencing libraries were prepared using the New England Biolabs' (NEB, Ipswich, Massachusetts, United States) NEBNext Ultra II DNA Library Prep Kit and amplified with 10 PCR cycles. Libraries were then quantified and pooled for sequencing on the NovaSeq X (Illumina) using 150-nucleotide (nt) paired-end sequencing.

Host reads were removed using a multi-step approach. First, human-derived reads were removed using the STAR aligner against the human reference genome 38 (hg38) and the *Pantroglodytes* genome (panTro4, 2011, UCSC). Low-quality reads were subsequently filtered using PriceSeqfilter with parameters set to remove reads below a quality threshold of 85% at a 0.999 significance level and reads with >95% ambiguous nucleotides. PCR duplicate reads were collapsed using cd-hit dup, and low-information reads were removed using a LZW complexity filter. An additional host read removal step was performed with Bowtie2 in sensitive mode, aligning against the combined human and chimpanzee reference index. The resulting non-host sequences were used as input for all downstream microbiome and AMR analysis.

Kraken2 (version 2.1.2) was used for taxonomic classification for microbiome assessment and Bracken (version 2.5) was used for estimation of genus and species abundances.[51-53] Separately, non-host reads were aligned to the MEGARes database version 3.0.0, a comprehensive database of antimicrobial resistance markers.[54 55,56] Antibiotic resistance determinants were included if they met 80% nucleotide identity between the query sequencing read and the reference AMR sequence. SNP verification was enabled (--snp Y) to confirm resistance determinants that require specific point mutations. Any hits that failed SNP confirmation were excluded. Additionally, the duplication flag (--deduped Y) is used to identify and remove potential PCR duplicate reads prior to resistance gene

quantification. If there were 0 hits across all samples, the respective ARG was excluded from analysis.

Statistical analysis

We modeled the impact of recent MDA in the previous 2-year study phase with a negative binomial Wald test from the DESeq2 package in R. Analysis was at the community-visit level. The counts for ARGs grouped by antibiotic class were modeled using recent MDA, cumulative MDAs before the previous study phase, and calendar time as predictors. Custom size factors, that account for differences in sequencing depth, were calculated using the total number of non-human reads as an offset. Recent MDA indicates the treatment (Placebo or Azithromycin) received by the community in the most recent study phase (approximately the last two years). Cumulative MDA represents the total number of azithromycin treatments received, excluding those given in the most recent study phase. Calendar time was expressed in years. The same analysis was performed on Beta-lactam resistance, and for 18 other non-macrolide antibiotic classes. Classes with zero reads in over 80% of communities (a prespecified threshold) were excluded from the final analysis.

The relationship between burden of resistance, recent MDA, cumulative MDA, and calendar time was also assessed for each ARG with a negative binomial Wald test. Models were fit to the number of reads per ARG, offset by the total non-human reads in that

sample. ARGs with zero reads in over 80% of communities were excluded from the final analysis.

We assessed Spearman's correlation coefficients between *ermF* and *ermA*, with ARG reads per million corresponding to non-macrolide antibiotic classes pre- and post-MDA. Post-MDA calculations were stratified by most recent treatment (i.e. placebo versus azithromycin). To account for multiple comparisons, we used a Bonferroni-Holm adjustment on the p-values corresponding to each ARG separately. Bootstrapping at the community level produced 95% confidence intervals for correlation coefficients.

Spearman's correlation coefficients were also calculated for *ermF* and *ermA* with genus reads per million pre- versus post-MDA. Bonferroni-Holm adjustment was applied to the p-values corresponding to each genus to account for multiple comparisons. Percent relative abundance of genus reads per million was calculated as the total number of reads per genus divided by the sum of the total bacterial reads. Bootstrapping at the community level produced 95% confidence intervals for correlation coefficients.

All analyses were completed with R version 4.4.1 (2024-06-14).

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Table 1: Predictors for Macrolide and Beta-Lactam resistance

Macrolides	Fold change (95% CI)*	*P*-value
Recent MDA**	1.25 (1.19, 1.30)	<0.00001
Cumulative MDA (#)***	1.01 (0.996, 1.02)	0.201
Time (years)	0.988 (0.969, 1.01)	0.204

Beta-Lactams	Fold change (95% CI)	*P*-value
Recent MDA	1.02 (0.955, 1.09)	0.559
Cumulative MDA (#)	1.00 (0.986, 1.02)	0.812
Time (years)	1.06 (1.03, 1.09)	0.00009

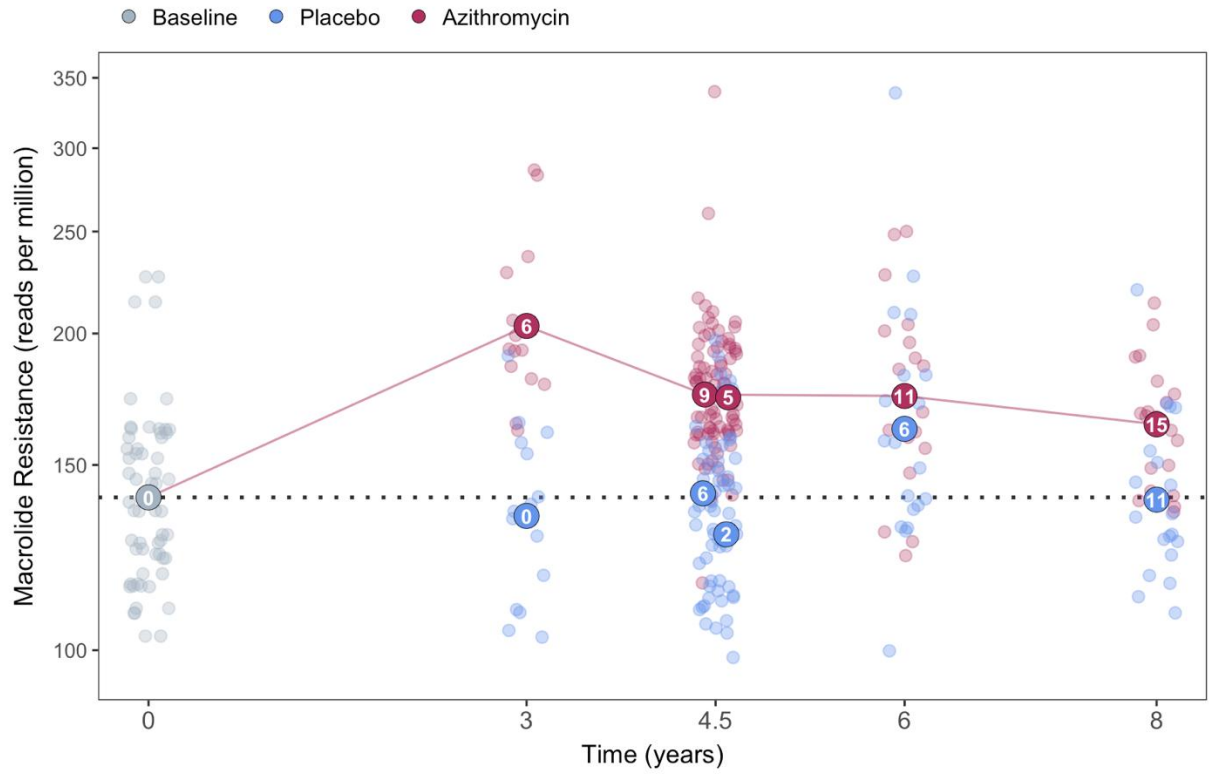
*Negative binomial regression with DESeq2
**Recent azithromycin MDA within the past 2 years
***Cumulative treatments with azithromycin excluding those received within the past 2 years

Table 2: Predictors for Antibiotic Resistance Genes

ARG (class)	Parameter*	Fold change (95% CI)	*P*-value
ermF (Macrolide)	Cumulative MDA (#)	0.970 (0.945, 0.994)	0.02
	Recent MDA	2.19 (1.97, 2.42)	<0.00001
	Time (year)	1.10 (1.05, 1.15)	0.00003
ermA (Macrolide)	Cumulative MDA (#)	0.953 (0.855, 1.06)	0.382
	Recent MDA	2.70 (1.73, 4.21)	0.00001
	Time (year)	0.926 (0.769, 1.12)	0.42
ant6 (Aminoglycoside)	Cumulative MDA (#)	0.965 (0.905, 1.03)	0.272
	Recent MDA	1.71 (1.31, 2.23)	0.00006
	Time (year)	1.16 (1.04, 1.3)	0.008
TET16S (Tetracycline)	Cumulative MDA (#)	0.977 (0.957, 0.998)	0.03
	Recent MDA	1.12 (1.03, 1.22)	0.009
	Time (year)	1.00 (0.969, 1.04)	0.798
cfx (Cephalosporin)	Cumulative MDA (#)	0.978 (0.962, 0.994)	0.007
	Recent MDA	1.08 (1.01, 1.16)	0.02
	Time (year)	1.10 (1.07, 1.13)	<0.00001

*Negative binomial regression with DESeq2

a


Baseline
Placebo
Azithromycin
Macrolide Resistance (reads per million)
350
300
250
200
150
100
0
3
4.5
6
8
Time (years)

b

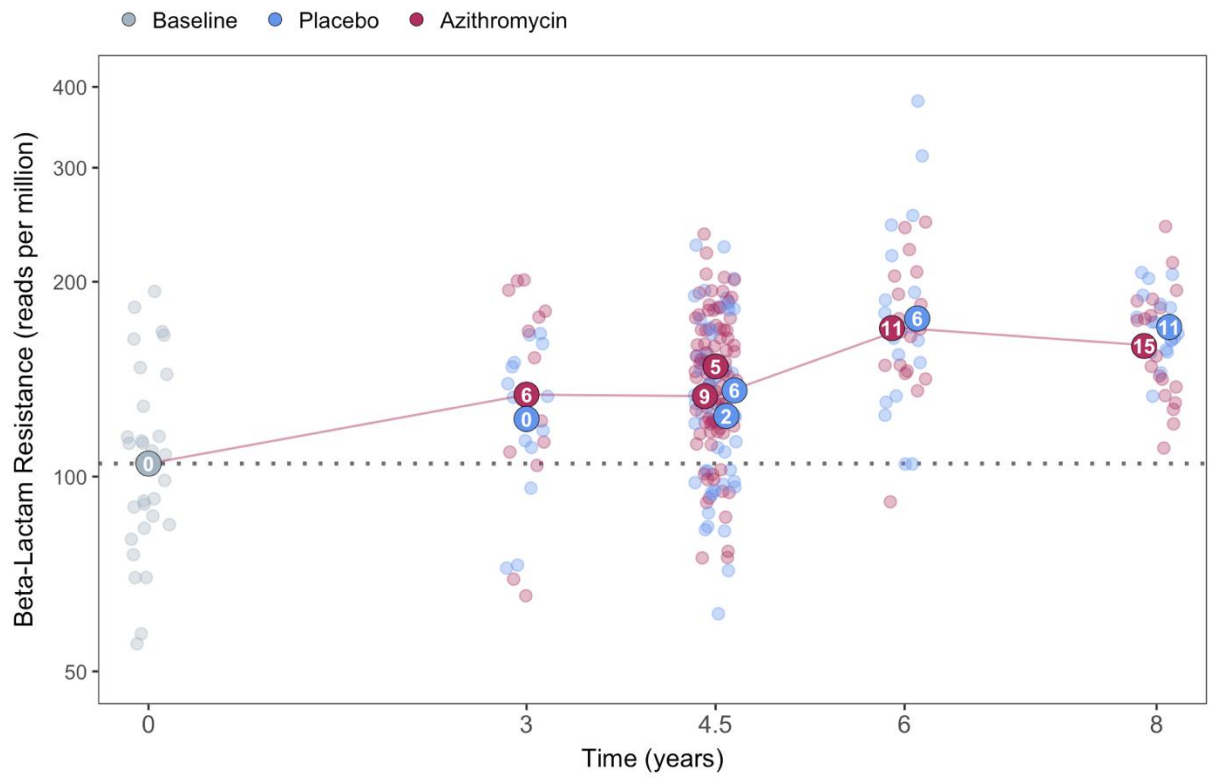

Baseline
Placebo
Azithromycin
Beta-Lactam Resistance (reads per million)
400
300
200
100
50
0
3
4.5
6
8
Time (years)

Figure 1: Community level antimicrobial resistance load over eight years following different randomized treatments. a. Macrolide resistance load expressed as normalized read number in communities over 8 years. **b.** Beta-lactam resistance load expressed as normalized read number in communities over 8 years. In both panels, communities that had received recent MDA with azithromycin within the past 2 years are shown in red, and communities that had received placebo MDA in blue. Points represent mean resistance reads per million at each timepoint. The cumulative number of azithromycin MDAs received by each treatment history overlays the point estimate. Communities that received azithromycin in the previous phase, the approximately 2-year period before sampling, are highlighted with a red line across time points. Note that at the 4.5-year time point, there are 4 possible treatment histories based on randomization to azithromycin or placebo at times 0 and 36 months.

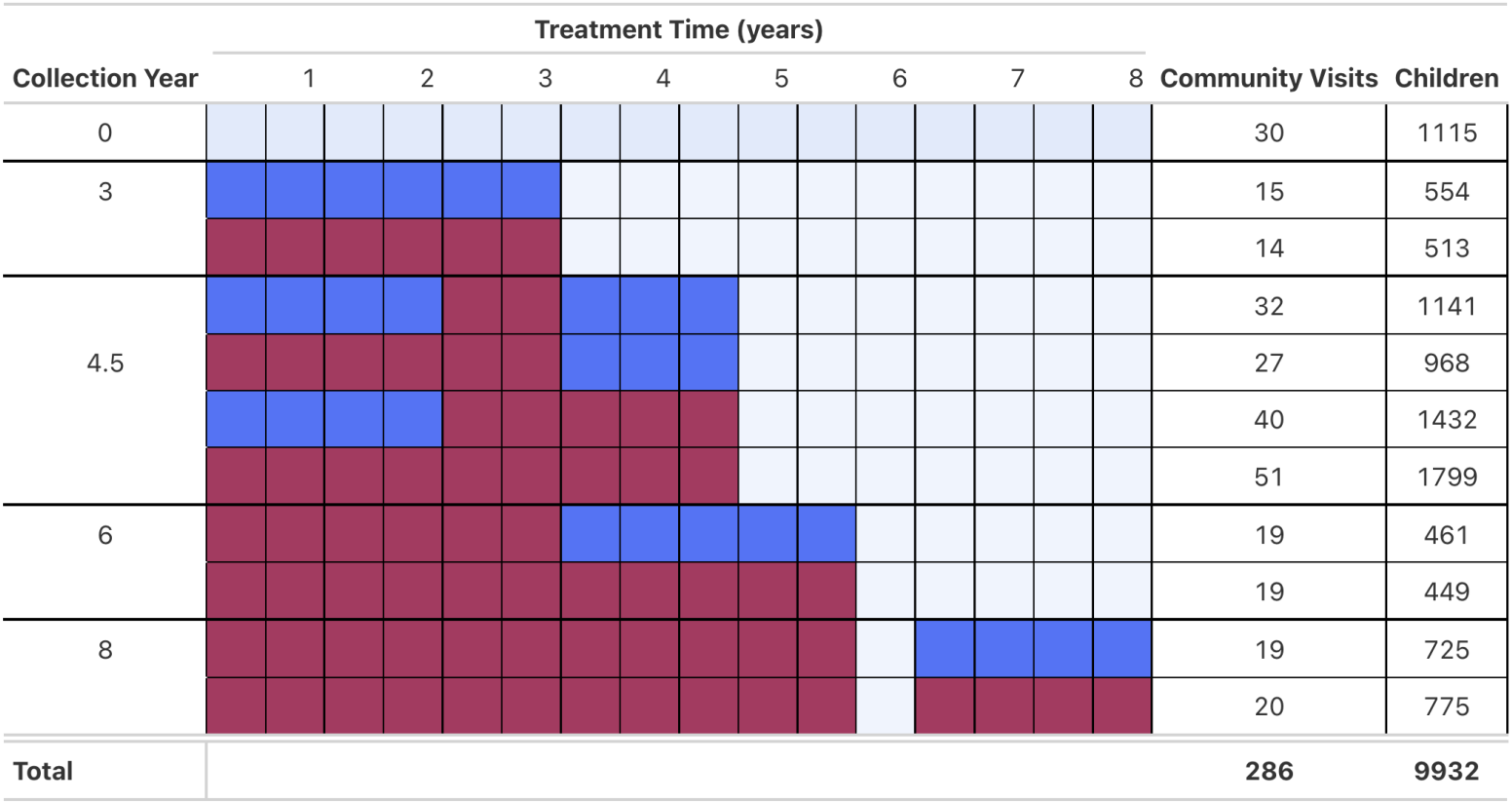


Figure 2: Community treatment schedules over the study period. Communities were randomized at baseline, 3 years, and 6 years to either azithromycin distributions (red cells) or placebo distributions (blue cells), resulting in multiple treatment histories. Those communities monitored at 3 years had been treated from baseline with the same intervention (azithromycin or placebo). Between 5 and 6 years, the SARS-CoV-2 pandemic necessitated a 6-month delay in interventions.

Antibiotic Class	FC (95% CI)	P-value
Aminoglycosides	0.99 (0.87, 1.13)	0.93
Bacitracin	0.59 (0.34, 1.02)	0.06
Beta-Lactams	1.02 (0.96, 1.09)	0.56
Cationic antimicrobial peptides	0.72 (0.53, 0.98)	0.04
Elfamycins	1.40 (0.96, 2.04)	0.08
Fosfomycin	0.75 (0.52, 1.06)	0.1
Lipopeptides	0.75 (0.51, 1.11)	0.15
Metronidazole	1.04 (0.91, 1.20)	0.56
Macrolides	1.25 (1.19, 1.30)	<0.0001
Mupirocin	0.27 (0.11, 0.64)	0.003
Rifampin	0.33 (0.15, 0.75)	0.008
Tetracyclines	1.03 (0.98, 1.07)	0.27
Trimethoprim	0.98 (0.90, 1.08)	0.74

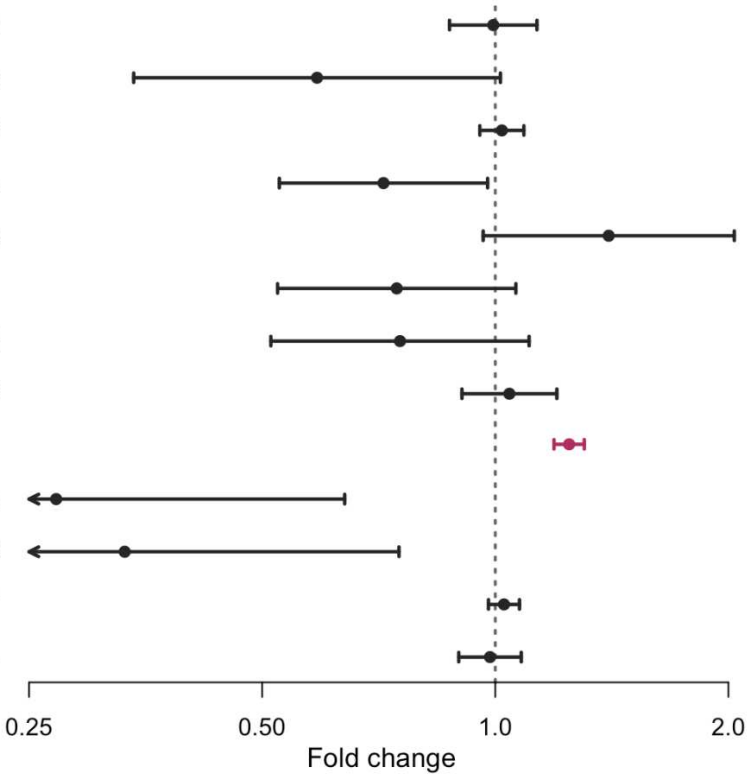


Figure 3: Effect of recent azithromycin MDA on resistance load for all antibiotic classes. Fold change and 95% confidence intervals were calculated using negative binomial regression in the DESeq2 package to explore possible co-selection for resistance to non-macrolide antibiotic classes.

Supplementary Material

Table S1: Effect of randomization to placebo in year 6 on year 8 isolates

Resistance	Fold change (95% CI)	*P*-value
Macrolides	0.854 (0.771, 0.946)	0.003
ermA	0.162 (0.017, 1.54)	0.113
ermF	0.527 (0.436, 0.637)	<0.00001

Table S2A: Recent Placebo *ermF* correlated ARGs

ARG	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
ant6	0.55	(0.40, 0.67)	<0.0001	<0.0001
cfx	0.51	(0.36, 0.64)	<0.0001	<0.0001
nimA	0.33	(0.14, 0.50)	0.0003	0.01

Table S2B: Recent Azithromycin *ermF* correlated ARGs

ARG	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
tetQ	0.39	(0.25, 0.50)	<0.0001	<0.0001
ant6	0.33	(0.17, 0.47)	<0.0001	0.002
tet32	0.32	(0.18, 0.47)	<0.0001	0.002
cfx	0.32	(0.16, 0.47)	<0.0001	0.002
dfrF	0.29	(0.13, 0.44)	0.0004	0.008
tet40	0.28	(0.13, 0.42)	0.0008	0.02
ermA	0.25	(0.07, 0.41)	0.002	0.04
tetO	0.25	(0.10, 0.39)	0.002	0.04
aci	-0.26	(-0.41, -0.09)	0.002	0.03
APH(6)	-0.26	(-0.41, -0.09)	0.001	0.03
MLS23S	-0.28	(-0.42, -0.11)	0.0007	0.01
ampCR	-0.29	(-0.45, -0.13)	0.0003	0.008
asmA	-0.29	(-0.44, -0.13)	0.0003	0.008
mphB	-0.3	(-0.44, -0.14)	0.0003	0.007
aph3-dprime	-0.3	(-0.44, -0.13)	0.0003	0.007
dfrA	-0.3	(-0.45, -0.13)	0.0003	0.007
bacA	-0.32	(-0.45, -0.16)	0.0001	0.003
kdpE	-0.33	(-0.46, -0.16)	<0.0001	0.002
pmrAR	-0.34	(-0.49, -0.17)	<0.0001	0.0008
ileS	-0.36	(-0.49, -0.19)	<0.0001	0.0004
PBP2	-0.36	(-0.50, -0.21)	<0.0001	0.0003
pmrF	-0.37	(-0.52, -0.22)	<0.0001	0.0002
rpoB	-0.38	(-0.51, -0.23)	<0.0001	<0.0001
PBP4b	-0.39	(-0.53, -0.24)	<0.0001	<0.0001
ampH	-0.4	(-0.54, -0.25)	<0.0001	<0.0001
ugd	-0.4	(-0.53, -0.26)	<0.0001	<0.0001
TetRM	-0.41	(-0.54, -0.26)	<0.0001	<0.0001
eptA	-0.44	(-0.56, -0.31)	<0.0001	<0.0001
msbA	-0.46	(-0.59, -0.30)	<0.0001	<0.0001

Table S3A: Recent Placebo *ermA* correlated ARGs

ARG	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
ugd	-0.3	(-0.45, -0.13)	0.001	0.04
asmA	-0.3	(-0.46, -0.12)	0.001	0.04
H-NS	-0.3	(-0.46, -0.12)	0.001	0.04
pmrF	-0.31	(-0.46, -0.14)	0.0009	0.04
AmpH	-0.36	(-0.53, -0.17)	0.0001	0.004
eptA	-0.37	(-0.52, -0.20)	<0.0001	0.003
MLS23S	-0.38	(-0.54, -0.20)	<0.0001	0.002
ileS	-0.47	(-0.59, -0.34)	<0.0001	<0.0001
rpoB	-0.51	(-0.63, -0.37)	<0.0001	<0.0001

Table S3B: Recent Azithromycin *ermA* correlated ARGs

ARG	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
ERF	0.25	(0.07, 0.41)	0.002	0.08
tet32	0.24	(0.07, 0.39)	0.004	0.109
dfrF	0.2	(0.03, 0.35)	0.01	0.341
tet40	0.19	(0.01, 0.35)	0.02	0.471
TET16S	0.19	(0.02, 0.35)	0.02	0.471
aph3-dprime	-0.17	(-0.33, -0.01)	0.04	0.644
APH(6)	-0.18	(-0.33, -0.01)	0.03	0.557
Tet(W)	-0.19	(-0.34, -0.02)	0.02	0.471
rrsA	-0.19	(-0.35, -0.03)	0.02	0.463
TetR	-0.2	(-0.35, -0.03)	0.02	0.421
pmrAR	-0.21	(-0.37, -0.04)	0.01	0.272
msbA	-0.22	(-0.37, -0.05)	0.01	0.238
MLS23S	-0.22	(-0.37, -0.07)	0.007	0.194
mphB	-0.23	(-0.38, -0.06)	0.005	0.142
ugd	-0.23	(-0.39, -0.07)	0.005	0.142
ACI	-0.24	(-0.39, -0.07)	0.004	0.112
PBP2	-0.25	(-0.40, -0.08)	0.003	0.09
CTX	-0.26	(-0.41, -0.10)	0.001	0.05
ampCR	-0.27	(-0.42, -0.10)	0.001	0.04
ampH	-0.29	(-0.44, -0.15)	0.0003	0.01
bacA	-0.3	(-0.44, -0.15)	0.0002	0.008
kdpE	-0.31	(-0.46, -0.15)	0.0001	0.005
eptA	-0.32	(-0.46, -0.16)	<0.0001	0.003

pmrF	-0.32	(-0.45, -0.17)	<0.0001	0.003
PBP4b	-0.33	(-0.47, -0.18)	<0.0001	0.002
asmA	-0.36	(-0.49, -0.21)	<0.0001	0.0003
ileS	-0.53	(-0.63, -0.41)	<0.0001	<0.0001
rpoB	-0.54	(-0.65, -0.42)	<0.0001	<0.0001

Table S4A: Recent Placebo *ermF*-genus correlations

Genus	Relative abundance (%)	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
Prevotella	26.74	0.47	(0.32, 0.60)	<0.0001	<0.0001
Faecalibacterium	6.71	0.42	(0.24, 0.56)	<0.0001	0.0002
Bifidobacterium	4.46	-0.36	(-0.52, -0.21)	<0.0001	0.002
Simiaoa	2	0.3	(0.12, 0.48)	0.001	0.04
Roseburia	1.27	0.38	(0.21, 0.51)	<0.0001	0.001
Ruminococcus	1.14	0.38	(0.20, 0.53)	<0.0001	0.002
Lachnospira	0.91	0.35	(0.17, 0.53)	0.0002	0.006
Alistipes	0.45	0.34	(0.15, 0.51)	0.0002	0.008
Phascolarctobacterium	0.45	0.33	(0.16, 0.49)	0.0004	0.02
Limosilactobacillus	0.14	-0.33	(-0.48, -0.17)	0.0004	0.02
Enterococcus	0.13	-0.34	(-0.50, -0.17)	0.0003	0.008
Corynebacterium	0.1	-0.39	(-0.54, -0.21)	<0.0001	0.001
Sodaliphilus	0.04	0.35	(0.18, 0.50)	0.0001	0.006
Dysosmobacter	0.04	0.36	(0.18, 0.52)	0.0001	0.004
Proteus	0.02	-0.35	(-0.49, -0.18)	0.0001	0.005
Coprobacter	0.007	0.33	(0.15, 0.49)	0.0004	0.02
Butyrivibrio	0.006	0.43	(0.25, 0.58)	<0.0001	0.0001
Massilistercora	0.003	0.35	(0.18, 0.50)	0.0002	0.008
Methylorubrum	<0.001	-0.33	(-0.50, -0.14)	0.0004	0.02

Table S4B: Recent Azithromycin *ermF*-genus correlations

Genus	Relative abundance (%)	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
Prevotella	26.74	0.4	(0.25, 0.54)	<0.0001	<0.0001
Bacteroides	20.17	-0.25	(-0.40, -0.08)	0.002	0.04
Bifidobacterium	4.46	-0.37	(-0.50, -0.21)	<0.0001	0.0002
Escherichia	2.85	-0.37	(-0.50, -0.21)	<0.0001	0.0001
Roseburia	1.27	0.34	(0.17, 0.48)	<0.0001	0.001
Ruminococcus	1.14	0.26	(0.11, 0.41)	0.001	0.04
Lachnospira	0.91	0.42	(0.26, 0.55)	<0.0001	<0.0001
Parabacteroides	0.83	-0.28	(-0.44, -0.10)	0.0006	0.02
Wujia	0.54	0.31	(0.15, 0.45)	0.0002	0.008
Megasphaera	0.48	-0.27	(-0.41, -0.11)	0.001	0.03
Phascolarctobacterium	0.45	0.3	(0.14, 0.44)	0.0003	0.01

Weissella	0.32	0.32	(0.16, 0.47)	0.0001	0.005
Sutterella	0.29	-0.3	(-0.43, -0.16)	0.0003	0.006
Sarcina	0.28	0.26	(0.11, 0.42)	0.001	0.04
Anaerotruncus	0.21	0.31	(0.15, 0.45)	0.0002	0.008
Enterococcus	0.13	-0.3	(-0.45, -0.13)	0.0003	0.009
Schaalia	0.05	-0.28	(-0.43, -0.12)	0.0006	0.03
Sodaliphilus	0.04	0.45	(0.32, 0.58)	<0.0001	<0.0001
Prevotellamassilia	0.03	0.32	(0.15, 0.46)	0.0001	0.004
Rothia	0.02	0.43	(0.27, 0.56)	<0.0001	<0.0001
Eggerthella	0.02	-0.42	(-0.54, -0.27)	<0.0001	<0.0001
Proteus	0.02	-0.29	(-0.43, -0.13)	0.0004	0.01
Terrisporobacter	0.02	0.35	(0.19, 0.49)	<0.0001	0.0008
Pseudobutyrivibrio	0.009	0.26	(0.10, 0.41)	0.002	0.05
Butyrivibrio	0.006	0.28	(0.12, 0.42)	0.0006	0.02
Romboutsia	0.005	0.3	(0.13, 0.45)	0.0003	0.01
Leuconostoc	0.004	0.29	(0.13, 0.43)	0.0005	0.02
Faecalitalea	0.002	0.31	(0.16, 0.47)	0.0001	0.006
Candidatus Nanosynbacter	0.001	0.29	(0.14, 0.44)	0.0004	0.02
Abiotrophia	<0.001	0.29	(0.13, 0.43)	0.0004	0.01
Capnocytophaga	<0.001	0.28	(0.10, 0.44)	0.0008	0.04
Candidatus Saccharimonas	<0.001	0.3	(0.15, 0.43)	0.0003	0.01
Cupriavidus	<0.001	-0.28	(-0.43, -0.11)	0.0008	0.03

Table S5A: Recent Placebo *ermA*-genus correlations

Genus	Relative abundance (%)	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
Bifidobacterium	4.46	-0.56	(-0.67, -0.42)	<0.0001	<0.0001
Escherichia	2.85	-0.34	(-0.50, -0.16)	0.0003	0.005
Porphyromonas	1.66	0.46	(0.30, 0.60)	<0.0001	<0.0001
Roseburia	1.27	0.37	(0.18, 0.51)	<0.0001	0.002
Lachnospira	0.91	0.33	(0.15, 0.48)	0.0004	0.02
Collinsella	0.34	-0.37	(-0.54, -0.20)	<0.0001	0.001
Sarcina	0.28	0.45	(0.29, 0.59)	<0.0001	<0.0001
Ezakiella	0.18	0.57	(0.40, 0.69)	<0.0001	<0.0001
Limosilactobacillus	0.14	-0.3	(-0.46, -0.12)	0.001	0.04
Enterococcus	0.13	-0.41	(-0.56, -0.24)	<0.0001	0.0003
Finegoldia	0.09	0.56	(0.40, 0.69)	<0.0001	<0.0001
Ndongobacter	0.09	0.51	(0.37, 0.64)	<0.0001	<0.0001
Anaerococcus	0.08	0.59	(0.44, 0.71)	<0.0001	<0.0001
Negativicoccus	0.06	0.56	(0.41, 0.70)	<0.0001	<0.0001
Peptoniphilus	0.06	0.54	(0.38, 0.67)	<0.0001	<0.0001
Schaalia	0.05	0.3	(0.12, 0.47)	0.001	0.05
Sneathia	0.05	0.32	(0.13, 0.47)	0.0007	0.03
Dialister	0.04	0.39	(0.22, 0.53)	<0.0001	0.001
Fenollaria	0.04	0.55	(0.40, 0.68)	<0.0001	<0.0001
Agrobacterium	0.04	0.57	(0.42, 0.69)	<0.0001	<0.0001
Dysosmobacter	0.04	0.34	(0.17, 0.50)	0.0002	0.008
Granulicatella	0.03	0.41	(0.24, 0.56)	<0.0001	0.0002
Mageeibacillus	0.03	0.49	(0.34, 0.63)	<0.0001	<0.0001
Murdochiella	0.03	0.62	(0.48, 0.73)	<0.0001	<0.0001
Mobiluncus	0.02	0.6	(0.46, 0.70)	<0.0001	<0.0001
Staphylococcus	0.02	0.36	(0.18, 0.51)	0.0001	0.004
Proteus	0.02	-0.35	(-0.51, -0.17)	0.0001	0.005
Gemella	0.01	0.42	(0.25, 0.57)	<0.0001	0.0001
Pseudomonas	0.01	0.43	(0.26, 0.57)	<0.0001	<0.0001
Cruoricaptor	0.01	0.57	(0.40, 0.68)	<0.0001	<0.0001
Fastidiosipila	0.007	0.58	(0.42, 0.71)	<0.0001	<0.0001
Lawsonella	0.005	0.46	(0.28, 0.60)	<0.0001	<0.0001
Varibaculum	0.004	0.51	(0.34, 0.64)	<0.0001	<0.0001
Mogibacterium	0.003	0.4	(0.23, 0.55)	<0.0001	0.0004
Achromobacter	0.002	0.41	(0.22, 0.57)	<0.0001	0.0002
Kocuria	0.002	0.39	(0.22, 0.54)	<0.0001	0.001
Rhizobium	0.002	0.4	(0.23, 0.54)	<0.0001	0.0005
Burkholderia	0.002	0.34	(0.17, 0.49)	0.0002	0.008
Helcococcus	0.001	0.51	(0.35, 0.64)	<0.0001	<0.0001

Vaginimicrobium	0.001	0.36	(0.17, 0.51)	0.0001	0.005
Dolosigranulum	<0.001	0.36	(0.20, 0.51)	<0.0001	0.003
Erysipelothrix	<0.001	0.37	(0.17, 0.53)	<0.0001	0.003
Micrococcus	<0.001	0.44	(0.27, 0.60)	<0.0001	<0.0001
Suicoccus	<0.001	0.33	(0.15, 0.50)	0.0004	0.02
Jeotgalibacillus	<0.001	0.34	(0.15, 0.51)	0.0002	0.009
Aerococcus	<0.001	0.35	(0.17, 0.53)	0.0001	0.006
Brevibacterium	<0.001	0.41	(0.22, 0.56)	<0.0001	0.0004
Salinicoccus	<0.001	0.32	(0.13, 0.49)	0.0006	0.03

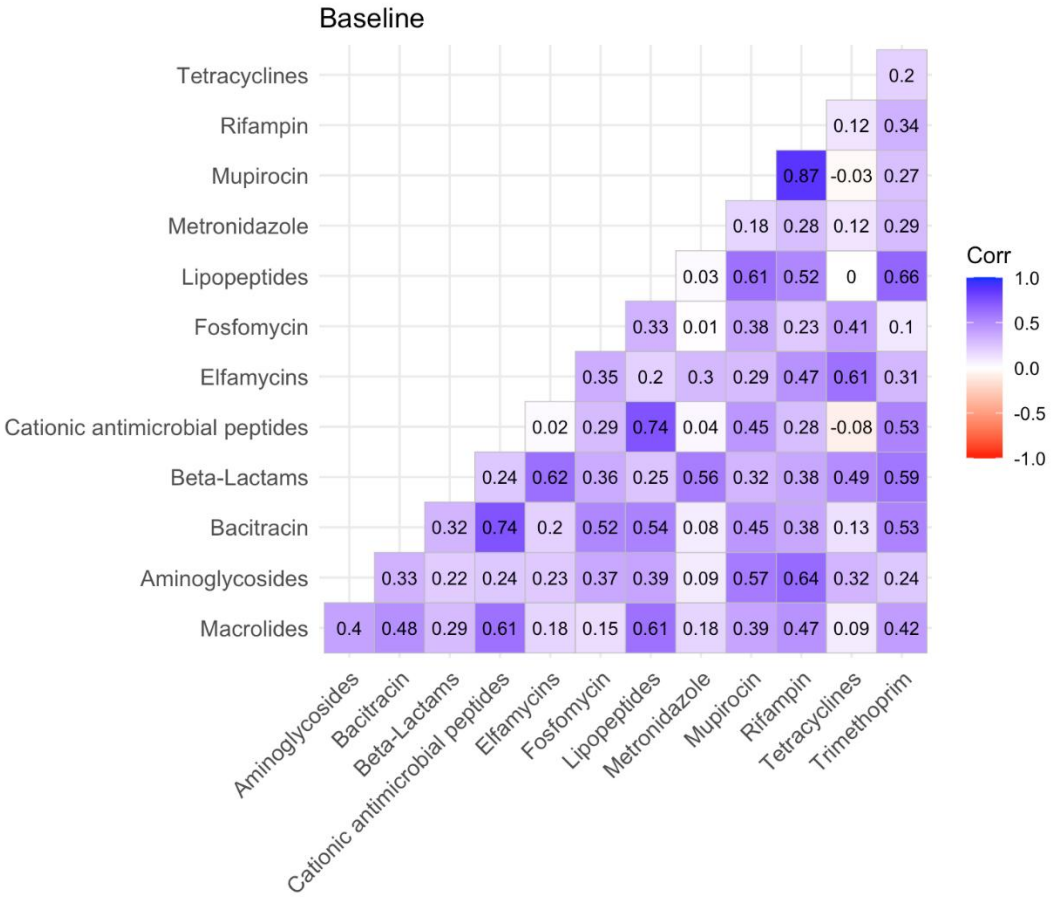
Table S5B: Recent Azithromycin *ermA*-genus correlations

Genus	Relative abundance (%)	Correlation coefficient	95% CI	P-value	Bonferroni-holm adjusted p-value
Bifidobacterium	4.46	-0.51	(-0.62, -0.37)	<0.0001	<0.0001
Escherichia	2.85	-0.29	(-0.42, -0.15)	0.0005	0.006
Porphyromonas	1.66	0.56	(0.43, 0.68)	<0.0001	<0.0001
Coprococcus	1.23	0.35	(0.20, 0.49)	<0.0001	0.0007
Ruminococcus	1.14	0.39	(0.24, 0.53)	<0.0001	<0.0001
Megasphaera	0.48	-0.54	(-0.65, -0.42)	<0.0001	<0.0001
Veillonella	0.45	0.27	(0.12, 0.42)	0.0009	0.04
Sutterella	0.29	-0.26	(-0.42, -0.12)	0.001	0.03
Sarcina	0.28	0.3	(0.14, 0.45)	0.0003	0.009
Odoribacter	0.21	0.3	(0.13, 0.46)	0.0003	0.01
Ezakiella	0.18	0.8	(0.72, 0.85)	<0.0001	<0.0001
Enterococcus	0.13	-0.37	(-0.51, -0.22)	<0.0001	0.0001
Corynebacterium	0.1	0.31	(0.16, 0.46)	0.0002	0.007
Eubacterium	0.1	0.37	(0.22, 0.50)	<0.0001	0.0002
Finegoldia	0.09	0.78	(0.69, 0.85)	<0.0001	<0.0001
Ndongobacter	0.09	0.54	(0.39, 0.67)	<0.0001	<0.0001
Anaerococcus	0.08	0.78	(0.70, 0.85)	<0.0001	<0.0001
Streptobacillus	0.08	0.3	(0.15, 0.45)	0.0002	0.009
Negativicoccus	0.06	0.76	(0.66, 0.84)	<0.0001	<0.0001
Peptoniphilus	0.06	0.71	(0.61, 0.80)	<0.0001	<0.0001
Brevundimonas	0.05	0.45	(0.30, 0.58)	<0.0001	<0.0001
Schaalia	0.05	0.35	(0.20, 0.51)	<0.0001	0.0006
Fenollaria	0.04	0.83	(0.77, 0.89)	<0.0001	<0.0001
Agrobacterium	0.04	0.67	(0.55, 0.77)	<0.0001	<0.0001

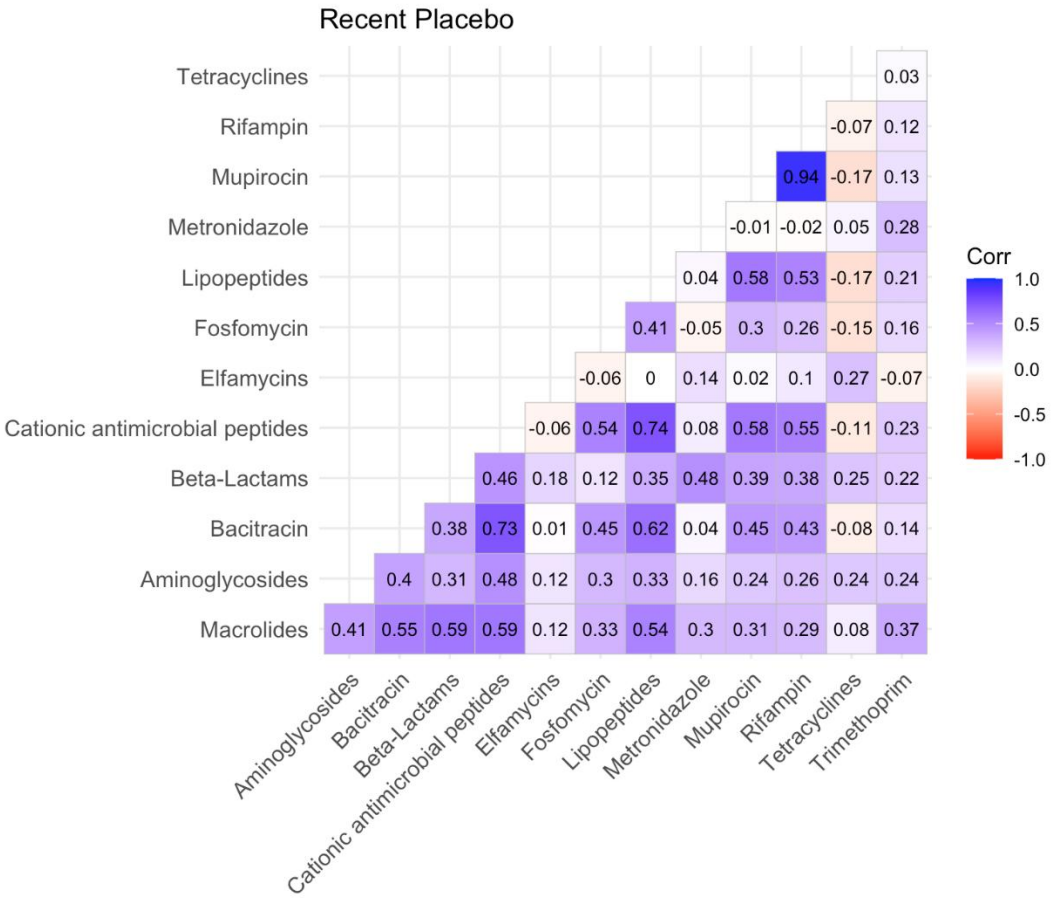
Morganella	0.03	-0.28	(-0.42, -0.14)	0.0007	0.02
Granulicatella	0.03	0.45	(0.31, 0.58)	<0.0001	<0.0001
Mageeibacillus	0.03	0.48	(0.33, 0.61)	<0.0001	<0.0001
Caproicibacterium	0.03	0.27	(0.11, 0.42)	0.0009	0.04
Murdochiella	0.03	0.8	(0.72, 0.87)	<0.0001	<0.0001
Parolsenella	0.03	-0.28	(-0.43, -0.12)	0.0007	0.02
Mobiluncus	0.02	0.73	(0.65, 0.80)	<0.0001	<0.0001
Rothia	0.02	0.29	(0.13, 0.44)	0.0004	0.01
Staphylococcus	0.02	0.57	(0.44, 0.68)	<0.0001	<0.0001
Eggerthella	0.02	-0.32	(-0.47, -0.16)	<0.0001	0.002
Acidaminococcus	0.02	-0.33	(-0.46, -0.18)	<0.0001	0.002
Methylobacterium	0.02	0.35	(0.19, 0.50)	<0.0001	0.0007
Delftia	0.01	0.42	(0.27, 0.56)	<0.0001	<0.0001
Gemella	0.01	0.53	(0.39, 0.65)	<0.0001	<0.0001
Pseudomonas	0.01	0.3	(0.13, 0.44)	0.0003	0.01
Cruoricaptor	0.01	0.73	(0.63, 0.81)	<0.0001	<0.0001
Gardnerella	0.01	0.39	(0.23, 0.52)	<0.0001	<0.0001
Fastidiosipila	0.007	0.75	(0.66, 0.83)	<0.0001	<0.0001
Lawsonella	0.005	0.54	(0.40, 0.68)	<0.0001	<0.0001
Leuconostoc	0.004	0.33	(0.17, 0.48)	<0.0001	0.002
Alcaligenes	0.004	0.38	(0.23, 0.52)	<0.0001	<0.0001
Varibaculum	0.004	0.64	(0.52, 0.74)	<0.0001	<0.0001
Acinetobacter	0.004	0.3	(0.14, 0.45)	0.0003	0.01
Mogibacterium	0.003	0.49	(0.35, 0.61)	<0.0001	<0.0001
Rhodococcus	0.003	0.37	(0.22, 0.51)	<0.0001	0.0002
Massilistercora	0.003	0.27	(0.12, 0.42)	0.001	0.04
Achromobacter	0.002	0.58	(0.44, 0.69)	<0.0001	<0.0001
Kocuria	0.002	0.46	(0.30, 0.59)	<0.0001	<0.0001
Rhizobium	0.002	0.55	(0.41, 0.68)	<0.0001	<0.0001
Burkholderia	0.002	0.43	(0.29, 0.56)	<0.0001	<0.0001
Helcococcus	0.001	0.75	(0.65, 0.83)	<0.0001	<0.0001
Vaginimicrobium	0.001	0.57	(0.44, 0.68)	<0.0001	<0.0001
Dolosigranulum	<0.001	0.52	(0.38, 0.65)	<0.0001	<0.0001
Abiotrophia	<0.001	0.29	(0.14, 0.42)	0.0005	0.02
Bradyrhizobium	<0.001	0.48	(0.33, 0.61)	<0.0001	<0.0001
Erysipelothrix	<0.001	0.44	(0.30, 0.57)	<0.0001	<0.0001
Micrococcus	<0.001	0.47	(0.32, 0.61)	<0.0001	<0.0001
Cutibacterium	<0.001	0.37	(0.21, 0.52)	<0.0001	0.0003
Capnocytophaga	<0.001	0.32	(0.17, 0.45)	0.0001	0.005
Suicoccus	<0.001	0.39	(0.24, 0.53)	<0.0001	<0.0001
Caldibacillus	<0.001	-0.31	(-0.45, -0.15)	0.0002	0.009
Moraxella	<0.001	0.37	(0.23, 0.49)	<0.0001	0.0003

Aerococcus	<0.001	0.48	(0.34, 0.61)	<0.0001	<0.0001
Brucella	<0.001	0.44	(0.29, 0.58)	<0.0001	<0.0001
Faecalibaculum	<0.001	0.27	(0.11, 0.42)	0.001	0.05
Bulleidia	<0.001	0.39	(0.25, 0.53)	<0.0001	<0.0001
Aminobacter	<0.001	0.4	(0.26, 0.53)	<0.0001	<0.0001
Methylorubrum	<0.001	0.36	(0.20, 0.50)	<0.0001	0.0004
Latilactobacillus	<0.001	0.29	(0.15, 0.43)	0.0004	0.02
Stenotrophomonas	<0.001	0.35	(0.19, 0.49)	<0.0001	0.0006
Arthrobacter	<0.001	0.37	(0.23, 0.50)	<0.0001	0.0002
Arcanobacterium	<0.001	0.28	(0.13, 0.43)	0.0005	0.02
Kytococcus	<0.001	0.31	(0.15, 0.46)	0.0002	0.008
Janibacter	<0.001	0.27	(0.11, 0.42)	0.001	0.04

a.


Baseline
Tetracyclines
Rifampin
Mupirocin
Metronidazole
Lipopeptides
Fosfomycin
Elfamycins
Cationic antimicrobial peptides
Beta-Lactams
Bacitracin
Aminoglycosides
Macrolides
0.2
0.12 0.34
0.87 -0.03 0.27
0.18 0.28 0.12 0.29
0.03 0.61 0.52 0 0.66
0.33 0.01 0.38 0.23 0.41 0.1
0.35 0.2 0.3 0.29 0.47 0.61 0.31
0.02 0.29 0.74 0.04 0.45 0.28 -0.08 0.53
0.24 0.62 0.36 0.25 0.56 0.32 0.38 0.49 0.59
0.32 0.74 0.2 0.52 0.54 0.08 0.45 0.38 0.13 0.53
0.33 0.22 0.24 0.23 0.37 0.39 0.09 0.57 0.64 0.32 0.24
0.4 0.48 0.29 0.61 0.18 0.15 0.61 0.18 0.39 0.47 0.09 0.42
Aminoglycosides
Bacitracin
Beta-Lactams
Cationic antimicrobial peptides
Elfamycins
Fosfomycin
Lipopeptides
Metronidazole
Mupirocin
Rifampin
Tetracyclines
Trimethoprim
Corr
1.0
0.5
0.0
-0.5
-1.0

b.


Recent Placebo
Tetracyclines
Rifampin
Mupirocin
Metronidazole
Lipopeptides
Fosfomycin
Elfamycins
Cationic antimicrobial peptides
Beta-Lactams
Bacitracin
Aminoglycosides
Macrolides
0.03
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0.04 0.58 0.53 -0.17 0.21
0.41 -0.05 0.3 0.26 -0.15 0.16
-0.06 0 0.14 0.02 0.1 0.27 -0.07
-0.06 0.54 0.74 0.08 0.58 0.55 -0.11 0.23
0.46 0.18 0.12 0.35 0.48 0.39 0.38 0.25 0.22
0.38 0.73 0.01 0.45 0.62 0.04 0.45 0.43 -0.08 0.14
0.4 0.31 0.48 0.12 0.3 0.33 0.16 0.24 0.26 0.24 0.24
0.41 0.55 0.59 0.59 0.12 0.33 0.54 0.3 0.31 0.29 0.08 0.37
Aminoglycosides
Bacitracin
Beta-Lactams
Cationic antimicrobial peptides
Elfamycins
Fosfomycin
Lipopeptides
Metronidazole
Mupirocin
Rifampin
Tetracyclines
Trimethoprim
Corr
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-0.5
-1.0

c.

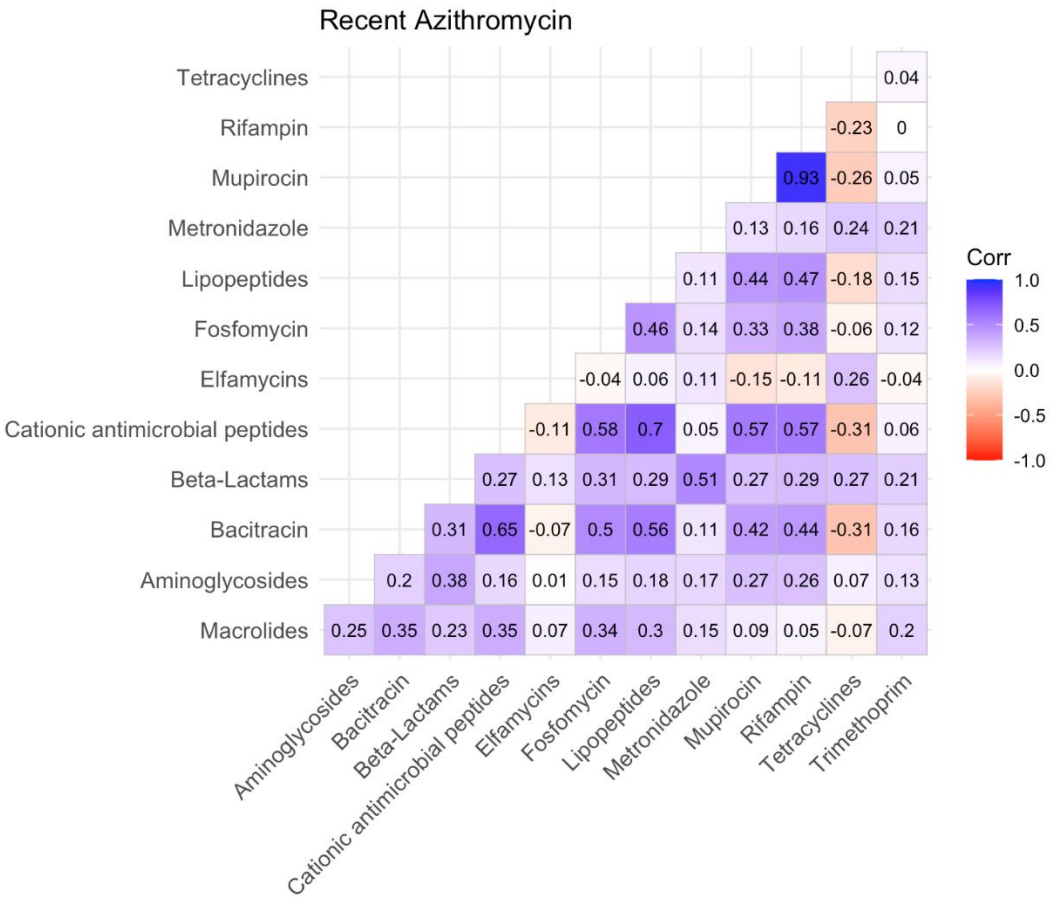


Figure S1: Correlation of antibiotic resistance by class. **a**) Antibiotic class correlation matrix at baseline, prior to any treatment. Antibiotic class correlation matrices stratified by recent treatment with placebo (**b**) or azithromycin (**c**) in the approximately two-year period before sample collection.

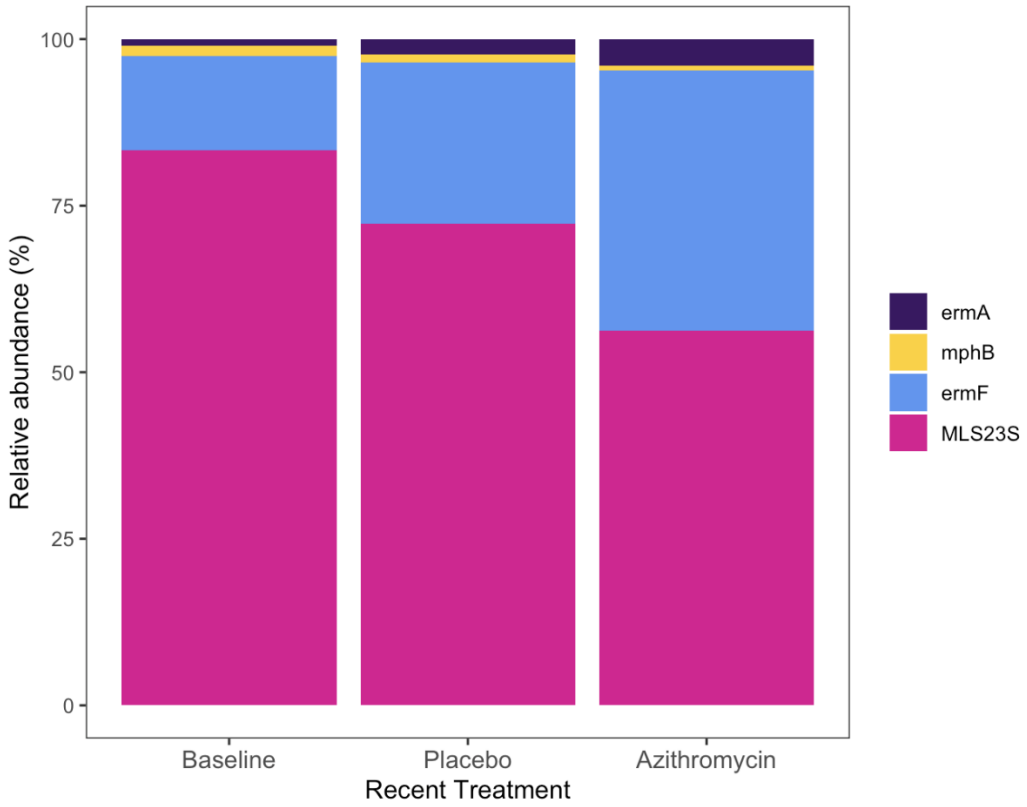


Figure S2: Relative abundance of macrolide ARGs. Relative abundance of macrolide ARGs and baseline compared to communities that had received treatment with placebo for the previous study period and those who had received azithromycin for the previous study period.