

polymorphic regions were associated with village-level prevalence of infection ([Supplementary Figure 4](#)).

No SNPs were associated with village-level prevalence of TF ([Figure 3A](#)). However, 8 polymorphic regions from positions 774 000–791 000 were associated with TF prevalence ([Figure 3B](#)). SNPs in these regions were focused in CTA0743/*pbpB* (harboring 29 SNPs), CTA0747/*sufD* (10 SNPs), and CTA0742/*ompA* (7 SNPs). All SNP

Simpson D and adjusting for number of genomes sampled per district. We used published *Ct* infection, TF, and TI prevalence estimates [6, 28] (Table 3). *Ct* infection and TI prevalence were significantly higher with increasing *ompA* diversity; a similar trend was found for TF prevalence. In a multivariate model, only *Ct* infection prevalence was associated with increasing *ompA* diversity.

DISCUSSION

This study sequenced *Ct* from ocular samples collected from districts in Amhara, Ethiopia, which had received approximately 5 years of the SAFE strategy, as part of trachoma control efforts. We found that sequences were typical of ocular *Ct*, at both the whole-genome level and in tropism-associated genes, yet phylogenetically distinct from most previously sequenced *Ct* genomes. There was no evidence of macrolide-resistance alleles in this ocular *Ct* population. Greater *ompA* diversity

at the district level was associated with increased *Ct* infection prevalence. A continued commitment to the implementation of the SAFE strategy with consideration of enhanced MDA accompanied by further longitudinal investigation is warranted in Amhara.

Almost 900 million doses of azithromycin have been distributed by trachoma control programs since 1999, and in Amhara 15 million doses are administered annually [3]. Mass distribution (u)-5 (t)-5 (m).9 (f)0.5 (en)-5 9-4.9 ((d a)9 (nn)19 (u)-3 ()13 40

have been associated with trachoma; therefore, resistance in other bacteria may be important.

No *Ct* genomes in this study had acquired azithromycin-resistance alleles, but there may be other genomic factors that support *Ct* transmission after treatment. To explore this, we compared Amharan *Ct* genomes with previously sequenced *Ct* to find polymorphism(s) specific to this population that could explain continued transmission. The few SNPs identified as specific to Amhara were dispersed across the genome in known polymorphic genes, rather than being overrepresented in genes related to *Ct* survival. The typical nature of this *Ct* population was supported by phylogenetic clustering with other ocular 10 ()TJ0.109 Tw 0 -1.368 Td[(in g)8 (en)46id5(si1 (or)2 (p

can increase nasopharyngeal carriage of macrolide-resistant *Staphylococcus* [35] and *Streptococcus* [36] and alters the fecal microbiome [37, 38], with reports of increased macrolide-resistant *Escherichia coli* [39]. This study, in agreement with previous work [16–18], found no evidence of macrolide resistance in this *Ct* population. While encouraging, it does not rule out macrolide resistance as a potential problem in these communities. Carriage of macrolide-resistant pathogens in the gut and nasopharynx may be impacted by antibiotic treatment. Additionally, presence of additional species of *Chlamydia* [40, 41] and nonchlamydial bacteria [42–45] in the ocular niche

We identified several polymorphic regions associated with village-level TF prevalence. The polymorphisms were .9 (p)X

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References

1. Mariotti SP, Pararajasegaram R, Resnikoff S. Trachoma: looking forward to Global Elimination of Trachoma by 2020 (GET 2020). *Am J Trop Med Hyg* 2003; 69(5 Suppl):33–5.
2. Emerson PM, Burton M, Solomon AW, Bailey R, Mabey D. The SAFE strategy for trachoma control: using operational research for policy, planning and implementation. *Bull World Health Organ* 2006; 84:613–9.
3. Stewart AEP, Zerihun M, Gessese D, et al. Progress to eliminate trachoma as a public health problem in Amhara National Regional State, Ethiopia: results of 152 population-based surveys. *Am J Trop Med Hyg* 2019; 101:1286–95.
4. Astale T, Sata E, Zerihun M, et al. Population-based coverage survey results following the mass drug administration of azithromycin for the treatment of trachoma in Amhara, Ethiopia. *PLoS Negl Trop Dis* 2018; 12:e0006270.
5. Ebert CD, Astale T, Sata E, et al. Population coverage and factors associated with participation following a mass drug administration of azithromycin for trachoma elimination in Amhara, Ethiopia. *Trop Med Int Health* 2019; 24:493–501.
6. Nash SD, Stewart AEP, Zerihun M, et al. Ocular *Chlamydia trachomatis* infection under the SAFE strategy in Amhara, Ethiopia, 2011–2015. *Clin Infect Dis* 2018; 67:1840–6.
7. Geisler WM, Black CM, Bandea CI, Morrison SG. *Chlamydia trachomatis* OmpA genotyping as a tool for studying the natural history of genital chlamydial infection. *Sex Transm Infect* 2008; 84:541–4; discussion 544–5.
- 8.

22. Nash SD, Chernet A, Moncada J, et al. Ocular *Chlamydia trachomatis* infection and infectious load among pre-school aged children within trachoma hyperendemic districts receiving the SAFE strategy, Amhara region, Ethiopia. *PLoS Negl Trop Dis* **2020**; *14*:e0008226.
23. Butcher RM, Sokana O, Jack K, et al. Correction: low prevalence of conjunctival infection with *Chlamydia trachomatis* in a treatment-naïve trachoma-endemic region of the Solomon Islands. *PLoS Negl Trop Dis* **2016**; *10*:e0005051.
24. Butcher R, Houghton J, Derrick T, et al. Reduced-cost *Chlamydia trachomatis*-specific multiplex real-time PCR diagnostic assay evaluated for ocular swabs and use by trachoma research programmes. *J Microbiol Methods* **2017**; *139*:95–102.
25. Lutter EI, Bonner C, Holland MJ, et al. Phylogenetic analysis of *Chlamydia trachomatis* Tarp and correlation with clinical phenotype. *Infect Immun* **2010**; *78*:3678–88.
26. Caldwell HD, Wood H, Crane D, et al. Polymorphisms in *Chlamydia trachomatis* tryptophan synthase genes differentiate between genital and ocular isolates. *J Clin Invest* **2003**; *111*:1757–69.
27. Gomes JP, Nunes A, Bruno WJ, Borrego MJ, Florindo C, Dean D. Polymorphisms in the nine polymorphic membrane proteins of *Chlamydia trachomatis* across all serovars: evidence for serovar Da recombination and correlation with tissue tropism. *J Bacteriol* **2006**; *188*:275–86.
28. Nash SD, Stewart AEP, Astale T, et al. Trachoma prevalence remains below threshold in five districts after stopping mass drug administration: results of five surveillance surveys within a hyperendemic setting in Amhara, Ethiopia. *Trans R Soc Trop Med Hyg* **2018**; *112*:538–45.
29. Harrison MA, Harding-Esch EM, Marks M, et al. Impact of mass drug administration of azithromycin for trachoma elimination on prevalence and azithromycin resistance of genital *Mycoplasma genitalium* infection. *Sex Transm Infect* **2019**; *95*:522–8.
30. Coles CL, Seidman JC, Levens J, Mkocho H, Munoz B, West S. Association of mass treatment with azithromycin in trachoma-endemic communities with short-term reduced risk of diarrhea in young children. *Am J Trop Med Hyg* **2011**; *85*:691–6.
31. Whitty CA, Glasgow KW, Sadler M, et al. *PLoS Negl Trop Dis* **2019**; *13*:e0007111.

46. Su H, Watkins NG, Zhang YX, Caldwell HD. *Chlamydia trachomatis*–host cell interactions: role of the chlamydial major outer membrane protein as an adhesin. *Infect Immun* **1990**; *58*:1017–25.
47. Zhang J, Lietman T, Olinger L, Miao Y, Stephens RS. Genetic diversity of *Chlamydia trachomatis* and the prevalence of trachoma. *Pediatr Infect Dis J* **2004**; *23*:217–20.
48. Chin SA, Morberg DP, Alemayehu W, et al. Diversity of *Chlamydia trachomatis* in trachoma-hyperendemic communities treated with azithromycin. *Am J Epidemiol* **2018**; *187*:1840–5.
49. Dawson C, Wood TR, Rose L, Hanna L. Experimental inclusion conjunctivitis in man. 3. Keratitis and other complications. *Arch Ophthalmol* **1967**; *78*:341–9.
50. Tarizzo ML, Nataf R, Nabli B. Experimental inoculation of thirteen volunteers with agent isolated from inclusion conjunctivitis. *Am J Ophthalmol* **1967**; *63*(Suppl 1):1120–8.