Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review

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Mass azithromycin distribution is a core component of trachoma control programmes and could reduce mortality in children younger than 5 years in some settings. In this systematic review we synthesise evidence on the emergence of antimicrobial resistance after mass azithromycin distribution. We searched electronic databases for publications up to June 14, 2018. We included studies of any type (excluding modelling studies, surveillance reports, and review articles) on community-wide distribution of oral azithromycin for the prevention and treatment of trachoma that assessed macrolide resistance, without restrictions to the type of organism. We extracted prevalence of resistance from published reports and requested unpublished data from authors of included studies. Of 213 identified studies, 19 met inclusion criteria (12 assessed *Streptococcus pneumoniae*) and were used for qualitative synthesis. Macrolide resistance after azithromycin distribution was reported in three of the five organisms studied. The lack of resistance in *Chlamydia trachomatis* suggests that azithromycin might remain effective for trachoma programmes, but evidence is scarce. As mass azithromycin distribution for trachoma continues and is considered for other indications, ongoing monitoring of antimicrobial resistance will be required.

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Introduction

WHO recommends 3–5 years of annual mass azithromycin distribution (single directly observed oral dose at 20 mg/kg) to control trachoma in communities with more than 10% prevalence of active trachoma among children aged 1–9 years.[1] Mass azithromycin distribution reduces the prevalence of trachoma and can eliminate infection with ocular strains of *Chlamydia trachomatis*, trachoma's causative agent.[2–8] Mass azithromycin distribution for trachoma may also reduce the burden of other childhood infectious diseases, including respiratory tract infections, diarrhoea, and malaria.[9–15] Studies have even found reductions in all-cause mortality after mass azithromycin distribution.[16–18] A large cluster-randomised trial showed that biannual azithromycin distribution reduced under-5 mortality by 14%, compared with placebo.[18] The results of this study have initiated a global conversation on the role of mass azithromycin distribution in areas with high child mortality, and may lead to the inclusion of mass azithromycin distribution in child survival programmes.

Although mass azithromycin distribution reduces trachoma prevalence and improves child survival, it might select for macrolide resistance in the target (*C trachomatis*) and non-target organisms. Resistance selection could decrease the effectiveness of azithromycin for trachoma treatment over time. Moreover, bystander selection could affect other potentially pathogenic organisms and treatment for various conditions.[19,20] For example, in some settings, macrolides are recommended as first-line drugs for community-acquired pneumonia.[21] Selection of resistance in *Streptococcus pneumoniae* from azithromycin distribution for trachoma could therefore detrimentally affect management of this condition. A systematic review published in 2015 assessed resistance in *S pneumoniae* after mass distribution of azithromycin in articles published up to 2013.[22] The authors found a correlation between baseline and after-treatment prevalence of macrolide resistance. In communities in which baseline *S pneumoniae* resistance to azithromycin was low, mass distribution of azithromycin for trachoma increased resistance temporarily, with the prevalence of resistance subsequently declining after the cessation of treatment. In communities in which baseline resistance or frequency of azithromycin administration was high, resistance remained high after treatment.

Here, we update the previous systematic review[22] with articles published since 2013 on *S pneumoniae* resistance to azithromycin, and expand the scope to include reports of azithromycin resistance in other organisms. Our goal was to summarise the existing evidence on the prevalence of antimicrobial resistance following mass azithromycin distribution for trachoma and define future research priorities.

Methods

Search strategy and selection criteria

We systematically reviewed the literature without language restrictions for all years possible. We searched Cochrane Library, Embase, MEDLINE, and Web of Science for studies published from database inception until July 10, 2017. A second search was done on June 14, 2018, to capture recently published literature. We also searched all conference abstracts available online from the Association for Research in Vision and Ophthalmology and the American Society for Tropical Medicine and Hygiene, paper and electronic versions of the conference proceedings for all meetings of the International Symposium on Human Chlamydial Infections since 1998, and electronic versions of abstracts for all meetings of the WHO's Trachoma Scientific Informal Workshop (obtained from the conference organisers). We searched two online databases, the Grey Literature Report and Open Grey, for grey literature. We manually reviewed the reference lists from all included articles. In addition, all first and corresponding authors of included articles were contacted by email to collect additional unpublished data from studies that met our inclusion criteria.

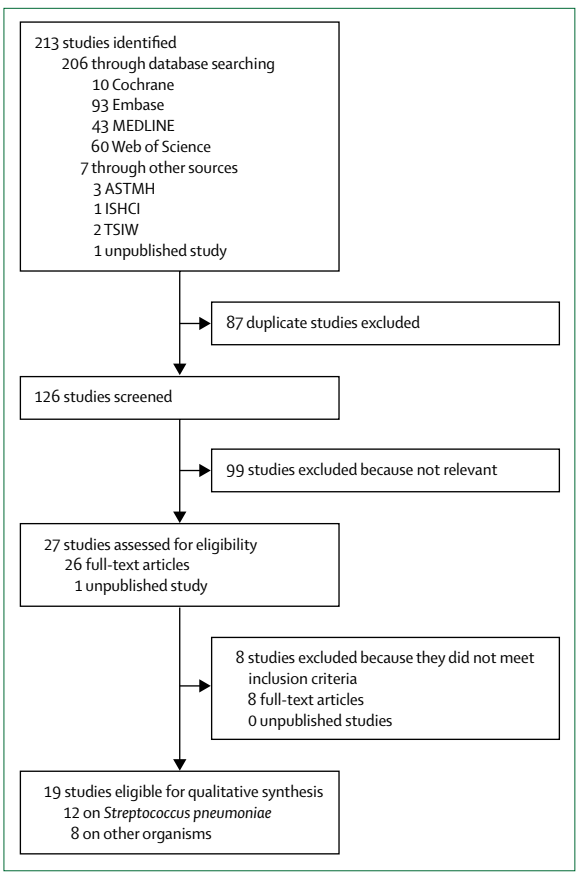

Figure 1: **Flow diagram of the study selection process**
ASTMH=American Society for Tropical Medicine and Hygiene. ISHCI=International Symposium on Human Chlamydial Infections. TSIW=Trachoma Scientific Informal Workshop.

We included the terms azithromycin, resistance, and trachoma in all electronic searches. We used variations of the following search string, when appropriate: (azithromycin OR Zithromax) AND resistan* AND trachoma. Full search strategy details are provided in the appendix.

See Online for appendix

We screened titles and abstracts of all retrieved citations for relevance, removed duplicates, and reviewed full articles that passed the initial screening for eligibility. Two independent researchers (KSO and EGD) screened titles and abstracts and reviewed full texts, and a third (CEO) adjudicated discrepancies.

For more on the **PRISMA guidelines** see http://www.prisma-statement.org/

We included studies on the community-wide distribution of oral azithromycin for trachoma that measured the prevalence of carriage of any organism and their sensitivity to macrolides. We excluded studies on the use of azithromycin for purposes other than the prevention and treatment of trachoma, mathematical modelling studies, surveillance reports, and review articles.

Outcomes, data extraction, and quality

KSO and EGD independently extracted data from all full articles included. KSO adjudicated discrepancies. KSO and EGD used REDCap electronic data capture tools hosted at the University of California, San Francisco (USA), for data extraction and adjudication.[23] The main outcomes assessed were the prevalence of carriage and of macrolide resistance in all organisms before and after mass distribution of oral azithromycin for trachoma. Other variables extracted from each study included geographical location, study design, sample size, treatment coverage, frequency and duration of mass administration of azithromycin, subject sampling method, specimen sampling method, and resistance assessment method. We used the Cochrane Collaboration's risk of bias tool for randomised studies and the ROBINS-I tool for non-randomised studies to assess the risk of bias.[24,25] We slightly modified these tools' rating scales to make them more similar to each other, and rated all included studies as having high, moderate, low, or unclear risk of bias in each category (confounding, selection, misclassification, performance, detection, attrition, and reporting). We only assessed the risk of bias for those elements of the studies regarding prevalence of carriage and of resistance estimation. For papers describing secondary analyses from studies whose full study details are reported in separate publications, we attempted to obtain the original publication and incorporated information from the original publication into the qualitative synthesis.

Data synthesis and statistical analysis

We completed the qualitative synthesis of included studies by organism. The pre-specified analysis plan indicated meta-analysis of prevalence of resistance to azithromycin and carriage by organism, across included studies and in pre-specified subgroups. However, because of the wide variation in study design, frequency of azithromycin distribution, and follow-up timing across studies, we did not do the intended meta-analysis. We instead identified studies on *S pneumoniae* that included a 6-month follow-up timepoint and graphed the prevalence of macrolide resistance at 6 months after the final treatment against treatment frequency. We calculated the average resistance for each treatment frequency, weighted by number of included communities. We did a qualitative synthesis of the design and results of studies on organisms other than *S pneumoniae,* by organism. We completed and reported this systematic review according to the PRISMA 2009 guidelines (checklist in the appendix). The study protocol was registered in PROSPERO (CRD42017071592). The full protocol is included in the appendix.

Results

We retrieved 213 records through our database search and from other sources (figure 1). After duplicate removal, we screened the title and abstract of 126 records. 27 of the screened records met the criteria for full review, including 26 full-text articles and one study unpublished

	Country	Design	Communities treated	Treatment population	Treatment coverage, % (individuals treated/total treatment population)*	Untreated control	Treatment frequency	Baseline	Follow-up†	Sample population (size at first collection in treated group)	Resistance testing
Leach et al, 1997[33]	Australia	Longitudinal (single group)	1	Children aged <15 years with trachoma and household contacts aged <15 years	59% (130/221)	No	Single	Yes	2 weeks, 2 months, 6 months	Children aged <15 years with trachoma (79)	E-test
Fry et al, 2002[11]	Nepal	Repeated cross-sectional	3	Children aged 1–10 years	NR	No	Single	Yes	10 days, 6 months	Randomly selected children aged 1–10 years (167)	Broth dilution MIC
Gaynor et al, 2003[26]	Nepal	Cross-sectional	1	Children aged 1–10 years with trachoma and household contacts	NR	No	Single	No	12 months	Randomly selected children aged 1–10 years (57)	E-test
Batt et al, 2003[30]	Tanzania	Repeated cross-sectional	1	Non-pregnant residents aged >1 year	85% (4782/5619)	No	Single	Yes	2 months, 6 months	All children aged ≤7 years (1315)	E-test
Gaynor et al, 2005[27]	Nepal	Cross-sectional	1	Children aged 1–10 years	80% (NR)	Yes	Annual ×3	No	6 months	All children aged 1–7 years (194)	Sensititre
Haug et al, 2010[28]	Ethiopia	Repeated cross-sectional‡	8	Non-pregnant residents aged >1 year	90% (2488/2765)	Yes	Biannual ×6	No	6 months, 12 months, 24 months	Randomly selected children aged 1–5 years (120)	Sensititre
Skalet et al, 2010[29]	Ethiopia	Randomised controlled trial	12	Children aged 1–10 years	74% (3547/4764)	Yes	Quarterly ×4	Yes	3 months	Randomly selected children aged <10 years (110)	E-test
Coles et al, 2013[32]	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents aged ≥6 months	90% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	Randomly selected children aged <5 years (486)	Kirby-Bauer disk diffusion and E-test
Burr et al, 2014[34]	The Gambia	Repeated cross-sectional‡	8	Non-pregnant residents aged ≥6 months	89% (715/799)	No	Annual ×3	No	0 months, 1 month, 6 months	All children aged <15 years and randomly selected individuals aged ≥15 years (415)	Disk diffusion and E-test
Burr et al, 2014[34]	The Gambia	Repeated cross-sectional‡	8	Non-pregnant residents aged ≥6 months	91% (1019/1124)	No	Single	No	30 months	All children aged <15 years and randomly selected individuals aged ≥15 years (400)	Disk diffusion and E-test
Bloch et al, 2017[31]	Tanzania	Cross-sectional	32	Non-pregnant residents aged ≥6 months	NR	No	Annual ×4	No	48 months	Randomly selected children aged 1–59 months (1047)	Kirby-Bauer disk diffusion and E-test
Keenan et al, 2018[35]	Niger	Randomised controlled trial	24	Non-pregnant residents aged >6 months	86% (2508/2916)	No	Annual ×3	Yes	12 months	Randomly selected children aged 0–5 years (180)	Targeted PCR (ermB and mefA/E)
Keenan et al, 2018[35]	Niger	Randomised controlled trial	24	Biannual: children aged 6 months to 12 years	82% (2556/3132)	No	Biannual ×6	Yes	6 months	Randomly selected children aged 0–5 years (168)	Targeted PCR (ermB and mefA/E)
TEF trial§	Ethiopia	Cross-sectional‡	8	Non-pregnant residents aged >1 year	87% (2302/2645)	Yes	Annual ×3	No	6 months	Randomly selected children aged 1–5 years (120)	Sensititre

NR=not reported. MIC=minimum inhibitory concentration. TEF=Trachoma Elimination Follow-up.*Treatment at baseline; where possible, numbers exclude tetracycline-treated subjects. †Follow-up timepoints presented as time after final treatment. ‡Subset of communities included in a cluster-randomised trial. §NCT00221364.

Table 1: **Characteristics of studies on macrolide resistance in *Streptococcus pneumoniae***

at the time of the search. The full review resulted in the exclusion of eight records for the following reasons: six presented data already included in other full-text articles, one did not assess community-wide distribution of azithromycin for trachoma, and one assessed serotype distribution in *S pneumoniae* isolates. Our qualitative synthesis covered data from a total of 19 studies, including 18 published articles[11,15,26–41] and unpublished data from one trial (the Trachoma Elimination Follow-up trial [TEF; NCT00221364]). Study design details for the unpublished data were obtained through personal communication with the investigators and from other publications.[5,28] 12 studies (including the TEF trial) were on *S pneumoniae*[11,26–35] and eight on other organisms[15,31,36–41] (tables 1, 2). Of the eight studies that report macrolide resistance, and in some cases other outcomes, in organisms other than *S pneumoniae*, two assessed resistance in *Staphylococcus aureus*,[31,41] three in *C trachomatis*,[36–38] three in *Escherichia coli*,[31,39,40] and one in *Plasmodium falciparum* (tables 2, 3).[15] Several included

	Country	Design	Communities treated[1]	Treatment population	Treatment coverage, % (individuals treated/ total treatment population)*	Untreated control	Treatment frequency	Baseline	Follow-up†	Sample population (sample size at first collection in treated group)	Resistance testing
Staphylococcus aureus											
Bojang et al, 2017[41]	The Gambia	Repeated cross-sectional‡	8	Non-pregnant residents aged ≥6 months	89% (715/799)	No	Annual ×3	No	1 month, 6 months	All children aged <15 years and randomly selected individuals aged ≥15 years (415)	Culture and disk diffusion
Bojang et al, 2017[41]	The Gambia	Repeated cross-sectional‡	8	Non-pregnant residents aged ≥6 months	90% (1019/1129)	No	Single	No	30 months	All children aged <15 years and randomly selected individuals aged ≥15 years (400)	Culture and disk diffusion
Bloch et al, 2017[31]	Tanzania	Cross-sectional	32	Non-pregnant residents aged ≥6 months	NR	No	Annual ×4	No	48 months	Randomly selected children aged 1 month to 5 years (1047)	Kirby-Bauer disk diffusion and E-test
Chlamydia trachomatis											
Solomon et al, 2005[36]	Tanzania	Longitudinal (single group)	1	Non-pregnant residents aged ≥1 year	94% (916/978)	No	Single	Yes	2 months	All individuals with active trachoma (174)	Culture
Hong et al, 2009[37]	Ethiopia	Cross-sectional	24	Non-pregnant residents aged ≥1 year	NR	Yes	Biannual ×4	No	18 months	All children aged 1–5 years (552)	Culture
West et al, 2014[38]	Tanzania	Longitudinal (single group)	32	Non-pregnant residents aged ≥6 months	NR	No	Annual ×4	Yes	2 months	All children aged <10 years with active trachoma (359)	Culture
Escherichia coli											
Seidman et al, 2014[39]§	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents aged ≥6 months	91% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	40 children aged <3 years per community (160)	E-test
Seidman et al, 2016[40]§	Tanzania	Longitudinal (cohort)	4	Non-pregnant residents aged ≥6 months	91% (NR)	Yes	Single	Yes	1 month, 3 months, 6 months	40 children aged <3 years per community (160) plus all children reporting diarrheal symptoms (NR)	Kirby-Bauer disk diffusion
Bloch et al, 2017[31]	Tanzania	Cross-sectional	32	Non-pregnant residents aged ≥6 months	NR	No	Annual ×4	No	48 months	Randomly selected children aged 1 month to 5 years (1048)	E-test
Plasmodium falciparum											
Schachterle et al, 2014[15]	Tanzania	Longitudinal (cohort)	4	All residents	91% (6252/6894)	Yes	Single	Yes	1 month, 3 months, 4 months, 6 months	Randomly selected parent–child pairs, children aged <5 years (1045)	Targeted PCR

NR=not reported. *Treatment at baseline; where possible, numbers exclude tetracycline-treated participants. †Follow-up timepoints presented as time after final treatment. ‡Subset of communities included in a cluster-randomised trial. §Similar study population.

Table 2: **Characteristics of studies on macrolide resistance in organisms other than *Streptococcus pneumoniae***

studies[15,39,40] were part of the PRET Plus study in Tanzania, and additional publications[9,12] describing methods for that study were consulted in the synthesis.

Characteristics of studies on macrolide resistance in *S pneumoniae*

Of the 12 included studies on macrolide resistance in *S pneumoniae* (table 1), three focused on communities in Nepal,[11,26,27] three (including the TEF trial) in Ethiopia,[28,29] three in Tanzania,[30–32] and one each in Australia,[33] The Gambia,[34] and Niger.[35] Most studies included fewer than ten communities (median six, range 1–32) and none of them reported mass distribution of azithromycin preceding the distribution whose effect on *S pneumoniae* resistance profiles was being assessed. Five studies targeted treatment to children,[11,26,27,29,33] six (including the TEF trial) to all non-pregnant individuals older than 6 or 12 months,[28,30–32,34] and one study assessed both mass treatment and treatment targeted to children.[35] Seven studies (including the TEF trial) reported the number of individuals in the study population.[28–30,33–35] The number of individuals who received treatment ranged from 221 to 5619 (median 2765).

The frequency of azithromycin distribution varied across studies: a single treatment (six studies),[11,26,30,32–34] annual treatment for 3–4 years (five studies, including the TEF trial),[27,31,34,35] biannual treatment for 3 years (two studies),[28,35] and quarterly treatment for 1 year (one study).[29] Two studies[34,35] compared different treatment frequencies. Overall treatment coverage was reported in nine studies

	Outcomes	Results*	Summary	Conclusions
Staphylococcus aureus				
Bojang et al, 2017[41]	Prevalence of carriage and resistance	Prevalence of carriage (annual): 25% (102/414, 1 month), 39% (161/417, 3 months), 9% (30/343, 6 months). Prevalence of resistance (annual): 9% (37/414, 1 month), 34% (142/417, 3 months), 7% (25/343, 6 months). Prevalence of carriage (single): 7% (25/375, 30 months). Prevalence of resistance (single): 2% (6/375, 30 months).	The prevalence of carriage at the final survey was similar between treatment arms (aOR 1·47, 95% CI 0·72–3·00, p=0·286); the prevalence of resistance increased over time in annually treated communities, with a significant difference between treatment groups found at the final survey (aOR 5·22, 1·49–18·34, p=0·010)	..
Bloch et al, 2017[31]	Prevalence of carriage, prevalence of resistance, MIC	Prevalence of carriage, prevalence of resistance, and MIC ranges 48 months after MDA	Prevalence of: carriage (treated) 13% (138/1047); resistance (treated) 29% (40/138); MICs ≥8 μg/mL: 80% (32/40).	Some resistance found 4 years after MDA
Chlamydia trachomatis				
Solomon et al, 2005[36]	MCC	Mean MCC at baseline and 2 months; MCC for individuals with positive culture at both timepoints	Prevalence of culture positive (treated): 82% (46/56, baseline), 89% (8/9, 2 months). Mean MCC (treated): 0·6 μg/mL (baseline), 1·0 μg/mL (2 months).	No evidence of increased azithromycin resistance after single MDA
Hong et al, 2009[37]	MIC, MCC	MIC and MCC by serotype and treatment group 18 months after MDA	Prevalence of culture positive: 70% (7/10, treated) and 60% (6/10, untreated). Mean MIC: 0·5 μg/mL (treated) and 0·4 μg/mL (untreated). Mean MCC: 0·5 μg/mL (treated) and 0·6 μg/mL (untreated).	MICs and MCCs similar between biannually treated and untreated communities (MIC p=0·76, MCC p=1·00); no evidence of increased azithromycin resistance between biannually treated and untreated communities
West et al, 2014[38]	MIC, MBC	MIC and MBC by individual for baseline and 2 months	Mean MIC (treated): 0·26 μg/mL (baseline) and 0·20 μg/mL (2 months). Mean MBC (treated): 0·27 μg/mL (baseline) and 0·26 μg/mL (2 months).	No evidence of resistance to azithromycin in any sample
Escherichia coli				
Seidman et al, 2014[39]†	Prevalence of carriage; prevalence of resistance; aOR for resistance by treatment	Prevalence of carriage and resistance to azithromycin and erythromycin at baseline, 1 month, 3 months, and 6 months by treatment group and compared in logistic regression models	Prevalence of resistance (treated): 10% (NR/300, baseline), 44% (NR/347, 1 month), 30% (NR/347, 3 months), 23% (NR/191, 6 months). Prevalence of resistance (untreated): 19% (NR/205, baseline), 14% (NR/325, 1 month), 10% (NR/324, 3 months), 12% (NR/118, 6 months).	Initial increase in prevalence of carriage of macrolide-resistant *E coli* after MDA, then decrease over time to stable above baseline levels 6 months after treatment; significantly increased odds of carriage of macrolide resistant isolates over time in communities treated with azithromycin than untreated (1 month aOR 11·21, 95% CI 7·13–17·63, p<0·001; 3 month aOR 10·64, 3·79–29·92, p<0·001; 6 month aOR 4·76, 1·52–14·90, p<0·001)
Seidman et al, 2016[40]†	Pathogenic status of isolates; prevalence of resistance; aOR for resistance by treatment	Prevalence of resistance to multiple antibiotics by pathogenic status and compared by treatment group in logistic regression models	Prevalence of resistance: 35% (243/687, pathogenic) and 27% (491/1805, non-pathogenic)	Prevalence of antibiotic resistance significantly higher in pathogenic than in non-pathogenic *E coli*; azithromycin treatment significantly associated with increased odds of carriage of macrolide-resistant isolates (aOR 3·64, 95% CI 2·38–5·78, p<0·001)
Bloch et al, 2017[31]	Prevalence of carriage; prevalence of resistance; MIC	Prevalence of carriage, prevalence of resistance, and MIC ranges 48 months after MDA	Prevalence of: carriage (treated) 62% (646/1047); resistance 17% (107/644); MICs ≥32 μg/mL: 83% (86/103)	Some resistance found 4 years after MDA
Plasmodium falciparum				
Schachterle et al, 2014[15]	Prevalence of infection; sequencing of *P falciparum* ribosomal L4 protein	Prevalence of infection at baseline, 1 month, 3 months, 4 months, and 6 months after MDA by treatment group; sequencing of full-length *P falciparum* ribosomal L4 protein for samples from 12 patients	Prevalence of infection (treated): 6% (53/854, baseline), 2% (14/851, 1 month), 2% (15/715, 3 months), 1% (5/637, 4 months), 1% (4/625, 6 months). Prevalence of infection (untreated): 6% (54/894, baseline), 5% (37/779, 1 month), 3% (17/670, 3 months), 2% (8/531, 4 months), 1% (4/593, 6 months); sequencing, no evidence of resistance.	Short-term reduction in the prevalence of malaria without selecting for azithromycin resistance after single MDA

aOR=adjusted odds ratio. MIC=minimum inhibitory concentration. MDA=mass drug administration. MCC=minimum chlamydicidal concentration. MBC=minimum bactericidal concentration. NR=not reported. *Unless otherwise indicated, reported as % prevalence (number of isolates identified with carriage or resistance/total number of samples). †Same study population.

Table 3: **Reported results from studies on macrolide resistance in organisms other than *Streptococcus pneumoniae***

(including the TEF trial)[27–30,32–35] and ranged from 59% to 91% (median 86%). Five studies (including the TEF trial)[27–29,32] included an untreated control group, and six studies[11,29,30,32,33,35] included a baseline assessment of the prevalence of azithromycin resistance before treatment.

Follow-up assessments ranged from 2 weeks to 4 years after the final treatment. All studies sampled *S pneumoniae* in children, either including all children of a given age range or a random selection of them. One study[34] also assessed randomly selected individuals aged 15 years or older. 11 studies (including the TEF trial) assessed nasopharyngeal samples,[11,26–29,31–35] and one assessed oropharyngeal samples.[30] 11 studies (including the TEF trial)[11,26–34] assessed phenotypic

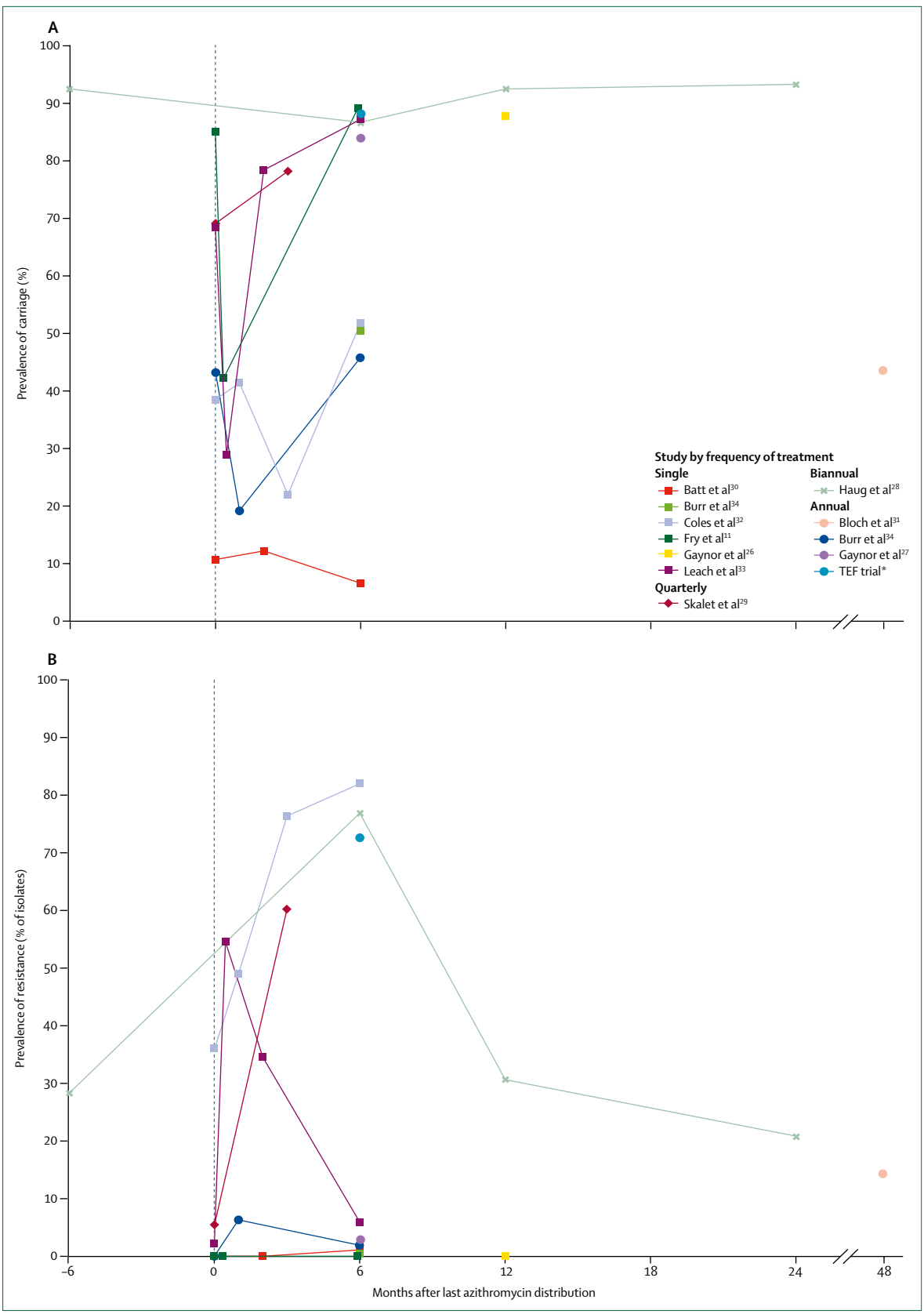

Figure 2: **Prevalence of carriage and of macrolide resistance of *Streptococcus pneumoniae* over time among groups treated with azithromycin**

Prevalence of *S pneumoniae* carriage (A) and of macrolide resistance (B) by the number of months after the last distribution of azithromycin is shown for all time points assessed in the 12 different azithromycin-treated groups studied in the 11 unique studies that used microbiological methods to assess phenotypic resistance. TEF=Trachoma Elimination Follow-up. *NCT00221364.

resistance of *S pneumoniae* to azithromycin, erythromycin, or both, using disk diffusion or minimum inhibitory concentration (MIC). Two studies[29,30] also used targeted PCR to test for common genetic resistance determinants associated with macrolide resistance—*ermB*, *mefA/E*, or both—and one study[35] only assessed genotypic resistance. Among the 11 phenotypic resistance studies, four studies used E-test strips (AB Biodisk, Sweden and USA)[26,29,30,33] and three (including the TEF trial) used broth dilution Sensititre MIC plates (Trek Diagnostics Inc, USA)[27,28] to establish the resistance status and the MIC. One study[11] used broth dilution MIC testing but did not specify a commercial product. Three studies[31,32,34] used Kirby-Bauer disk diffusion to establish the resistance status and E-test (AB Biodisk, Sweden or Biomerieux, Marcy l'Etoile, France) to assess MIC values among isolates classified as resistant. Nine of the 11 studies defined breakpoints explicitly or by referencing the manufacturer's instructions, the National Committee for Clinical Laboratory Standards,[42] or the Clinical and Laboratory Standards Institute.[43] The remaining two studies[26,34] did not include a reference.

Outcomes of studies on azithromycin resistance in *S pneumoniae*

Among the six studies that conducted baseline assessments before distribution of azithromycin, prevalence of carriage of *S pneumoniae* ranged from 10·7% to 85·0% (median 55·9% before the first treatment, figure 2A).[11,29,30,32,33,35] Prevalence of carriage estimated 6 months after the last treatment (nine studies, including the TEF trial) ranged from 6·6% to 89·3% (median 84·0%).[11,27,28,30,32–35] Five of the six studies with more than two follow-up timepoints saw transient decreases in carriage shortly after the final distribution of azithromycin, with returns to initial carriage prevalence over time.[11,28,30,32–34] Prevalence of carriage remained below 52% in four studies[30–32,34] at all timepoints, including in two different treatment frequency groups in one study (figure 2A).

Baseline values of prevalence of resistance, assessed before azithromycin distribution (six studies), ranged from 0·0% to 35·8% (median 1·0%, figure 2B).[11,29,30,32,33,35] 6 months after the final treatment (nine studies, including the TEF trial), prevalence of resistance ranged from 0·0% to 81·9% (median 3·1%).[11,27,28,30,32–35] Of the six studies with more than two follow-up visits, three showed transient increases in prevalence of resistance, followed by decreases to near baseline levels over time;[28,33,34] one showed increases in prevalence at all timepoints;[32] one showed no resistance at the first two timepoints and a small increase at the final timepoint;[30] and one showed no resistance at any timepoint.[11] In the study of genotypic resistance, prevalence of genetic determinants of resistance was identical in annual and biannual treatment groups at baseline (median 20%, IQR 10–40).[35] After 2 years of mass distributions, resistance increased to a median of 40% (20–40) in the annual group and 60% (50–80) in the biannually treated group (p<0·001).

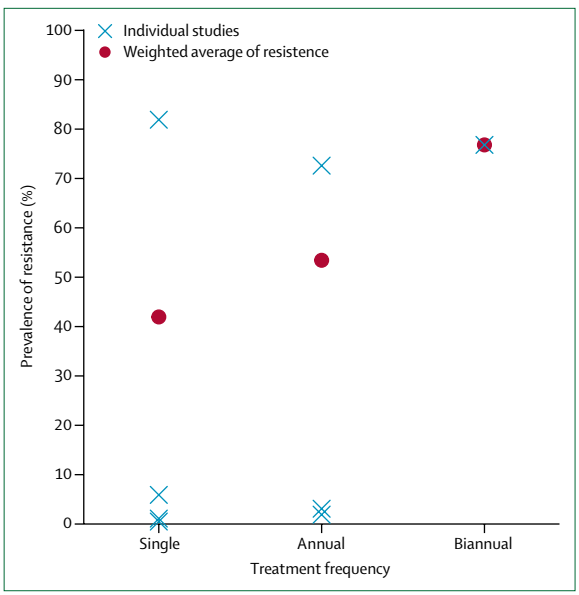

Figure 3: **Macrolide resistance in *Streptococcus pneumoniae* 6 months after treatment and average of resistance weighed by number of treated communities**

Prevalence of resistance reported in individual studies 6 months after the final mass azithromycin distribution by treatment frequency. Additionally, a weighted average of resistance by treatment frequency is shown. Each study was weighted by the number of communities included in the study and the average reported resistance was calculated for each treatment frequency.

Of the eight studies that measured prevalence of phenotypic resistance 6 months after treatment, four[11,30,32,33] gave a single treatment, three (including the TEF trial)[27,34] gave three annual treatments, and one[28] gave six biannual treatments. The weighted average of resistance 6 months after the final treatment was 42·0% for single treatment, 53·4% for annual treatments, and 76·8% for biannual treatments (figure 3).

Characteristics and outcomes of studies on macrolide resistance in *S aureus*

We included two studies on resistance in *S aureus* in this review.[31,41] A repeated cross-sectional study[41] in The Gambia compared three annual distributions to a single distribution in eight communities, with follow-ups at 1 and 6 months after the annual distributions and 30 months after the single distribution. This study included neither a baseline assessment nor an untreated control group. The second study[31] used a cross-sectional design in Tanzania to assess resistance 48 months after four annual distributions in 32 communities and included neither a baseline assessment nor an untreated control group.

The Gambia study[41] sampled 415 individuals from the annually treated communities and 400 individuals from the communities receiving a single distribution, including all children younger than 15 years and a random sample

of adults aged 15 years or older. The Tanzania study[31] included 1047 randomly selected children aged 1–5 years. Resistance was assessed with culture and disk diffusion in The Gambia study, and with Kirby-Bauer disk diffusion and E-test in the Tanzania study. Both studies found macrolide resistance after treatment with azithromycin. The longitudinal study in The Gambia reported a transient increase in resistance after three annual distributions of azithromycin, from 8·9% at 1 month after treatment to 34·1% 3 months after treatment, followed by a decrease tox·3% 6 months after treatment.[41] The final prevalence of resistance 6 months after annual distributions was significantly higher than 30 months after a single distribution (7·3% *vs* 1·6%; adjusted odds ratio [aOR] 5·2, 95% CI 1·5–18·3, p=0·010).[41]

Characteristics and outcomes of studies on macrolide resistance in *C trachomatis*

Of the three included studies on *C trachomatis,* two were cohort studies in Tanzanian communities,[36,38] and one was a cross-sectional study on Ethiopian communities.[37] One study distributed a single treatment in one community,[36] one distributed four annual treatments to 32 communities,[38] and another distributed four biannual treatments to 24 communities.[37] One study included an untreated control group,[37] and two studies included a baseline assessment,[36,38] but none of the studies included both. Follow-up occurred 2 months[36,38] or 18 months[37] after the final treatment.

To assess resistance, the studies sampled 174 individuals with trachoma,[36] 552 children aged 1–5 years,[37] and 354 children younger than 10 years.[38] Non-standard microbiological protocols were used to assess resistance in *C trachomatis* in all studies. None of the studies found evidence of clinically significant azithromycin resistance in *C trachomatis* when comparing groups before and after treatment or treated and untreated groups.

Characteristics and outcomes of studies on macrolide resistance in *E coli*

We included three studies on *E coli*, based in Tanzania: two examined the same population of four treated communities included in a cohort study,[39,40] and the third used a cross-sectional design in 32 communities.[31] The two studies[39,40] from the same population assessed resistance at baseline and at 1, 3, and 6 months after a single distribution of azithromycin and included an untreated control group. The other study[31] assessed resistance 48 months after four annual distributions of azithromycin and included neither a baseline assessment nor an untreated control group.

The two studies[39,40] from the same population each included 160 children younger than 3 years, with one of the studies also including an additional sample of children reporting diarrhoea. The other study included 1048 randomly selected children aged 1–5 years.[31] Resistance was assessed with E-test in two studies,[31,39] and Kirby-Bauer disk diffusion in the third.[40] All studies on *E coli* found macrolide resistance after treatment. One of the cohort studies reported a short-term increase in macrolide resistant *E coli* isolates immediately after the single treatment, followed by decreasing resistance over 6 months, with the final assessment at 6 months showing macrolide resistance greater than baseline levels.[39] At all post-treatment timepoints, treated communities in this study had an increased odds of carriage of macrolide-resistant isolates compared with untreated communities (1 month aOR 11·2, 95% CI 7·1–17·6, p<0·001; 3 month aOR 10·6, 3·8–29·9, p<0·001; 6 month aOR 4·8, 1·5–14·9, p<0·001).[39]

Characteristics and outcomes of studies on macrolide resistance in *P falciparum*

The one included study on *P falciparum* was done on a sample from the cohort study in Tanzania that included measurements of resistance in *S pneumoniae* and *E coli*.[15] This study treated four communities with a single distribution of azithromycin and assessed resistance in the treated communities and in an untreated control group at baseline and at 1, 3, 4, and 6 months after treatment. The investigators randomly selected children younger than 5 years and their parents, including 1045 samples in the resistance assessment. Resistance was assessed with targeted PCR. The study found a 73% reduction in malaria infection 1 month after treatment, when comparing treated with untreated groups (95% CI 43–89). The difference between groups waned over time, and no evidence of azithromycin resistance was identified.

Risk of bias

We assessed the risk of bias due to confounding, selection, misclassification, performance, detection, attrition, and reporting for all studies included in the review, when possible (appendix). Of the 19 studies included in this review, two were randomised controlled trials.[29,35] One trial compared resistance in communities treated with mass azithromycin with resistance in untreated communities;[29] the other compared groups treated with different frequencies of mass azithromycin distribution.[35] Of the remaining 17 studies, seven were not designed to attribute causal effects to the intervention: five used uncontrolled before-and-after designs,[26,30,33,36,38] and two (including the TEF trial) did not include any comparison group.[31] Five studies used a cohort design and attempted to include controls for confounding,[15,32,34,39,40] whereas five did not include enough information to assess risk of bias from confounding.[11,27,28,37,41] No study reported masking of participants and personnel, but five studies (including the TEF trial) reported masking outcome assessors.[28,29,35,37] Risk of attrition bias was low for nine studies (including the TEF trial);[11,28–30,34–36,41] the other ten studies did not provide enough information on follow-up to assess risk.[15,26,27,31–33,37–40] The two trials had a

low risk of bias from selective reporting since outcomes were prespecified.[29,35] The other studies did not provide enough information to fully assess risk of bias in this category, although these papers generally reported the same types of outcomes for all available timepoints, suggesting that selective reporting is unlikely.

Discussion

This systematic review documents selection for macrolide resistance in three of the five studied organisms after mass azithromycin distribution for the elimination of trachoma. A previous review summarised the evidence on macrolide resistance in *S pneumoniae* after mass azithromycin distribution from eight articles published before 2013.[22] Here, we updated this earlier review to include literature available up to June 2018 and sought unpublished data, enabling us to include 50% more data on *S pneumoniae* from 12 studies. We further expanded on the previous review by widening our inclusion criteria to studies on macrolide resistance in any organism after mass azithromycin distribution. Finally, as the results of the MORDOR trial[18] spur discussion on the role of the mass distribution of azithromycin in child survival, our review not only synthesises the available evidence on emergent antimicrobial resistance, but also outlines research priorities for future work on the effects of mass azithromycin distributions.

Three studies evaluating the effect of mass azithromycin distribution found no evidence of selection for macrolide resistance in the target organism *C trachomatis*.[36–38] Mass azithromycin distribution is very effective in reducing the prevalence of the ocular strains of *C trachomatis* that cause trachoma.[45] Given concerns about the potential for azithromycin's reduced effectiveness for trachoma elimination in the face of increasing resistance, the lack of macrolide resistance in *C trachomatis* found in these studies is encouraging. However, as the number of doses of distributed azithromycin increases each year, continued vigilance will be required to monitor for emergence of macrolide resistance in *C trachomatis*.

The most commonly studied organism was *S pneumoniae*, with 12 studies (including the TEF trial) reporting resistance in isolates of this bacterium after mass azithromycin distribution for trachoma treatment.[11,26–35] *S pneumoniae* is an important commensal organism that colonises the nasopharynx and can cause pneumonia. Overall, these studies showed an increase in macrolide resistance in *S pneumoniae* immediately after treatment, which appears to dissipate with time. Five studies (including the TEF trial)[27–29,32] included an untreated control group and showed a substantially increased resistance in *S pneumoniae* in communities that received azithromycin compared with those that did not. This finding indicates that the increase is probably due to the azithromycin intervention rather than secular trends. Although heterogeneity in study design, setting, population, treatment frequency, and follow-up time precluded formal meta-analysis, trends in the included studies suggested that increasing treatment frequency (eg, single, annual, and biannual) increased selection for macrolide resistance in *S pneumoniae*. Studies and programmes that are planning greater frequencies of azithromycin distribution should consider the potential for increased selection for macrolide resistance.

Some evidence of selection for macrolide resistance following mass azithromycin distribution was noted in other organisms, including *E coli* and *S aureus*. Enterotoxigenic *E coli* strains are a major cause of childhood diarrhoea, although macrolides are not typically used against them. No studies assessed resistance in *Campylobacter* spp, a common cause of childhood diarrhoea for which azithromycin is first-line therapy.[46] In mass azithromycin programmes for yaws eradication, emergence of azithromycin-resistant *Treponema pallidum* has been reported.[47] Assessment of additional potentially pathogenic organisms in areas with mass azithromycin distribution will be important to fully understand the impact of mass azithromycin distributions on the emergence of macrolide resistance.

We found that most studies evaluating resistance selection following mass azithromycin distribution for trachoma focused on Gram-positive organisms, including *S pneumoniae* and *S aureus*. Azithromycin remains an important line of treatment for several pathogenic Gram-negative bacteria, including *Neisseria gonorrhoeae*, *Salmonella* spp, and *Shigella* spp. *N gonorrhoeae* has developed resistance to many available antimicrobials. The US Centers for Disease Control and Prevention currently recommends dual therapy for gonorrhoea treatment with ceftriaxone and azithromycin.[48] However, transmission of azithromycin-resistant strains of *N gonorrhoeae* has been reported.[49] Previous work has shown a non-significant decrease in the prevalence of *N gonorrhoeae* in women after mass azithromycin distribution for trachoma control,[50] but the effect of the mass distribution of azithromycin on resistance selection in *N gonorrhoeae* has not been studied in depth. Typhoid and non-typhoidal invasive salmonella infections are major causes of morbidity. Increased resistance to fluoroquinolones and third-generation cephalosporins has been reported, and azithromycin is commonly used for enteric fever.[51–54] Exclusion of azithromycin from the options for enteric fever treatment would represent a substantial global health challenge. Furthermore, azithromycin is first-line treatment for shigellosis, another major cause of childhood diarrhoea.[55] Hence, understanding the effect of mass azithromycin distribution in these and other Gram-negative organisms is a research priority.

Some evidence suggests that resistance prevalence may decrease after cessation of antibiotic pressure.[28,33,34,39,41] The development of macrolide resistance may result in a fitness cost, which could explain this observation.[56–59] Minimum concentrations of azithromycin for inhibiting growth of *Shigella* spp and *Salmonella* spp are relatively

high, and the mechanism of acquisition and transfer of resistance in Gram-negative organisms differs from that of Gram-positive organisms.[60,61] Differences in the mechanism of resistance acquisition could affect the extent of resistance reversibility after selection pressure removal.[56] For example, the use of azithromycin for the treatment of sexually transmitted infections in men who have sex with men in high-income settings has selected for azithromycin resistant *Shigella* spp, which are transmitted even among individuals who have not previously been given azithromycin.[62] Future research in Gram-negative organisms should also evaluate trends in resistance after the removal of azithromycin selection pressure.

Given continued mass azithromycin treatments for trachoma and the potential role of azithromycin in child survival programmes,[18] these results underscore several important research priorities. First, we recommend continued longitudinal surveillance of multiple organisms, including short-term and long-term assessments. Short-term assessments will yield information about the immediate impact of azithromycin distributions, whereas long-term assessments will provide evidence of long-term effects after the cessation of azithromycin selection pressure. As trachoma is eliminated in some geographical regions and mass azithromycin distributions are stopped, monitoring for several years after cessation of treatment will provide important data on the long-term effects of mass azithromycin distribution. Second, the assessment of selection for resistance after multiple mass azithromycin distributions of increased duration, particularly against *C trachomatis*, could offer important insights on the impact of many years of annual mass treatment. In some districts of Ethiopia, a decade of annual mass azithromycin distribution has not consistently led to elimination of infection.[63] Whether resistance contributes to persistent infection in some geographical regions or clusters is unknown. Third, we recommend research continue to include susceptibility testing on antimicrobials to which increased resistance might have particularly deleterious population-level effects. Macrolide use has been associated with increased *S pneumoniae* resistance to other antimicrobial drugs in some studies.[64–66] Here, we focused solely on macrolide resistance, though many studies included in this review also examined resistance to other key antibiotics such as penicillin. Fourth, we recommend adequately powered randomised study designs with baseline assessments and masked laboratory personnel to better understand whether changes in selection for resistant organisms are due to secular trends (eg, increased community antibiotic consumption) or periodic azithromycin treatments.

Although there has been enormous success in controlling ocular *C trachomatis* infection with mass azithromycin distribution, available evidence suggests that these distributions select for macrolide resistance in some potentially pathogenic organisms. There might also be a dose response causing increased resistance selection as the number of distributions increases. The scarce available evidence suggests that when antibiotic selection pressure is removed, the prevalence of resistance may return to baseline levels over time, though most studies followed populations for 6 months or less and results were mixed in studies with shorter follow-up periods. As azithromycin distribution programmes are continued for trachoma and potentially for child survival, monitoring of resistance in multiple organisms will be required to ensure that any unintended consequences of mass azithromycin distribution can be identified and mitigated.

Contributors

All authors contributed to the study design. KSO, EGD, and CEO conducted the literature search and data extraction. KSO and CEO conducted the data synthesis, created the tables and figures, and wrote the manuscript. All authors contributed to the interpretation of the data and revision of the manuscript.

Declaration of interests

We declare no competing interests.

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