Mass administration of azithromycin and *Streptococcus pneumoniae* carriage: cross-sectional surveys in the Gambia

Sarah E Burr,[a] Sally Milne,[b] James Jafali,[c] Ebrima Bojang,[c] Megha Rajasekhar,[a] John Hart,[a] Emma M Harding-Esch,[d] Martin J Holland,[a] David CW Mabey,[a] Ansumana Sillah,[e] Robin L Bailey[a] & Anna Roca[c]

Objective To evaluate the effect of repeated mass drug administration (MDA) of azithromycin in the Gambia on the nasopharyngeal carriage of *Streptococcus pneumoniae* and on the emergence of antibiotic-resistant strains.

Methods This study involved villages that participated in a cluster randomized trial comparing the effect of one versus three azithromycin MDA rounds on the prevalence of trachoma. Only villages in which most children received 7-valent pneumococcal conjugate vaccine were included. Three cross-sectional surveys were performed in two villages that received three annual MDA rounds: the first immediately before the third MDA round and the second and third, 1 and 6 months, respectively, after the third MDA round. The third survey also covered six villages that had received one MDA round 30 months previously. Pneumococcal carriage was assessed using nasopharyngeal swabs and azithromycin resistance was detected using the Etest.

Findings The prevalence of pneumococcal carriage decreased from 43.4% to 19.2% between the first and second surveys ($P < 0.001$) but rebounded by the third survey (45.8%; $P = 0.591$). Being a carrier at the first survey was a risk factor for being a carrier at the second (odds ratio: 3.71; $P < 0.001$). At the third survey, the prevalence of carriage was similar after one and three MDA rounds (50.3% versus 45.8%, respectively; $P = 0.170$), as was the prevalence of azithromycin resistance (0.3% versus 0.9%, respectively; $P = 0.340$).

Conclusion Three azithromycin MDA rounds did not increase the prevalence of nasopharyngeal carriage of azithromycin-resistant *S. pneumoniae* strains compared with one round.

Abstracts in عربي, 中文, Français, Русский and Español at the end of each article.

Introduction

Trachoma, which is due to ocular infection with the intracellular bacterium *Chlamydia trachomatis*, is the leading infectious cause of blindness worldwide. The discovery that ocular *C. trachomatis* infection can be successfully treated with a single, oral dose of azithromycin marked a significant advance in trachoma control.[1] Today, mass drug administration (MDA) with azithromycin is a key component of a multifaceted strategy designed to control all phases of trachoma and recommended by the World Health Organization (WHO): the SAFE strategy, where S stands for surgery for trichiasis; A, for antibiotics to reduce the reservoir of *C. trachomatis* infection; F, for facial cleanliness to reduce transmission from ocular and nasal secretions; and E, for environmental improvements to interrupt transmission of the bacterium and prevent re-emergence of the infection.[2,3]

Current WHO guidelines suggest that three annual rounds of azithromycin MDA should be completed before the prevalence of trachoma is reassessed.[4] However, evidence from the Gambia and the United Republic of Tanzania indicates that three rounds are unnecessary in low prevalence settings.[5–8] In such situations, repeated azithromycin MDA may be detrimental if it results in the selection of macrolide-resistant pathogens. Although currently there is no evidence that repeated MDA increases the prevalence of azithromycin-resistant *C. trachomatis*,[9] there is epidemiological evidence suggesting that pharyngeal carriage of macrolide-resistant *Streptococcus pneumoniae* increases following repeated MDA for trachoma control.[10,11] Because *S. pneumoniae* is a leading cause of childhood mortality, especially in Africa, and because asymptomatic nasopharyngeal carriage is the initial step in the pathogenesis of pneumococcal disease, increased carriage of macrolide-resistant strains is a public health concern.

The Gambia, which is situated in western Africa and is in the Sahel belt, has seen a decline in the prevalence of follicular trachoma in children in recent decades: the prevalence (adjusted for the 1- to 9-year-old age group) derived from national surveys fell from 20% in 1986 to 7% in 1996.[12,13] The country's National Eye Health Programme, which was established in 1986, has implemented all aspects of the SAFE strategy.[14] An evaluation of the implementation of SAFE interventions indicated that Gambian households have good access to water and latrines but a low awareness of community health education programmes that promote face washing.[15] Recent survey work carried out in four health districts following the national azithromycin MDA campaign that ran from 2007 to 2010 suggests that the prevalence of follicular trachoma in the country is now nearing 5%, which is the threshold for elimination.[8]

The Partnership for the Rapid Elimination of Trachoma (PRET) study was a cluster randomized trial embedded within the Gambia's MDA campaign that compared the prevalence of active trachoma in Gambian communities which had received azithromycin annually for three years with that in communities which received a single treatment round.[8] The aim of the present study was to determine whether the nasopharyngeal

[a] Department of Clinical Research, London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT, England.
[b] Faculty of Life Sciences, University of Manchester, Manchester, England.
[c] Medical Research Council Unit, Fajara, Banjul, Gambia.
[d] Public Health England, Colindale, London, England.
[e] National Eye Health Programme, Ministry of Health and Social Welfare, Kanifing, Gambia.
Correspondence to Sarah E Burr (email: Sarah.Burr@lshtm.ac.uk).
(*Submitted: 20 November 2013 – Revised version received: 25 February 2014 – Accepted: 2 March 2014 – Published online: 29 April 2014*)

carriage of azithromycin-resistant *S. pneumoniae* was more prevalent in communities that received three MDA rounds than in those that received one. We considered only data from villages in which children had been vaccinated with a 7-valent pneumococcal conjugate vaccine (PCV-7) because we wanted our results to be relevant to the increasing number of African countries that include these vaccines in their vaccination schedules.

Methods

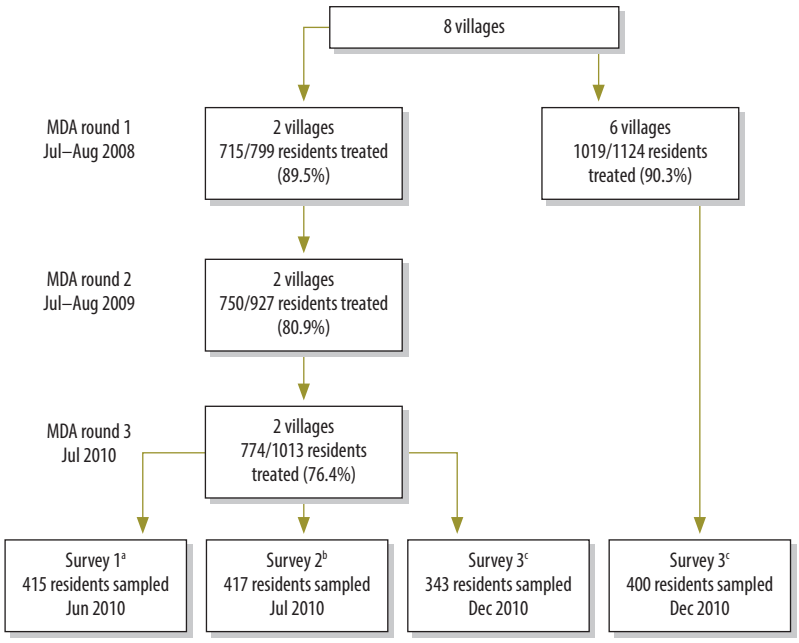
The study included the residents of eight villages in a rural region of western Gambia where the population predominantly comprises subsistence farmers who grow millet and maize for home consumption and groundnuts as a cash crop. The people belong mainly to the Jola and Mandinka ethnic groups. The climate of the Gambia is tropical and there is one rainy season, lasting from June to October.

All study villages had participated in a cluster randomized trial of the impact of PCV-7 on pneumococcal nasopharyngeal carriage that ran from July 2006 to July 2008.[16] All children who were under the age of 30 months when the vaccine trial started, who were born during the trial period or who moved into a study village during the trial period received PCV-7.[16] In August 2009, PCV-7 was introduced into the Gambian Expanded Programme on Immunization.

The PRET study was a cluster randomized, controlled trial whose design has been described elsewhere.[8,17] Briefly, enumeration areas with a population of 600 to 800 individuals were randomized either to receive MDA with azithromycin annually for three years or to have treatment discontinued if the prevalence of either active trachoma or ocular *C. trachomatis* infection in children aged 5 years or younger fell below 5% (i.e. the stopping rule). Six months after the first MDA round, the prevalence of active trachoma and of *C. trachomatis* infection in communities randomized to the stopping rule were 2.4% and 0%, respectively, and MDA therefore ceased in all those communities.[8]

Our study comprised two treatment arms and involved only villages in which children had received PCV-7 (Fig. 1). The first arm included two villages that had been randomized to three annual MDA rounds. Three cross-sectional surveys were conducted in these villages: the first took place 11 months after the second MDA round, the second took place 1 month after the third MDA round and the third took place 6 months after the third MDA round. The second arm included six villages that had been randomized to the stopping rule and where MDA had been carried out only once. In these villages, one cross-sectional survey was conducted 30 months after the single MDA round – it took place at the same time as the third cross-sectional survey in villages that received three MDA rounds (Fig. 1).

Fig. 1. **Flowchart for the study of the effect of mass azithromycin administration on nasopharyngeal carriage of *Streptococcus pneumoniae*, Gambia, 2008–2010**



MDA: mass drug administration.

[a] The first cross-sectional survey was carried out 11 months after the second azithromycin MDA round in villages that took part in three rounds.

[b] The second survey was carried out 1 month after the third MDA round.

[c] The third survey was carried out 6 months after the third MDA round in villages that took part in three rounds and, at the same time, 30 months after the single MDA round in villages that received only one round.

Census data were gathered in the week before the first cross-sectional survey. All children under the age of 15 years who were included in the census and who were present at the time of the survey were invited to participate. In addition, 150 individuals aged 15 years or more were randomly selected to participate in each cross-sectional survey, including the single survey conducted in villages randomized to the stopping rule. Although random selection was carried out independently for each survey, the second and third surveys in villages that received three MDA rounds included only individuals who had received azithromycin during the third MDA round in July 2010.

During each survey, a questionnaire was administered and a nasopharyngeal specimen was taken from each participant by means of a calcium alginate swab, which was then inoculated in a transport medium of skimmed milk, tryptone, glucose and glycerol in a sterile vial. In the field, the samples were kept on wet ice. They were transferred to a refrigerator set to 4 °C within 8 hours of collection and moved to long-term storage at −70 °C within 24 hours of collection.

For analysis, the nasopharyngeal swab samples were thawed at room temperature and 10 µL of the transport medium was inoculated onto Columbia agar supplemented with 5% sheep's blood and 5 µg/mL of gentamicin. The agar plates were then incubated for 18 to 24 hours at 35 °C in an atmosphere containing 5% carbon dioxide. Presumptive *S. pneumoniae* colonies were identified on the basis of their morphology and optochin sensitivity and the presence of *S. pneumoniae* was confirmed using a

Table 1. **Participants in study of the effect of mass azithromycin administration on nasopharyngeal carriage of *Streptococcus pneumoniae*, Gambia, 2010**

Characteristic	No. (%) of participants in villages exposed to three MDA rounds			No. (%) of participants in villages exposed to one MDA round[d]
	First survey[a]	Second survey[b]	Third survey[c]	
All participants	415 (100)	417 (100)	343 (100)	400 (100)
Age, years				
< 10	205 (49.4)	173 (41.5)	182 (53.1)	182 (45.5)
≥ 10	210 (50.6)	244 (58.6)	161 (46.9)	218 (54.5)
Sex				
Male	219 (52.8)	209 (50.1)	158 (46.1)	211 (52.8)
Jola ethnicity	409 (98.6)	407 (97.6)	333 (97.1)	369 (92.3)
Occupation				
None	185 (45.1)	162 (39.0)	169 (50.0)	155 (40.9)
Student	149 (36.3)	149 (35.9)	90 (26.6)	133 (35.1)
Agricultural worker	76 (18.5)	104 (25.1)	79 (23.4)	91 (24.0)
Other	5 (1.2)	2 (0.5)	5 (1.5)	21 (5.3)
Schooling, years				
0	260 (62.7)	258 (61.9)	242 (71.8)	260 (65.0)
1–3	73 (17.6)	67 (16.1)	36 (10.7)	63 (15.8)
4–6	51 (12.3)	48 (11.5)	25 (7.4)	11 (2.8)
> 6	31 (7.5)	44 (10.6)	34 (10.1)	66 (16.5)
Able to read	156 (37.6)	158 (37.9)	56 (16.3)	76 (19.0)
Able to write	155 (37.4)	158 (37.9)	63 (18.4)	94 (23.5)
Recent[e] health visit	14 (3.4)	12 (2.9)	30 (8.8)	19 (4.8)
Recent[e] antibiotic use	2 (0.5)	2 (0.5)	4 (1.2)	6 (1.5)
Smoker	20 (4.8)	12 (2.9)	13 (3.8)	13 (3.3)
Smoker in household	265 (63.9)	273 (65.4)	166 (48.7)	121 (30.3)

MDA: mass drug administration.
[a] The first survey was carried out 11 months after the second MDA round.
[b] The second survey was carried out 1 month after the third MDA round.
[c] The third survey was carried out 6 months after the third MDA round.
[d] Only the third survey was carried out in villages that received a single MDA round – 30 months after MDA, at the same time as the third survey in villages that took part in three MDA rounds.
[e] Within the last 30 days.

polymerase chain reaction (PCR) technique that targeted the *cpsA* gene.[18] The density of nasopharyngeal carriage was scored semiquantitatively as previously described.[19] For each nasopharyngeal swab sample, up to four *S. pneumoniae* colonies with different morphologies were screened by disc diffusion for their sensitivity to azithromycin. The minimal inhibitory concentration of azithromycin was determined using the Etest (Biomerieux, Marcy l'Etoile, France) in isolates with an intermediate or resistant phenotype. Nasopharyngeal swab samples that tested positive for *S. pneumoniae* were serotyped using a multiplex PCR assay optimized for African clinical samples.[20] Colonies were scraped from the first quadrant of the agar plate and total, genomic DNA was extracted using the QIAamp DNA Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The primer concentrations and cycling conditions were as recommended by the United States Centers for Disease Control and Prevention protocol.[20]

Statistical analysis

Data were double-entered into an OpenClinica database (OpenClinica, Waltham, United States of America). Differences between survey samples and between treatment groups in the proportion of patients who carried any pneumococci or azithromycin-resistant pneumococci were evaluated using Fisher's exact test. In addition, logistic regression analysis was used to identify risk factors for pneumococcal carriage, to control for confounders and to test for interactions. The results were reported in terms of odds ratios (ORs) and 95% confidence intervals (CIs). Both CIs and *P*-values were estimated using clustered (by village) robust standard errors.

The primary analysis considered the overall prevalence of pneumococcal nasopharyngeal carriage and the secondary analysis considered the prevalence of the carriage of azithromycin-resistant strains (i.e. those with a minimal inhibitory concentration of azithromycin of 16 μg/mL or more) and of individual serotypes and serotype groups. Five serotype groups were investigated: (i) the most frequent serotypes, which were defined as those detected in at least 10 individuals; (ii) PCV-7 serotypes, which included pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, and serotypes 6A and 6C; (iii) serotypes not in PCV-7, which included any other pneumococcal serotypes detectable by the multiplex PCR method; (iv) paediatric serotypes, which were defined as serotypes 4, 6, 7, 9, 14, 18, 19 and 23;[21] and (v) non-serotypeable pneumococci. All *P*-values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate procedure. Fisher's exact test was used to evaluate heterogeneity in the serotype distribution between villages.

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Gambian Government/Medical Research Council Unit and the Gambia Joint Ethics Committee. Written, informed consent was freely obtained from all participants of the PRET trail and additional written consent was obtained from participants in our ancillary study. For minors, informed consent was obtained from a parent or guardian.

Results

The first cross-sectional survey collected data on 415 individuals from villages that took part in three azithromycin MDA rounds and the second survey collected data on 417 similar individuals. The third survey included a total of 743 participants: 343 from villages that took part in three MDA rounds and 400 from villages randomized to the stopping rule

Table 2. **Prevalence of nasopharyngeal *Streptococcus pneumoniae* carriage in individuals from villages exposed to three mass azithromycin administration rounds, by survey, Gambia, 2010**

Age of survey participants	Cross-sectional survey	No. of survey participants	No. (%) of *S. pneumoniae* carriers	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
< 10 years	First[b]	205	124 (60.5)	1.0	1.0
	Second[c]	173	61 (35.3)	0.35 (0.23–0.53)	0.35 (0.23–0.53)
	Third[d]	182	116 (63.7)	1.14 (0.75–1.73)	1.14 (0.75–1.73)
≥ 10 years	First[b]	210	56 (26.7)	1.0	1.0
	Second[c]	244	19 (7.8)	0.22 (0.13–0.39)	0.23 (0.13–0.40)
	Third[d]	161	41 (25.5)	0.91 (0.57–1.47)	0.96 (0.59–1.55)
All	First[b]	415	180 (43.4)	1.0	1.0
	Second[c]	417	80 (19.2)	0.30 (0.22–0.41)	0.30 (0.22–0.42)
	Third[d]	343	157 (45.8)	1.09 (0.82–1.46)	1.09 (0.80–1.48)

CI: confidence interval; MDA: mass drug administration; OR: odds ratios.
[a] The OR was adjusted for age and sex and the within-village correlation of participants was taken into account using clustered robust standard errors.
[b] The first survey was carried out 11 months after the second MDA round.
[c] The second survey was carried out 1 month after the third MDA round.
[d] The third survey was carried out 6 months after the third MDA round.

Table 3. **Prevalence of nasopharyngeal *Streptococcus pneumoniae* carriage at the third survey,[a] by azithromycin treatment, Gambia, 2010**

Age of survey participants	No. of MDA rounds	No. of survey participants	No. (%) of *S. pneumoniae* carriers	Crude OR (95% CI)	Adjusted OR[b] (95% CI)
< 10 years	1	182	136 (74.7)	1.0	1.0
	3	182	116 (63.7)	0.59 (0.29–1.17)	0.59 (0.30–1.17)
≥ 10 years	1	218	65 (29.8)	1.0	1.0
	3	161	41 (25.5)	0.72 (0.36–1.5)	0.69 (0.34–1.39)
All	1	400	201 (50.3)	1.0	1.0
	3	343	157 (45.8)	0.77 (0.41–1.44)	0.65 (0.35–1.20)

CI: confidence interval; OR: odds ratios; MDA: mass drug administration.
[a] The third survey was carried out 6 months after the third mass drug administration round.
[b] The OR was adjusted for age and sex and the within-village correlation of risk factors was taken into account using clustered robust standard errors.

and that took part in one round (Fig. 1). The demographic characteristics of the participants sampled in each survey are listed in Table 1.

Table 2 reports the overall prevalence of nasopharyngeal *S. pneumoniae* carriage in individuals from villages that took part in three MDA rounds. In the first survey, 11 months after the second MDA round, the prevalence was 43.4%. By the second survey, 1 month after the third MDA round, the prevalence had decreased significantly, to 19.2%. However, by the third survey, 6 months after the third MDA round, it was 45.8%, similar to the initial level. In each survey, the prevalence of nasopharyngeal carriage was lower among individuals aged 10 years or older than among younger individuals. Table 3 shows that, in the third survey, there was no significant difference in prevalence between individuals from villages that took part in three MDA rounds and those from villages that took part in one: the prevalence was 45.8% and 50.3% in the two treatment arms, respectively.

No evidence of pneumococcal azithromycin resistance was found in the first cross-sectional survey among individuals from villages that took part in three MDA rounds. The prevalence of the pneumococcal nasopharyngeal carriage of azithromycin-resistant isolates among sampled individuals rose to 1.2% in the second survey but decreased to 0.9% in the third (Table 4). In the third survey, the prevalence of the carriage of azithromycin-resistant strains was similar in individuals from villages that took part in three MDA rounds and in those from villages that took part in one: 0.9% versus 0.3%, respectively (Table 4).

Serotyping of pneumococci isolated in the third survey showed no significant difference in the prevalence of the carriage of PCV-7 serotypes between the treatment arms (Table 5). Nor was there a significant difference in the prevalence of serotypes not in PCV-7, of paediatric serotypes or of non-serotypeable pneumococci. However, the prevalence of serotypes 15B and/or 15C was significantly higher in individuals from villages that took part in three MDA rounds than in those from villages that took part in one (9.0% versus 2.3%, respectively; $P = 0.001$); the prevalence of serotype 10A was also higher (6.1% versus 0.6%, respectively; $P < 0.001$), whereas that of serotype 35B was lower (1.2% versus 4.5%, respectively; $P = 0.031$; Table 5). The distribution of serotype 10A in villages that took part in three MDA rounds was significantly heterogeneous ($P = 0.012$), as was the distribution of serotype 35B in villages that took part in one round ($P = 0.006$). By contrast, there was no significant difference in the distribution of serotypes 15B and/or 15C between the two villages that took part in three MDA rounds ($P = 0.348$), which provides further evidence for the existence of a difference between the two treatment arms.

Further analysis indicated that individuals who were *S. pneumoniae* carriers in the first cross-sectional survey were significantly more likely than those who were not to be carriers in the second survey (adjusted OR, aOR: 3.71; Table 6). However, there was no significant association between the density of carriage at the first survey and carriage at the second survey: the aOR for carriage at the second survey for high-density versus low-density carriage at the first survey was 1.09 (95% CI: 0.66–1.80). In the second survey, pneumococcal carriage was less likely in participants aged 10 years or older than in younger children (aOR: 0.33) and less likely in students (aOR: 0.24) and agricultural workers (aOR: 0.32) than in individuals with no occupation (Table 6).

Table 4. **Prevalence of nasopharyngeal azithromycin-resistant *Streptococcus pneumoniae* carriage, by azithromycin treatment and survey, Gambia, 2010**

No. of MDA rounds	Cross-sectional survey	Survey participants								
		Aged < 10 years			Aged ≥ 10 years			All		
		No. of survey participants	No. (%) of carriers[a]	*P*[b]	No. of survey participants	No. (%) of carriers[a]	*P*[b]	No. of survey participants	No. (%) of carriers[a]	*P*[b]
Between-survey comparison										
3	First[c]	205	0 (0)	0.200	210	0 (0)	0.119	415	0 (0)	0.066
3	Second[d]	173	2 (1.2)	–	244	3 (1.2)	–	417	5 (1.2)	
3	Third[e]	182	3 (1.7)	–	161	0 (0)	–	343	3 (0.9)	
Between-treatment comparison										
1	Third[e]	182	1 (0.6)	0.311	218	0 (0)	NA	400	1 (0.3)	0.340
3	Third[e]	182	3 (1.7)	–	161	0 (0)	–	343	3 (0.9)	

MDA: mass drug administration; NA: not applicable.
[a] Carriers of azithromycin-resistant *Streptococcus pneumonia.*
[b] Differences between survey samples and between treatment groups were evaluated using Fisher's exact test.
[c] The first survey was carried out 11 months after the second MDA round in villages that took part in three rounds.
[d] The second survey was carried out 1 month after the third MDA round.
[e] The third survey was carried out 6 months after the third MDA round in villages that took part in three rounds and, at the same time, 30 months after the single MDA round in villages that had received one round.

Discussion

In communities where the initial prevalence of pneumococcal nasopharyngeal carriage was high but the prevalence of the carriage of azithromycin-resistant strains was low, we observed that receiving three annual azithromycin MDA rounds was associated with a short-term decrease in pneumococcal carriage that was not accompanied by a significant increase in azithromycin resistance. There was no evidence of azithromycin resistance at the first cross-sectional survey in communities that received two MDA rounds. Moreover, since a previous study carried out in the same geographical area showed no evidence of erythromycin resistance in individuals carrying pneumococcus,[22] we did not expect macrolide-resistant strains to be circulating before treatment. The absence of azithromycin resistance at the first cross-sectional survey is consistent with previous studies carried out in Australia, Nepal and the United Republic of Tanzania, all of which showed that substantial azithromycin resistance did not develop following a single treatment dose in areas where the baseline prevalence of the carriage of resistant strains was low.[23–25] The prevalence of the carriage of azithromycin-resistant pneumococci rose to just over 1% at the second cross-sectional survey. However, at the third survey, there was no significant difference in prevalence between villages that received three annual MDA rounds and those that received one.

Table 5. **Prevalence of carriage of *Streptococcus pneumoniae* serotypes, by azithromycin treatment, Gambia, 2010**

S. pneumoniae serotype	No. (%) of serotype carriers from villages		*P*[a]
	exposed to 1 MDA round (*n* = 400)	exposed to 3 MDA rounds (*n* = 343)	
16F	35 (8.8)	22 (6.4)	0.426
15B and/or 15C[b]	9 (2.3)	31 (9.0)	0.001
6A, 6B and/or 6C[c]	21 (5.3)	14 (4.1)	0.667
3	19 (4.8)	8 (2.3)	0.241
34	12 (3.0)	12 (3.5)	0.962
19A	12 (3.0)	10 (2.9)	1.000
35B	18 (4.5)	4 (1.2)	0.031
10A	1 (0.6)	21 (6.1)	< 0.001
19F	8 (2.0)	11 (3.2)	0.539
23B	8 (2.0)	5 (1.5)	0.927
14	8 (2.0)	4 (1.2)	0.586
PCV-7 serotypes[d]	48 (12.0)	38 (11.1)	0.896
Non-vaccine serotypes[e]	206 (51.5)	161 (46.9)	0.395
Paediatric serotypes[f]	76 (19.0)	53 (15.5)	0.360
Non-serotypeable pneumococci	33 (8.3)	17 (5.0)	0.177

MDA: mass drug administration; PCV-7: 7-valent pneumococcal conjugate vaccine.
[a] *P*-values were adjusted for multiple testing using the Benjamini and Hochberg false discovery rate procedure.
[b] The polymerase chain reaction method used could not distinguish between serotypes 15B and 15C.
[c] The polymerase chain reaction method used could not distinguish between serotypes 6A, 6B and 6C.
[d] PCV-7 serotypes included serotypes 4, 6B, 9V, 14, 18C, 19F and 23F plus serotypes 6A and 6C.
[e] Detectable serotypes not in PCV-7.
[f] Paediatric serotypes included serotypes 4, 6, 7, 9, 14, 18, 19 and 23.

In contrast to our findings, a study carried out in central areas of the United Republic of Tanzania reported that a single MDA round significantly increased the prevalence of azithromycin resistance, which was detectable 6 months later.[26] In that study, azithromycin-resistant pneumococcal strains

Table 6. **Risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* at the second survey[a] among individuals from villages exposed to three rounds of mass azithromycin administration, Gambia, 2010**

Characteristic	No. of participants[a]	No. (%) of carriers of *S. pneumoniae*	Unadjusted OR (95% CI)	Adjusted OR[b] (95% CI)
Carrier at first survey[c]				
No	154	15 (9.7)	1.0	1.0
Yes	124	45 (36.3)	5.28 (2.77–10.07)	3.71 (2.44–5.64)
Age				
< 10 years	173	61 (35.3)	1.0	
≥ 10 years	244	19 (7.8)	0.16 (0.12–0.20)	0.33 (0.28–0.38)
Occupation				
None	162	63 (38.9)	1.0	1.0
Student	149	13 (8.7)	0.15 (0.06–0.37)	0.24 (0.07–0.86)
Agricultural worker	104	4 (3.9)	0.06 (0.04–0.10)	0.32 (0.30–0.34)
Sex				
Male	209	39 (18.7)	1.0	NA
Female	208	41 (19.7)	1.07 (0.81–1.41)	NA
Village[d]				
1	217	32 (14.8)	1.0	NA
2	200	48 (24.0)	1.83 (1.83–1.83)	NA
Schooling, years				
0	258	67 (26.0)	1.0	NA
1	67	8 (11.9)	0.39 (0.18–0.84)	NA
2	48	2 (4.2)	0.12 (0.04–0.40)	NA
≥ 3	44	3 (6.8)	0.21 (0.18–0.24)	NA
Able to read				
No	259	67 (25.9)	1.0	NA
Yes	158	13 (8.2)	0.26 (0.14–0.48)	NA
Able to write				
No	259	67 (25.9)	1.0	NA
Yes	158	13 (8.2)	0.26 (0.14–0.48)	NA
Recent[e] health visit				
No	405	75 (18.5)	1.0	NA
Yes	12	5 (41.7)	3.14 (2.87–3.44)	NA
Recent[e] antibiotic use				
No	415	79 (19.0)	1.0	NA
Yes	2	1 (50.0)	4.25 (0.15–122.58)	NA
Smoker in household				
No	144	28 (19.4)	1.0	NA
Yes	273	52 (19.1)	0.97 (0.57–1.66)	NA

CI: confidence interval; MDA: mass drug administration; NA: not applicable; OR: odds ratio.
[a] The second cross-sectional survey was carried out 1 month after the third MDA round.
[b] The OR was adjusted for carriage of *S. pneumoniae* at the first cross-sectional survey, age and occupation.
[c] The first survey was carried out 11 months after the second MDA round.
[d] Individuals from two villages participated in the survey.
[e] Within the last 30 days.

were present in the community before treatment: 2.1% of pneumococcal strains isolated at baseline were resistant. Six months after the single MDA round, the proportion of resistant isolates was 35%. Since, in our study, we did not collect samples 6 months after MDA in areas that received a single MDA round, we cannot directly compare our results with those of the Tanzanian study and we cannot say what the immediate effect of a single MDA round was in the Gambian population. However, as we did not observe azithromycin resistance 6 months after the third MDA round in communities that received three rounds, it is unlikely that we would have observed resistance 6 months after the first MDA round. In addition, the Tanzanian study also reported a large variation in the proportion of individuals with azithromycin-resistant pneumococci in the untreated study arm: it ranged from 4.4% to 13.1%. Another difference between the two studies was that 65% of the participants in the Tanzanian study reported taking unspecified drugs to treat suspected infections in the 30 days before baseline azithromycin treatment, whereas only 2% of the participants in our study reported recent antibiotic use at the first survey. Moreover, a significant proportion of children in the Tanzanian study received amoxicillin for acute respiratory infections during the study period.

In the villages in our study that received three MDA rounds and where the prevalence of pneumococcal carriage and rates of transmission were high,[16,22] the third MDA round decreased the prevalence of pneumococcal carriage from 43.4% at the first survey 11 months after the second MDA round to 19.2% at the second survey 1 month after the third round. However, this effect had already waned 6 months after treatment, by the time of the third survey. This short-lived reduction in pneumococcal nasopharyngeal carriage may, at least in part, explain the decrease in child mortality that has been reported following MDA in communities in Ethiopia[27,28] since bacterial carriage is a proxy measure of invasive disease. Despite the significant reduction in the prevalence of pneumococcal carriage associated with the third MDA round, one month later, at the second survey, we were able to identify individuals who were pneumococcal carriers and who could still, therefore, transmit pneumococcus to the community. Being a carrier in the first cross-sectional survey was a risk factor for being a carrier one month after the third MDA round. The other risk factors for carriage after treatment were similar to known risk factors for pneumococcal carriage and included young age – which is consistent with previous findings indicating that children drive pneumococcal transmission.[16,22]

Serotype replacement is a concern in communities in which vaccines

with limited valency have been used to protect against pneumococcal disease. However, our data suggest that azithromycin MDA did not modify the carriage of the most prevalent *S. pneumoniae* serotypes, except for serotypes 15B and/or 15C, which had a higher prevalence at the third survey in the two villages that received three MDA rounds than in villages that received one round. Moreover, the prevalence of the carriage of PCV-7 serotypes was not affected by MDA.

One limitation of this study is the small number of villages included. This was a consequence of our decision to include only villages in which children had received PCV-7, as recommended by WHO,[29] because we wanted our findings to be relevant to countries that include PCV-7 in their national immunization programmes. As a result, we selected villages that had participated in the PRET trial and in a trial of the impact of PCV-7 on pneumococcal nasopharyngeal carriage.[16] Whether azithromycin would have had the same effect in the absence of PCV-7 vaccination cannot, therefore, be addressed directly by our study. However, if azithromycin has the same effect on all serotypes, as our data suggest, the initial serotype distribution in the population should not affect the outcome of treatment.

In summary, the high prevalence of pneumococcal carriage and the high pneumococcal transmission rate in our study population coupled with the administration of PCV-7 to young children provided a unique opportunity to evaluate the effect of azithromycin MDA on pneumococcal nasopharyngeal carriage. We found that three MDA rounds, administered according to WHO guidelines, did not increase the prevalence of the carriage of azithromycin-resistant pneumococcal strains over the long term and that children played an important role in pneumococcal transmission in the community following azithromycin MDA. ■

Acknowledgements

We thank Lamin Camara, Omar Camara, Robyn Homan Damry, Sarjo Dibba, Christopher Ford, Musukoi Jammeh, Omar Jarra, Hassan Joof, Tumani Kuyateh, Lamin Leigh, Pateh Makalo, Omar Manneh, Zenobe Reade, Sidu Sibi and Jane Whitton at the Medical Research Council Unit, the Gambia and community leaders and villagers who participated in the study. Sarah E Burr and Sally Milne were also affiliated with the Medical Research Council Unit, the Gambia.

Funding: This study was funded by the Bill & Melinda Gates Foundation and the Medical Research Council, United Kingdom of Great Britain and Northern Ireland.

Competing interests: None declared.

ملخص

توزيع دواء الأزيثرومايسين على نطاق جماهيري وانتقال العقدية الرئوية: مسوح متعددة القطاعات في غامبيا

الغرض تقييم تأثير تكرار توزيع دواء الأزيثرومايسين على نطاق جماهيري في غامبيا على انتقال العقدية الرئوية عن طريق البلعوم الأنفي وعلى ظهور سلالات مقاومة للمضادات الحيوية.

الطريقة اشتملت هذه الدراسة على القرى التي شاركت في تجربة عشوائية عنقودية تقارن تأثير جولة واحدة مقابل ثلاث جولات من توزيع دواء الأزيثرومايسين على نطاق جماهيري على معدل انتشار التراخوما. وتم فقط إدراج القرى التي تلقى معظم أطفالها اللقاح المتقارن السباعي المضاد للمكورات الرئوية. وتم إجراء ثلاثة مسوح متعددة القطاعات في قريتين تلقتا ثلاث جولات سنوية من توزيع دواء الأزيثرومايسين على نطاق جماهيري: أجري المسح الأول مباشرة قبل الجولة الثالثة من توزيع دواء الأزيثرومايسين على نطاق جماهيري والثانية والثالثة بعد شهر وستة أشهر من الجولة الثالثة لتوزيع دواء الأزيثرومايسين على نطاق جماهيري، على التوالي. وشمل المسح الثالث كذلك ست قرى تلقت جولة توزيع دواء الأزيثرومايسين على نطاق جماهيري قبل ثلاثين شهراً. وتم تقييم انتقال العقدية الرئوية باستخدام مسحات البلعوم الأنفي وتم اكتشاف مقاومة الأزيثرومايسين باستخدام اختبار مقياس إبسيلون.

النتائج انخفض معدل انتشار انتقال العقدية الرئوية من 43.4 ٪ إلى 19.2 ٪ بين المسحين الأول والثاني (الاحتمال < 0.001) ولكنه ارتد مع المسح الثالث (45.8 ٪؛ الاحتمال = 0.591). واعتبر الناقل في المسح الأول عامل اختطار كناقل في المسح الثاني (نسبة الاحتمال: 3.71؛ الاحتمال < 0.001). وكان معدل انتشار الانتقال في المسح الثالث متشابهاً بعد جولة واحدة وثلاث جولات من توزيع دواء الأزيثرومايسين على نطاق جماهيري (50.3 ٪ مقابل 45.8 ٪، على التوالي؛ الاحتمال = 0.170)، مع معدل انتشار مقاومة دواء الأزيثرومايسين (0.3 ٪ مقابل 0.9 ٪، على التوالي؛ الاحتمال = 0.340).

الاستنتاج لم تؤدِ الجولات الثلاث لتوزيع دواء الأزيثرومايسين على نطاق جماهيري إلى ازدياد معدل انتشار انتقال سلالات العقدية الرئوية المقاومة للأزيثرومايسين عن طريق البلعوم الأنفي مقارنة بجولة واحدة

摘要

阿奇霉素的集体服药和肺炎链球菌带菌：冈比亚横断面调查

目的 评估阿奇霉素在冈比亚的重复大规模药物治疗（MDA）对肺炎链球菌鼻咽带菌和耐药菌株出现的影响。

方法 本研究涉及参加比较沙眼流行率一轮和三轮阿奇霉素 MDA 效果集群随机试验的村庄。仅纳入其大多数儿童接受 7 价肺炎球菌结合疫苗的村庄。对接受三轮年度 MDA 的两个村庄进行三次横断面调查：在第三轮 MDA 前不久完成第一次调查，第二次和第三次调查分别在第三轮 MDA 之后的 1 个月和 6 个月进行。第三次调查还涉及在 30 个月之前接受过一轮 MDA 的六个村庄。使用鼻咽拭子评估肺炎球菌带菌，使用浓度梯度法检测阿奇霉素耐药性。

结果 在第一次和第二次调查之间肺炎球菌带菌流行率从 43.4% 下降至 19.2%（P<0.001），但是到第三次调查则有所反弹（45.8%；P=0.591）。第一次调查的携带者是第二次调查中成为携带者的风险因素（优势比：3.71；P< 0.001）。在第三次调查时，一轮和三轮 MDA 之后

的带菌流行率（分别为 50.3% 和 45.8%；P=0.170）相似，阿齐霉素耐药流行率（分别为 0.3% 和 0.9%；P=0.340）也相似。

结论 较之一轮治疗，三轮阿奇霉素 MDA 没有提高阿奇霉素耐药肺炎球菌菌株鼻咽带菌的流行率。

Résumé

Administration massive d'azithromycine et portage du *Streptococcus pneumoniae*: études transversales en Gambie

Objectif Évaluer l'effet de l'administration de médicament massive (AMM) répétée en Gambie sur le portage du *Streptococcus pneumoniae* dans le nasopharynx et sur l'émergence de souches résistantes aux antibiotiques.

Méthodes Cette étude a impliqué des villages qui ont participé à un essai randomisé par grappes, comparant l'effet d'un cycle d'AMM d'azithromycine par rapport à 3 cycles d'AMM d'azithromycine sur la prévalence du trachome. Seuls les villages dans lesquels la plupart des enfants ont reçu le vaccin antipneumococcique conjugué heptavalent ont été inclus dans l'étude. Trois études transversales ont été menées dans 2 villages ayant reçu 3 cycles d'AMM: la première étude juste avant la troisième AMM, et les deuxième et troisième études 1 et 6 mois, respectivement, après la troisième AMM. La troisième étude a également couvert 6 villages qui avaient reçu une seule AMM 30 mois auparavant. Le portage pneumococcique a été évalué par le biais de prélèvements nasopharyngés, et la résistance à l'azithromycine a été détectée à l'aide de l'Etest.

Résultats La prévalence du portage pneumococcique a diminué de 43,4% à 19,2% entre la première et la deuxième étude ($P < 0,001$), mais elle a rebondi à la troisième étude (45,8%; $P = 0,591$). Le fait d'être porteur lors de la première étude était un facteur de risque pour être porteur lors de la deuxième étude (rapport des cotes: 3,71; $P < 0,001$). Dans la troisième étude, la prévalence du portage était similaire après un et 3 cycles d'AMM (50,3% contre 45,8%, respectivement; $P = 0,170$). Il en est de même pour la prévalence de la résistance à l'azithromycine (0,3% contre 0,9%, respectivement; $P = 0,340$).

Conclusion Trois cycles d'AMM n'augmentent pas la prévalence du portage de souches *Streptococcus pneumoniae* résistantes à l'azithromycine dans le nasopharynx, par rapport à un seul cycle.

Резюме

Массовое назначение азитромицина и носительство пневмококков: перекрестные исследования в Гамбии

Цель Оценить влияние повторного массового применения препарата (МПП) азитромицина в Гамбии на носоглоточное носительство пневмококков и возникновение штаммов, устойчивых к антибиотикам.

Методы Данное исследование охватывало деревни, участвующие в кластерном рандомизированном исследовании, в котором сравнивался эффект одного против трех циклов массового применения препарата азитромицина на распространенность трахомы. В исследование были включены только те деревни, в которых большинство детей получало семивалентную пневмококковую конъюгированную вакцину. Было проведено три перекрестных исследования в двух деревнях, принимавших три ежегодных курса МПП: первое исследование было проведено сразу перед третьим курсом МПП, а второе и третье – через 1 и 6 месяцев соответственно, после завершения третьего курса МПП. В третье исследование также было включено шесть деревень, в которых прием одного курса МПП был осуществлен 30 месяцами ранее. Носительство пневмококковой инфекции оценивалось с помощью мазков из носоглотки, а устойчивость к азитромицину — с помощью Е-тестов.

Результаты Распространенность носительства пневмококков снизилась с 43,4% до 19,2% между первым и вторым исследованиями ($P < 0,001$), но вновь повысилась к третьему исследованию (45,8%, $P = 0,591$). Носитель в первом исследовании являлся фактором риска для носительства во втором исследовании (отношение рисков: 3,71; $P < 0,001$). В третьем исследовании распространенность носительства была аналогичной после одного и трех курсов МПП (50,3% против 45,8% соответственно, $P = 0,170$), как и распространенность устойчивости к азитромицину (0,3% против 0,9% соответственно, $P = 0,340$).

Вывод Три курса МПП азитромицина не приводили к увеличению распространенности носоглоточного носительства пневмококковых штаммов, резистентных к азитромицину, по сравнению с первым курсом МПП.

Resumen

La administración en masa de la azitromicina y el transporte del Streptococcus pneumoniae: encuestas transversales en Gambia

Objetivo Evaluar el efecto de una administración masiva repetida (MDA) de azitromicina en Gambia sobre el transporte nasofaríngeo del Streptococcus pneumoniae y sobre la aparición de cepas resistentes a los antibióticos.

Métodos Este estudio involucró a pueblos que participaron en un ensayo aleatorio por grupos que comparó el efecto de una MDA de azitromicina de una sola ronda frente a una MDA de tres rondas sobre la prevalencia del tracoma. Solo se incluyeron aquellos pueblos en los que la mayoría de niños habían recibido la vacuna conjugada antineumocócica 7-valente. Se realizaron tres encuestas transversales en dos pueblos que recibieron tres rondas anuales de MDA: la primera inmediatamente antes de la tercera ronda de MDA y la segunda y la tercera, 1 y 6 meses, respectivamente, después de la tercera ronda de MDA. La tercera encuesta también incluyó seis pueblos que habían recibido una ronda de MDA 30 meses antes. Se evaluó el transporte neumocócico por medio de frotis nasofaríngeos y se detectó la resistencia a la azitromicina por medio del Etest.

Resultados La prevalencia del transporte neumocócico disminuyó del 43,4 % al 19,2 % entre la primera y la segunda encuesta ($P <$ 0,001), pero se recuperó en el momento de la tercera encuesta (45,8

%; $P = 0{,}591$). Ser portador en la primera encuesta fue un factor de riesgo para seguir siendo portador de la segunda (cociente de posibilidades: 3,71; $P < 0{,}001$). En la tercera encuesta, la prevalencia del transporte fue similar tras una y tres rondas de MDA (50,3 % frente a 45,8 %, respectivamente; $P = 0{,}170$), al igual que la prevalencia de la resistencia a la azitromicina (0,3 % frente a 0,9 %, respectivamente; $P = 0{,}340$).

Conclusión Las tres rondas de MDA de azitromicina no aumentaron la prevalencia del transporte nasofaríngeo de cepas de S. pneumoniae resistentes a la azitromicina en comparación con una única ronda.

References

1. Bailey RL, Arullendran P, Whittle HC, Mabey DC. Randomised controlled trial of single-dose azithromycin in treatment of trachoma. Lancet. 1993;342(8869):453–6. doi: http://dx.doi.org/10.1016/0140-6736(93)91591-9 PMID: 8102427
2. Report of the first meeting of the: WHO Alliance for the Global Elimination of Trachoma.1997 Jun 30-Jul 1; Geneva, Switzerland. Geneva: World Health Organization; 1997. Available from: http://www.who.int/pbd/publications/trachoma/en/get_1997.pdf [cited 2012 Aug 31].
3. Report of the third meeting of the: WHO Alliance for the Global Elimination of Trachoma. 1998 Oct 19-20; Ouarzazate, Morocco. Geneva: World Health Organization; 1999. Available from: http://www.who.int/pbd/publications/trachoma/en/get_oct1998.pdf [cited 2013 Aug 31].
4. Making progress toward the global elimination of blinding trachoma. Geneva: WHO Alliance for the Global Elimination of Blinding Trachoma by 2020, World Health Organization; 2006. Available from: http://www.who.int/blindness/GET 10 REPORT FORMATED FINAL.pdf [cited 2013 Sep 30].
5. Solomon AW, Holland MJ, Alexander NDE, Massae PA, Aguirre A, Natividad-Sancho A, et al. Mass treatment with single-dose azithromycin for trachoma. N Engl J Med. 2004;351(19):1962–71. doi: http://dx.doi.org/10.1056/NEJMoa040979 PMID: 15525721
6. Burton MJ, Holland MJ, Makalo P, Aryee EA, Alexander ND, Sillah A, et al. Re-emergence of Chlamydia trachomatis infection after mass antibiotic treatment of a trachoma-endemic Gambian community: a longitudinal study. Lancet. 2005;365(9467):1321–8. doi: http://dx.doi.org/10.1016/S0140-6736(05)61029-X PMID: 15823382
7. Burton MJ, Holland MJ, Makalo P, Aryee EAN, Sillah A, Cohuet S, et al. Profound and sustained reduction in Chlamydia trachomatis in The Gambia: a five-year longitudinal study of trachoma endemic communities. PLoS Negl Trop Dis. 2010;4(10):e835. doi: http://dx.doi.org/10.1371/journal.pntd.0000835 PMID: 20957147
8. Harding-Esch EM, Sillah A, Edwards T, Burr SE, Hart JD, Joof H, et al.; Partnership for Rapid Elimination of Trachoma (PRET) study group. Mass treatment with azithromycin for trachoma: when is one round enough? Results from the PRET Trial in the Gambia. PLoS Negl Trop Dis. 2013;7(6):e2115. doi: http://dx.doi.org/10.1371/journal.pntd.0002115 PMID: 23785525
9. Hong KC, Schachter J, Moncada J, Zhou Z, House J, Lietman TM. Lack of macrolide resistance in Chlamydia trachomatis after mass azithromycin distributions for trachoma. Emerg Infect Dis. 2009;15(7):1088–90. doi: http://dx.doi.org/10.3201/eid1507.081563 PMID: 19624926
10. Haug S, Lakew T, Habtemariam G, Alemayehu W, Cevallos V, Zhou Z, et al. The decline of pneumococcal resistance after cessation of mass antibiotic distributions for trachoma. Clin Infect Dis. 2010;51(5):571–4. doi: http://dx.doi.org/10.1086/655697 PMID: 20649409
11. Skalet AH, Cevallos V, Ayele B, Gebre T, Zhou Z, Jorgensen JH, et al. Antibiotic selection pressure and macrolide resistance in nasopharyngeal Streptococcus pneumoniae: a cluster-randomized clinical trial. PLoS Med. 2010;7(12):e1000377. doi: http://dx.doi.org/10.1371/journal.pmed.1000377 PMID: 21179434
12. Faal H, Minassian D, Sowa S, Foster A. National survey of blindness and low vision in The Gambia: results. Br J Ophthalmol. 1989;73(2):82–7. doi: http://dx.doi.org/10.1136/bjo.73.2.82 PMID: 2784691
13. Dolin PJ, Faal H, Johnson GJ, Ajewole J, Mohamed AA, Lee PS. Trachoma in The Gambia. Br J Ophthalmol. 1998;82(8):930–3. doi: http://dx.doi.org/10.1136/bjo.82.8.930 PMID: 9828780
14. Elimination of blinding trachoma: only 10 years to go. Report of the fourteenth meeting of the WHO Alliance for the Global Elimination of Blinding Trachoma. Geneva: World Health Organization; 2010. Available from: http://www.who.int/blindness/publications/GET14_FINAL_REPORT.pdf [cited 2014 Feb 18].
15. Harding-Esch EM, Edwards T, Mkocha H, Munoz B, Holland MJ, Burr SE, et al.; PRET Partnership. Trachoma prevalence and associated risk factors in the Gambia and Tanzania: baseline results of a cluster randomised controlled trial. PLoS Negl Trop Dis. 2010;4(11):e861. doi: http://dx.doi.org/10.1371/journal.pntd.0000861 PMID: 21072224
16. Roca A, Hill PC, Townend J, Egere U, Antonio M, Bojang A, et al. Effects of community-wide vaccination with PCV-7 on pneumococcal nasopharyngeal carriage in the Gambia: a cluster-randomized trial. PLoS Med. 2011;8(10):e1001107. doi: http://dx.doi.org/10.1371/journal.pmed.1001107 PMID: 22028630
17. Stare D, Harding-Esch E, Munoz B, Bailey R, Mabey D, Holland M, et al. Design and baseline data of a randomized trial to evaluate coverage and frequency of mass treatment with azithromycin: the Partnership for Rapid Elimination of Trachoma (PRET) in Tanzania and The Gambia. Ophthalmic Epidemiol. 2011;18(1):20–9. doi: http://dx.doi.org/10.3109/09286586.2010.545500 PMID: 21275593
18. Morona JK, Morona R, Paton JC. Analysis of the 5′ portion of the type 19A capsule locus identifies two classes of cpsC, cpsD, and cpsE genes in Streptococcus pneumoniae. J Bacteriol. 1999 Jun;181(11):3599–605. PMID: 10348877
19. Roca A, Bottomley C, Hill PC, Bojang A, Egere U, Antonio M, et al. Effect of age and vaccination with a pneumococcal conjugate vaccine on the density of pneumococcal nasopharyngeal carriage. Clin Infect Dis. 2012;55(6):816–24. doi: http://dx.doi.org/10.1093/cid/cis554 PMID: 22700830
20. da Gloria Carvalho M, Pimenta FC, Jackson D, Roundtree A, Ahmad Y, Millar EV, et al. Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. J Clin Microbiol. 2010;48(5):1611–8. doi: http://dx.doi.org/10.1128/JCM.02243-09 PMID: 20220175
21. Brito DA, Ramirez M, de Lencastre H. Serotyping Streptococcus pneumoniae by multiplex PCR. J Clin Microbiol. 2003;41(6):2378–84. doi: http://dx.doi.org/10.1128/JCM.41.6.2378-2384.2003 PMID: 12791852
22. Hill PC, Akisanya A, Sankareh K, Cheung YB, Saaka M, Lahai G, et al. Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers. Clin Infect Dis. 2006;43(6):673–9. doi: http://dx.doi.org/10.1086/506941 PMID: 16912937
23. Leach AJ, Shelby-James TM, Mayo M, Gratten M, Laming AC, Currie BJ, et al. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of Streptococcus pneumoniae. Clin Infect Dis. 1997;24(3):356–62. doi: http://dx.doi.org/10.1093/clinids/24.3.356 PMID: 9114185
24. Fry AM, Jha HC, Lietman TM, Chaudhary JSP, Bhatta RC, Elliott J, et al. Adverse and beneficial secondary effects of mass treatment with azithromycin to eliminate blindness due to trachoma in Nepal. Clin Infect Dis. 2002;35(4):395–402. doi: http://dx.doi.org/10.1086/341414 PMID: 12145722
25. Batt SL, Charalambous BM, Solomon AW, Knirsch C, Massae PA, Safari S, et al. Impact of azithromycin administration for trachoma control on the carriage of antibiotic-resistant Streptococcus pneumoniae. Antimicrob Agents Chemother. 2003;47(9):2765–9. doi: http://dx.doi.org/10.1128/AAC.47.9.2765-2769.2003 PMID: 12936971
26. Coles CL, Mabula K, Seidman JC, Levens J, Mkocha H, Munoz B, et al. Mass distribution of azithromycin for trachoma control is associated with increased risk of azithromycin-resistant Streptococcus pneumoniae carriage in young children 6 months after treatment. Clin Infect Dis. 2013;56(11):1519–26. doi: http://dx.doi.org/10.1093/cid/cit137 PMID: 23487375
27. Porco TC, Gebre T, Ayele B, House J, Keenan J, Zhou Z, et al. Effect of mass distribution of azithromycin for trachoma control on overall mortality in Ethiopian children: a randomized trial. JAMA. 2009;302(9):962–8. doi: http://dx.doi.org/10.1001/jama.2009.1266 PMID: 19724043
28. Keenan JD, Ayele B, Gebre T, Zerihun M, Zhou Z, House JI, et al. Childhood mortality in a cohort treated with mass azithromycin for trachoma. Clin Infect Dis. 2011;52(7):883–8. doi: http://dx.doi.org/10.1093/cid/cir069 PMID: 21427395
29. World Health Organization. Pneumococcal conjugate vaccine for childhood immunization–WHO position paper. Wkly Epidemiol Rec. 2007;82(12):93–104. PMID: 17380597