

## Alterations in the conjunctival bacterial flora following a single dose of azithromycin in a trachoma endemic area

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### Abstract

**Background/claims**—The World Health Organisation has recommended repeated mass treatment of children in trachoma endemic areas with oral azithromycin. While chlamydia, the causative agent of trachoma, remains universally sensitive to azithromycin, there is concern that large scale programmes may alter the bacterial flora and induce resistance in streptococcal species. In this study the effect of a single dose of azithromycin on the prevalence, species distribution, and resistance of conjunctival bacterial flora was determined.

**Methods**—Baseline and 14 day follow up bacterial cultures were taken from the conjunctivae of 121 children who reside in a trachoma endemic area of Nepal. 91 children were treated with azithromycin at baseline and 31 children received deferred treatment at the 14 day follow up. **Results**—Although the prevalence of bacterial pathogens decreased significantly with azithromycin treatment, a significant change in the distribution of specific bacterial pathogens could not be demonstrated. Streptococcal resistance to azithromycin was found significantly more frequently after treatment. No change in the prevalence, distribution, or resistance pattern was found in the untreated control group.

**Conclusion**—Repeated mass treatment of trachoma endemic areas with oral azithromycin will have an effect on bacterial flora. However, further work needs to be done to determine if this will have any clinical relevance.

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The World Health Organisation (WHO), in collaboration with numerous non-governmental organisations and public health departments, has already begun to implement a global initiative to eliminate blinding trachoma by the year 2020.<sup>1</sup> A major part of this programme is the mass distribution of systemic antibiotics including azithromycin (Zithromax, Pfizer International, NY, USA). A single dose of azithromycin is extremely effective in eliminating the causative agent of trachoma, *Chlamydia trachomatis*, from an individual.<sup>2</sup> In addition to *C trachomatis*, azithromycin has antibacterial activity against many bacterial species including pathogenic bacteria that are

routinely found in the conjunctiva. While *Chlamydia* has remained sensitive to macrolides and azalides including azithromycin, other bacteria may develop resistance, and there is some concern that large scale trachoma programmes may interfere with bacterial flora.<sup>3</sup> In this study, we investigated the effect of azithromycin on the bacterial flora of children in a trachoma endemic area. In particular, we addressed the following questions. Does a single dose of azithromycin decrease the prevalence of conjunctival bacteria? Does a single dose of azithromycin alter the distribution of bacterial species found in the conjunctival flora? Does a single dose of azithromycin result in resistant *Streptococcus pneumoniae* or *Haemophilus influenzae* in the conjunctiva?

Trachoma is the second leading cause of blindness worldwide and is the leading cause of infectious blindness.<sup>4</sup> Recurrent infection of children with *C trachomatis* can lead to a cascade of conjunctival scarring, trichiasis, corneal ulceration, and ultimately blindness. Trachoma is endemic in poor, arid areas of Africa and Asia. The WHO has declared Nepal a high priority country for trachoma control.<sup>1</sup> The Lumbini zone in mid-western Nepal is an area known from previous studies to have endemic trachoma.<sup>5,6</sup>

Azithromycin is an azalide antibiotic with a broad spectrum of activity against Gram positive and Gram negative bacteria. It has unique pharmacokinetic properties including a long half life, and concentration of the antibiotic in local tissue reservoirs.<sup>7</sup> After oral dosing, tissue specimens from the conjunctiva have concentrations of azithromycin over 100 times the level in the serum.<sup>8</sup> Azithromycin is also more highly concentrated in inflamed tissues such as the sinuses,<sup>9</sup> which may be due, in part, to the preferential accumulation of azithromycin by polymorphonuclear leucocytes and other phagocytic cells.<sup>10</sup> Moreover, the minimum inhibitory concentration of azithromycin in conjunctival tissue remains above that necessary to inhibit *C trachomatis* after 6 days.<sup>11</sup> By the eighth day following oral dosing, the serum drug level is undetectable while the tissue levels remained above the MIC90 for *S pneumoniae* and *H influenzae*.<sup>12</sup>

Azithromycin has in vitro activity against many bacterial species commonly found on the conjunctiva, including *S pneumoniae*, *H influenzae*, *Staphylococcus* species, *S pyogenes*, and *C trachomatis*.<sup>7</sup> The low incidence of side effects and the high compliance with the dosing

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schedule make azithromycin an ideal drug for public health purposes. A single oral dose of azithromycin has been found to be as effective against active trachoma as 4–6 weeks of topical tetracycline ointment.<sup>2 13</sup>

## Materials and methods

### STUDY POPULATION

A village and a nearby elementary school in the Lumbini zone of mid-western Nepal were chosen by recommendation of the local healthcare officials. Informed consent was obtained for the children through consultation with guardians and the village chief. Baseline and 14 day follow up evaluations were performed on children aged 1–10 years at each location. On the baseline visit, trained examiners screened children for evidence of early trachoma by grading the right upper conjunctiva according to the simplified WHO grading scale for trachoma.<sup>14</sup> Children were considered clinically active if their right conjunctivae were scored as follicular trachoma (TF) or intense trachoma (TI). The right upper conjunctivae of all children were swabbed for bacterial cultures on blood agar plates. Ninety one children were randomised to receive oral azithromycin (Zithromax paediatric suspension, Pfizer International) at a dose of 20 mg/kg (the treated group) at baseline, and 31 children were randomised to receive the same dose of azithromycin deferred until the 14 day follow up visit (the control group). Randomisation (by choosing marked cards from a shuffled deck) was performed after the examination and culture. There was no use of antibiotic ointment or any new introduction of lid hygiene between baseline and the 14 day follow up.

Table 1 Characteristics of treated and control groups

	Treated	Control	Total
Children examined at baseline visit	91	31	122
Children examined at 14 day visit	84	25	109
Mean age at baseline visit	7.1	7.0	7.1
Active trachoma at baseline visit*	35 (39%)	11 (35%)	46 (38%)

\*Active trachoma is defined as follicular trachoma (TF) or intense trachoma (TI) on clinical examination according to the WHO simplified trachoma grading scale.<sup>13</sup>

Table 2 Prevalence of pathogenic bacteria\*

	Baseline visit		14 day visit	
Treated	36/91	(40%)	13/84	(15%)
Control	5/31	(16%)	4/25	(16%)
Total	41/122	(34%)	17/109	(16%)

\*Pathogenic bacteria are defined, for the purposes of this study, as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella* sp, *Neisseria* sp, and other Gram negative rods such as *Escherichia coli*.

Table 3 Bacterial species isolated

	Baseline visit (122 children examined)			14 day visit (109 children examined)		
	Treated	Control	Total	Treated	Control	Total
<i>S pneumoniae</i>	25	2	27	7	3	10
<i>Haemophilus</i>	8	1	9	5	1	6
<i>Moraxella</i>	4	2	6	0	0	0
<i>Neisseria</i>	2	0	2	0	0	0
<i>E coli</i>	0	0	0	1	0	1
Total	39	5	44	13	4	17

The conjunctivae of some children had growth of more than one bacterial species.

### LABORATORY ANALYSIS

The bacterial cultures were incubated and processed using standard microbiological techniques at the microbiology laboratory at the Lumbini-Rana Eye Hospital in Bhairahawa, Nepal. A dot of *Staphylococcus epidermidis* was placed on each plate to allow for growth of *Haemophilus* species.<sup>15</sup> Pathogenic bacteria (defined for the purposes of this study as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella* sp, *Neisseria* sp, and other Gram negative rods such as *Escherichia coli*) were isolated for identification and further testing. Antibiotic sensitivity for azithromycin and penicillin G was performed using the E-Test (AB Biodisk, Sweden) on all isolates of *S pneumoniae* and *H influenzae*.

Fourteen days after the initial visit, we returned to the school and village and recultured the conjunctivae of the same children who were previously examined. Children randomised to deferred azithromycin treatment were treated at this time.

### DATA ANALYSIS

The  $\chi^2$  statistic was used to compare the baseline and follow up prevalence of bacteria, distribution of bacteria, and prevalence of *S pneumoniae* resistance. When the expected number in a cell was five or fewer, a Fisher's exact test was used.

## Results

### PATIENT POPULATION

On the first visit, we examined 54 children at the school and 68 children at the village. The children ranged in age from 6–10 years of age in the school and 1–10 years of age in the village, with an overall average age of 7.1 years. Ninety one (75%) of the 122 children were randomised to receive azithromycin treatment at the first visit. One hundred and nine (89%) of the 122 children examined on the first visit were located and recultured on the 14 day visit (Table 1).

### TRACHOMA GRADING

In total, 46 (38%) of 122 of children were graded as clinically active; 11 (20%) of 54 from the school were active, and 35 (51%) of 68 children in the village. The proportion of active cases in the treated (35 of 91, or 38%) and in the control group (22 of 31, or 37%) were not significantly different ( $p > 0.90$ ,  $\chi^2$  test).

### CULTURE RESULTS

Pathogenic bacteria were recovered from the conjunctivae of 36 (40%) of 91 children in the treated group on the initial visit (Table 2). At the 14 day follow up, 13 (18%) of the 84 children had pathogenic bacteria isolated from their conjunctiva. There was a statistically significant decline in the number of pathogenic bacteria from before to after azithromycin treatment ( $p < 0.01$ ,  $\chi^2$  test). In the control group, the percentage of children with pathogenic bacteria remained unchanged in the 2 week period.

The distribution of bacterial isolates in the school and village is summarised in Table 3. In

Table 4 Children with recovery of bacteria on initial and subsequent cultures

Baseline culture	Sensitivity to:		14 day culture	Sensitivity to:	
	Azithromycin	Penicillin		Azithromycin	Penicillin
<i>Haemophilus</i> sp	Sensitive	Not tested	<i>Haemophilus</i> sp	Sensitive	Not tested
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Sensitive	Sensitive
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Sensitive	Sensitive
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Sensitive	Sensitive
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Sensitive	Sensitive
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Resistant	Sensitive
<i>S pneumoniae</i>	Sensitive	Sensitive	<i>S pneumoniae</i>	Resistant	Sensitive

the treated group, *S pneumoniae* was isolated from 25 children; *Haemophilus* species in eight children, *Moraxella* species in four children, and *Neisseria* species in two children. At the 14 day visit, *S pneumoniae* was again the most frequent isolate occurring in seven patients; *Haemophilus* species in five patients; and *E coli* in one patient. The difference in the distribution of organisms at baseline and at 14 days did not reach statistical significance ( $p=0.10$ ,  $\chi^2$  test). Also there was no significant difference in the distribution of bacterial species in the control group over the 14 day period.

#### SENSITIVITIES

Antibiotic sensitivities to azithromycin and penicillin G were performed on all isolates of *S pneumoniae* using the E-test (AB Biodisk, Sweden). The *Haemophilus* species were tested for azithromycin sensitivity. Of the 27 *S pneumoniae* isolates tested from the first visit (from both the treated and the control groups), all were sensitive to both azithromycin and penicillin G. Likewise, all nine *Haemophilus* isolates were sensitive to azithromycin. From the 14 day collection in the control group, all three isolates of *S pneumoniae* remained sensitive to the antibiotics. However, in the treated group, three of seven isolates of *S pneumoniae* were resistant to azithromycin. There was no change in sensitivity in these isolates to penicillin G. The five *Haemophilus* isolates found on the day 14 visit were sensitive to azithromycin.

#### PAIRED CULTURE RESULTS

Seven children had growth of the same bacterial species on the initial and subsequent culture (Table 4). Six of the seven children grew *S pneumoniae* on both cultures and one child grew *Haemophilus* species. Whereas all initial *S pneumoniae* isolates were sensitive to azithromycin by E-test, two isolates from the second visit were resistant to this antibiotic. All isolates were sensitive to penicillin G. The *Haemophilus* species was sensitive to azithromycin both before and after treatment.

#### Discussion

In the patients treated with azithromycin, we found a statistically significant decrease in the recovery of pathogenic bacteria from the conjunctiva, from 39% to 14%. Patients in the untreated control group had no change in the number of positive cultures over this 14 day time period. The systemic administration of azithromycin has also been shown to decrease bacterial flora elsewhere in the body. Adegbola

et al<sup>16</sup> reported a decrease of *S pneumoniae* recoverable from the oropharynx from 73% to 24% in children after azithromycin treatment. Leach et al<sup>17</sup> also found a statistically significant decrease in oropharyngeal *S pneumoniae* from 68% at baseline to 29% 2 weeks after azithromycin dosing. At 2 and 6 months after treatment, however, the rates of positive bacterial cultures had returned to baseline. The lower rate of *S pneumoniae* found in our study may be because of the culture source (conjunctiva rather than oropharynx), the study population (Leach et al sampled only children with active trachoma, who may tend to be at an age or in a socioeconomic group more likely to harbour pneumococcus as well), or the study location and season.

The most frequent pathogenic bacteria isolated in this study were *S pneumoniae* and *H influenzae*, and *Moraxella*. The prevalence of all three pathogens was reduced after azithromycin treatment. The distribution of bacterial species was not statistically different at baseline and 14 days after treatment. Previous studies of trachomatous eyes also had found *S pneumoniae* and *H influenzae* to be the most common non-chlamydial ocular pathogen.<sup>18</sup> Adegbola et al<sup>16</sup> found that *S pneumoniae* was less prevalent after azithromycin treatment, but that *H influenzae* was not significantly affected. In many countries, seasonal variation plays an important role in both the number and distribution of bacteria, as suggested by Peterson and Treadway.<sup>19</sup> It should be noted that the effect of azithromycin on the flora may also change during the course of the year, so care should be taken when generalising these results. Seasonal variation between the baseline and the follow up collections was relatively short (14 days) and was not thought to be a factor in the change in flora. In fact, there was no significant change in the frequency of bacterial infection in the control group, although the power to detect such a change was dependent on the relatively small size of this group.

On the 14 day follow up visit, we found that three of seven isolates of *S pneumoniae* from the treated group were resistant to azithromycin in vitro. Previously, two of these patients had on their initial cultures growth of *S pneumoniae* which was sensitive to azithromycin. The third patient initially had an  $\alpha$  haemolytic colony which could not be isolated from the background contamination, and in retrospect, may also have been *S pneumoniae*. No resistant isolates were present in the control group. Moreover, there was no change in the sensitivity of

the *S pneumoniae* isolates to an unrelated class of antibiotics, penicillin G. Leach *et al*<sup>17</sup> also found an increase in azithromycin resistant *S pneumoniae* from 1.3% to 15.8% following a single dose of azithromycin. In a large Finnish study,<sup>20</sup> higher rates of macrolide resistant *S pyogenes* occurred in parallel with the increased prescription of macrolide and azalide antibiotics at a national level. As the use of macrolides and azalides was reduced resistance decreased.

For several reasons, it is not known whether or not the acquired resistance found in this study is clinically relevant. Most colonising bacteria (resistant or sensitive) will not go on to cause a productive infection. Productive infections are often not treated with macrolide or azalide antibiotics, but instead with antibiotics to which *S pneumoniae* will presumably remain sensitive. Resistance found in vitro often does not correlate with clinical resistance. Resistant organisms tend to be less fit than sensitive organisms, presumably because they have had to make a compromise to become resistant, thus they may be less likely to cause infection and less likely to spread to other individuals<sup>21</sup>; it is not known how often resistant bacteria colonising either the conjunctiva or the oropharynx will produce infections in others (that is, cause initial resistance). The resistance may be transient, and it may be reversible when treatment programmes conclude, as suggested in Australian and Finnish studies.<sup>17 20</sup>

Mass treatment with azithromycin is recommended by the WHO for the treatment of endemic trachoma, and treatment programmes are already under way in some countries. We expect the use of any systemic antibiotic (including azithromycin) to have an effect on bacterial flora, both decreasing bacterial flora and increasing drug resistance in bacteria such as *S pneumoniae*. There is a large global programme already in place that monitors the development of resistance to azithromycin.<sup>1</sup> It will be particularly important to monitor the prevalence of resistance in areas receiving repeated mass administrations of azithromycin, in actual infections (not just in colonisation), and in both individuals who have received azithromycin (acquired resistance) and those who have not (initial resistance).

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