SCIENTIFIC REPORT

Topical ocular antibiotics induce bacterial resistance at extraocular sites

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Aim: To compare the prevalence of antibiotic resistance found in nasopharyngeal *Streptococcus pneumoniae* between villages treated with topical tetracycline or systemic azithromycin as part of a trachoma control programme.

Methods: All children aged 1–10 years were offered either single dose oral azithromycin treatment (20 mg/kg) or a course of topical 1% tetracycline ointment, depending on the area. Treatment was given annually for 3 years. Six months after the third annual treatment in each village, children were surveyed for nasopharyngeal carriage of *S pneumoniae* and resistance was determined using broth dilution MIC technique. Children in two additional villages, which had not yet been treated, were also surveyed.

Results: Nasopharyngeal carriage of *S pneumoniae* was similar in the tetracycline treated, azithromycin treated, and untreated areas (p=0.57). However, resistance to tetracycline and azithromycin was distributed differently between the three areas (p=0.004). The village treated with topical tetracycline had a higher prevalence of tetracycline resistance than the other villages (p=0.010), while the oral azithromycin treated village had a higher prevalence of macrolide resistance than the other villages (p=0.014).

Conclusions: Annual mass treatment with oral azithromycin may alter the prevalence of drug resistant *S pneumoniae* in a community. Surprisingly, topical tetracycline may also increase nasopharyngeal pneumococcal resistance. Topical antibiotics may have an effect on extraocular bacterial resistance.

Topical antibiotics are ideal for external ocular infections, such as conjunctivitis and keratitis, because they are easy to administer, achieve a high concentration at the site of infection, and have fewer potential systemic side effects.[1–4] The risk of developing bacterial resistance, in both ocular and non-ocular isolates, is believed to be lower with topically applied antibiotics. This is due to the relatively small number of bacteria in the eye and the small concentration of antibiotic that is absorbed systemically.[4] Topical antibiotics can clearly cause bacterial resistance in the conjunctiva, cornea, and lids.[5] It is also well known that ocular drops can be associated with systemic side effects, such as suppression of endogenous cortisol production with topical steroid use[6] and untoward respiratory and cardiovascular events with the use of topical beta blockers.[7] However, to the best of our knowledge, there are no reports of the emergence of drug resistance in bacteria at extraocular sites associated with topical ophthalmic antibiotics.

In this study, we distributed three annual mass antibiotic administrations to two villages in a trachoma endemic area of Western Nepal. The treated villages received either oral azithromycin or topical tetracycline. Six months after the last round of treatment, we surveyed young children for the carriage of antibiotic resistant pneumococcus in the nasopharynx. In addition, at this time point two further villages, as yet untreated, were also surveyed for nasopharyngeal pneumococcal carriage.

METHODS

This study was granted ethical approval by the Institutional Review Board of the University of California, San Francisco, and by the Nepal Health Research Council and Social Welfare Council. Verbal consent was obtained from the parents/guardian of all children who participated in this study. Three subdistricts (Village Development Committees) in the Kailali district of Western Nepal were surveyed for clinically active trachoma using the World Health Organization simplified grading system in May 1998.[8] The prevalence of clinically active trachoma (that is, TF and/or TI) in the right eye of all children aged 1–10 years was documented. Two subdistricts were chosen to be treated with oral azithromycin as part of the trachoma control programme beginning in November 1998; the other subdistrict was treated with topical tetracycline. An azithromycin treated village was matched to a tetracycline treated village, based on predominant ethnic group (Rana Tharu) and trachoma endemicity (the village with the highest disease prevalence was selected from the azithromycin treated subdistricts and also from the tetracycline treated subdistrict). Azithromycin treatment (single dose, 20 mg/kg, up to a maximum dose of 1 g) was distributed in one village, and 1% tetracycline ophthalmic ointment (twice a day for 6 weeks) distributed in the other. The full course of tetracycline ointment was given to the parent/guardian of each child for administration at home. All parents/guardians were instructed on how to apply the eye ointment and educated on the importance of regular application for the full 6 week treatment period. Treatment with topical tetracycline was not directly observed. A single round of antibiotic treatment was offered once per year to all children aged 1–10 years old in each village for a 3 year period. Two additional untreated villages from Kailali district were randomly selected to be surveyed for background pneumococcal carriage and resistance in May 2001. After this assessment, all children aged 1–10 years were offered oral azithromycin as part of a separate programme.

In May 2001, 6 months after the third annual treatment in each of the two treated villages, surveillance was performed for pneumococcal carriage and resistance, as well as assessment of trachoma clinical activity. Children aged 1–7 years were monitored, because the prevalence of pneumococcal carriage peaks in this age group and progressively decreases in older children.[9] Dacron nasopharyngeal swabs

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were obtained transnasally from all children aged 1–7 in order to determine the presence of *S pneumoniae*. Swabs were immediately placed into STGG media for preservation and transportation as previously described.[10–12] Samples were kept at 4℃ for up to 2–3 hours in the field, then placed at −20℃ at Geta Eye Hospital and frozen at −70℃ in Kathmandu before transport to the Proctor Foundation Microbiology Laboratory at the University of California, San Francisco, where they arrived frozen.[12] Samples were kept at −80℃ for up to 4 months before culture. All pneumococcal isolates were stored frozen at −80℃ until antibiotic sensitivity testing between October 2001 and November 2002. It has previously been shown that *S pneumoniae* collected from the nasopharynx can be safely transported and stored in STGG media for up to 2 years before analysis.[13]

Swabs were processed using standard laboratory techniques by laboratory personnel masked to the study arm of each sample. Susceptibility was determined using broth dilution minimum inhibitory concentration (Sensititre MIC plates, Trek Diagnostics Inc, Westlake, OH, USA). National Committee for Clinical Laboratory Standards (NCCLS) values for non-susceptibility to azithromycin, tetracycline, penicillin, and trimethoprim-sulfamethoxazole were used (M100-S9. Wayne, PA: National Committee for Clinical Laboratory Standards, 1999).

Cases in each of the azithromycin treated, tetracycline treated, and untreated areas were identified as azithromycin resistant, tetracycline resistant, or sensitive to both drugs, resulting in a 3×3 contingency table (note that all azithromycin resistant cases were also tetracycline resistant). Fisher's exact test was used to determine whether the observed distribution of pneumococcal antibiotic resistance between the three areas would likely have occurred by chance alone.[14] In order to explain the difference found in the overall distribution, the original 3×3 table was partitioned into the two relevant 2×2 post hoc comparisons, and a Bonferroni adjusted $p < 0.0125$ was considered significant (because the original table contains 4 degrees of freedom).

RESULTS

The prevalence of clinically active trachoma in children aged 1–10 years pretreatment was 50% in the tetracycline village and 39% in the azithromycin village. Six months after the final treatment, prevalence dropped to 20% in the tetracycline village and 4% in the azithromycin village.[15] The trachoma programme achieved an estimated antibiotic treatment coverage of 80%.[16]

Nasopharyngeal carriage was similar in the three study areas: 84% in the azithromycin treated village, 88% in the tetracycline village, and 85% in the untreated villages ($p = 0.57$, table 1). However, resistance to tetracycline and azithromycin was distributed differently between the three areas (3×3 Fisher's exact, $p = 0.004$, table 1). Post hoc testing revealed that the village treated with topical tetracycline had a higher prevalence of tetracycline resistance than the other villages (2×2 Fisher's exact, $p = 0.010$), whereas the village treated with oral azithromycin had a higher prevalence of macrolide resistance than the other villages (2×2 Fisher's exact, $p = 0.014$).

Resistance to penicillin was undetectable in both the antibiotic treated villages and the untreated villages. Resistance to trimethoprim-sulfamethoxazole was present in all of the treated and untreated villages, but was not significantly different between the three study areas (3×2 Fisher's exact, $p = 0.650$). Sulfonamides represent approximately 13% of total antibiotic use in this area of Nepal.[17]

DISCUSSION

Our data suggest that either topical tetracycline or oral azithromycin use can select for pneumococcal resistance in the nasopharynx. The selection for resistance to oral azithromycin has been a concern since mass trachoma distributions began.[11] [12] [18–20] Significant, transmissible macrolide resistance in chlamydia has not as yet been demonstrated. However, pneumococcal resistance has previously been shown to develop soon after mass azithromycin treatments.[18] [19] This resistance is reduced at 1 year unless subsequent treatments are offered.[11] [12] In this study, some resistance was identified after three annual azithromycin treatments. Although no pretreatment data are available, this resistance was apparently induced by the azithromycin distributions as no azithromycin resistance was found in the tetracycline treated village or in the two untreated villages. However, it is reassuring that even after three mass treatments, resistance was present at levels that are far below those found in Western countries.[21] This result suggests that three annual azithromycin treatments of children for trachoma may not result in a high prevalence of drug resistance, at least in an area where there is little to no previous resistance.

Topical tetracycline is still used for the treatment of trachoma, even though it has some drawbacks such as poor adherence because of irritation of the ocular surface, temporarily blurred vision, and lengthy treatment periods.[22] However, it has been argued that topical agents may be less likely to induce resistance at extraocular sites than a systemic antibiotic. Our study suggests this may not be true. Six months after antibiotic distribution, the village treated with topical tetracycline had significantly more tetracycline resistance than all the other villages. Even the untreated villages in our study area seemed to have a considerable amount of background tetracycline resistance. Interestingly, an argument could be made that the clinical implication of resistance from topical tetracycline is of greater concern than that from oral azithromycin, as tetracyclines are prescribed and used far more commonly in the community than are macrolides in this region of Western Nepal.[23] It should also be noted that penicillins make up about 40% of the antibiotic use in this area, and that no penicillin resistance was identified.[17]

Table 1 Distribution of antibiotic resistance in nasopharyngeal *S pneumoniae* in each of the three study areas

	Tetracycline treated (n = 143)	Azithromycin treated (n = 194)	Untreated (n = 107)
S pneumoniae carriage, (%)	126 (88%)	163 (84%)	91 (85%)
Antibiotic resistant *S pneumoniae* (% of isolates showing resistance)			
Tetracycline (≥8 μg/ml)	39 (31%)	28 (17%)	15 (16%)
Azithromycin* (≥2 μg/ml)	0 (0%)	5 (3%)	0 (0%)
Trimethoprim-sulfamethoxazole (≥4 & 76 μg/ml)	36 (29%)	39 (24%)	25 (27%)
Penicillin (≥2 μg/ml)	0 (0%)	0 (0%)	0 (0%)

*All azithromycin resistant isolates were also resistant to tetracycline.
MIC standard values for resistance (as per NCCLS) shown in parentheses.

It is expected that topical antibiotics select for resistance locally at the site of application. Our data suggest that ocular application of high concentrations of antibiotics likely affect the bacterial flora of the nasopharynx as well. This is not surprising, given that medications administered to the ocular surface can flow through the nasolacrimal duct into the nasopharynx, and that the nasopharynx in many children is colonised with pneumococcus. Regardless of the mechanism, emerging systemic resistance from topical antibiotic use, whether for primary treatment or secondary prevention, has to be considered—an effect not previously recognised.

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REFERENCES

1 **Davis SD**, Sarff LD, Hyndiuk RA. Corticosteroid in experimentally induced Pseudomonas keratitis: failure of prednisolone to impair the efficacy of tobramycin and carbenicillin therapy. Arch Ophthalmol 1978;**96**:126–8.
2 **Davis SD**, Sarff LD, Hyndiuk RA. Comparison of therapeutic routes in experimental pseudomonas keratitis. Am J Ophthalmol 1979;**87**:710–16.
3 **Baum J**, Barza M. Topical vs subconjunctival treatment of bacterial corneal ulcers. Ophthalmology 1983;**90**:162–8.
4 **Leeming JP**. Treatment of ocular infections with topical antibacterials. Clin Pharmacokinet 1999;**37**:351–60.
5 **Hwang DG**. Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol 2004;**49**(Suppl 2):S79–83.
6 **Krupin T**, Mandell AI, Podos SM, et al. Topical corticosteroid therapy and pituitary-adrenal function. Arch Ophthalmol 1976;**94**:919–20.
7 **Lama PJ**. Systemic adverse effects of beta-adrenergic blockers: an evidence-based assessment. Am J Ophthalmol 2002;**134**:749–60.
8 **Thylefors B**, Dawson CR, Jones BR, et al. A simple system for the assessment of trachoma and its complications. Bull World Health Organ 1987;**65**:477–83.
9 **Bogaert D**, van Belkum A, Sluijter M, et al. Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. Lancet 2004;**363**:1871–2.
10 **O'Brien KL**, Bronsdon MA, Dagan R, et al. Evaluation of a medium (STGG) for transport and optimal recovery of Streptococcus pneumoniae from nasopharyngeal secretions collected during field studies. J Clin Microbiol 2001;**39**:1021–4.
11 **Gaynor BD**, Holbrook KA, Whitcher JP, et al. Community treatment with azithromycin for trachoma is not associated with antibiotic resistance in Streptococcus pneumoniae at one year. Br J Ophthalmol 2003;**87**:147–8.
12 **Fry AM**, Jha HC, Lietman TM, 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**:395–402.
13 **Saukkoriipi A**, Leskela K, Herva E, et al. Streptococcus pneumoniae in nasopharyngeal secretions of healthy children: comparison of real-time PCR and culture from STGG-transport medium. Mol Cell Probes 2004;**18**:147–53.
14 **Stata**. Stata User's Guide, Release 6. College Station, TX: Stata Press, 1999.
15 **Gaynor BD**, Miao Y, Cevallos V, et al. Eliminating trachoma in areas with limited disease. Emerg Infect Dis 2003;**9**:596–8.
16 **Holm SO**, Jha HC, Bhatta RC, et al. Comparison of two azithromycin distribution strategies for controlling trachoma in Nepal. Bull World Health Organ 2001;**79**:194–200.
17 **Schiedler V**, Bhatta RC, Miao Y, et al. Pattern of antibiotic use in a trachoma-endemic region of Nepal: implications for mass azithromycin distribution. Ophthalmic Epidemiol 2003;**10**:31–6.
18 **Leach AJ**, Shelby-James TM, Mayo M, 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**:356–62.
19 **Chern KC**, Shrestha SK, Cevallos V, et al. Alterations in the conjunctival bacterial flora following a single dose of azithromycin in a trachoma endemic area. Br J Ophthalmol 1999;**83**:1332–5.
20 **Batt SL**, Charalambous BM, Solomon AW, et al. Impact of azithromycin administration for trachoma control on the carriage of antibiotic-resistant Streptococcus pneumoniae. Antimicrob Agents Chemother 2003;**47**:2765–9.
21 **Hyde T**, Gay K, Stephens D, et al. Macrolide resistance among invasive Sterptococcus pneumoniae isolates. JAMA 2001;**286**:1857–62.
22 **Schachter J**, West SK, Mabey D, et al. Azithromycin in control of trachoma. Lancet 1999;**354**:630–5.
23 **Chidambaram JD**, Bird M, Schiedler V, et al. Trachoma decline and widespread use of antimicrobial drugs. Emerg Infect Dis 2004;**10**:1895–9.