Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study

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Summary

Background Resistance to antibiotics is a major public-health problem, and studies that link antibiotic use and resistance have shown an association but not a causal effect. We used the macrolides azithromycin and clarithromycin to investigate the direct effect of antibiotic exposure on resistance in the oral streptococcal flora of healthy volunteers.

Methods Volunteers were treated with azithromycin (n=74), clarithromycin (74), or placebo (76) in a randomised, double-blind trial. Pharyngeal swabs were obtained before and after administration of study treatment through 180 days. The proportion of streptococci that were macrolide resistant was assessed and the molecular basis of any change in resistance investigated. Analyses were done on an intent-to-treat basis. This study is registered with ClinicalTrials.gov, number NCT00354952.

Findings The number of dropouts (n=20) was much the same in all groups until day 42; dropouts increased substantially at day 180 (105). Both macrolides significantly increased the proportion of macrolide-resistant streptococci compared with the placebo at all points studied, peaking at day 8 in the clarithromycin group (mean increase 50·0%, 95% CI 41·7–58·2; p<0·0001) and at day 4 in the azithromycin group (53·4%, 43·4–63·5; p<0·0001). The proportion of macrolide-resistant streptococci was higher after azithromycin treatment than after clarithromycin use, with the largest difference between the two groups at day 28 (17·4% difference, 9·2–25·6; p<0·0001). Use of clarithromycin, but not of azithromycin, selected for the *erm*(B) gene, which confers high-level macrolide resistance.

Interpretation This study shows that, notwithstanding the different outcomes of resistance selection, macrolide use is the single most important driver of the emergence of macrolide resistance in vivo. Physicians prescribing antibiotics should take into account the striking ecological side-effects of such antibiotics.

Introduction

Resistance to antibiotics is a major public-health problem.[1] Many ecological studies have shown a clear relation between antimicrobial use and resistance.[2] However, these studies are commonly confounded by a number of variables, and they show, at best, an association, not a causal effect. Moreover, they do not link antibiotic exposure in an individual to the outcome for that individual, which creates the so-called ecological fallacy.[3] Randomised clinical trials and, to some extent, observational studies that examine antibiotic-exposed versus non-exposed individuals are crucial to study definitively the link between antibiotic use and resistance as well as to provide in-vivo biological samples to study the molecular basis of resistance.[4–6] Previously, such evidence was provided solely by animal experimentation.[7,8]

Two macrolides—clarithromycin and azithromycin—are among the drugs of choice for the treatment of respiratory tract infections. Study of the link between antibiotic use and resistance—as well as the molecular mechanisms of resistance—is especially important because resistance to macrolides in common respiratory pathogens (eg, *Streptococcus pneumoniae* and *Streptococcus pyogenes*) is increasing,[9,10] and is most likely due to their inappropriate use.[2]

There is much debate as to which of these macrolides has greater potential for selecting resistant organisms,[11] one of the decisive factors for eventual preference for clinical use. Azithromycin has a long half-life,[12] and therefore a convenient dose regimen (once daily for 3 days, compared with twice daily for 7 days for clarithromycin). Theoretically, however, shorter drug exposure decreases the chance of the development of resistance, whereas higher tissue persistence and slowly receding azithromycin concentrations increase the chance of development of drug-resistant organisms. Some ecological studies have identified a strong relation between azithromycin use and macrolide resistance,[13] whereas others did not find a correlation.[14]

The few studies that have compared the effect of azithromycin and clarithromycin on the selection of resistance have also shown conflicting results.[15–17] Kastner and Guggenbichler[16] showed that a significantly higher proportion of paediatric patients carried resistant organisms for 6 weeks after azithromycin treatment than did those treated with clarithromycin. King and co-workers[17] were unable to show any difference between

the two macrolides because of a lack of power, whereas Matute and colleagues,[15] in a randomised double-blind study on 18 individuals, showed that neither clarithromycin nor azithromycin use selected for any resistant organisms in either the faecal or the oropharyngeal flora.

Resistance to macrolides in streptococci occurs via two main mechanisms. The first is active drug efflux mediated by a pump encoded by the *mef* (macrolide efflux) gene that confers low to moderate resistance against macrolides, with minimum inhibitory concentrations (MIC) to erythromycin (a prototype macrolide) ranging from 0·5 µg/mL to 32 µg/mL. In the second mechanism, a methylase encoded by the *erm*(B) gene modifies the macrolide binding site on the bacterial ribosome, generally conferring a high degree of resistance, with erythromycin MIC typically ranging from 32 µg/mL to more than 512 µg/mL.[18]

Various studies have shown that the oral commensal streptococcal flora endemically harbours the same macrolide resistance genes seen in the genetically related pathogenic streptococci.[19,20] Thus, we used the oral commensal streptococcal flora as model organisms to study the effect of different macrolides in selecting macrolide resistance in a healthy population. We did a randomised double-blind placebo-controlled trial with azithromycin and clarithromycin to investigate the direct effect of antibiotic exposure on resistance in the oral streptococcal flora of healthy volunteers. We also aimed to investigate the molecular basis for any differences in selection for resistance.

Methods

Participants

We did a randomised, double-blind, placebo-controlled trial between October, 2002, and May, 2003 (ie, during winter) and between March, 2003, and October, 2003 (ie, during summer) at the University of Antwerp, Belgium. Volunteers were selected on the basis of Belgian identity cards (to exclude those younger than 18 years) and a written questionnaire that included information on their sex, age, smoking status, previous antibiotic use, and employment in hospital with contact with patients. Of the 347 healthy adults who volunteered, 224 were eligible—ie, they were non-pregnant, free of any respiratory tract infection, and had not had any antibiotic treatment for at least the previous 3 months. These individuals were recruited after giving written informed consent. The study was approved by the medical ethics committee at the University Hospital of Antwerp, Belgium.

Procedures

On the basis of randomisation codes generated by Microsoft Excel, an administrator (who had no further role in the study) allocated volunteers to four groups: those who would receive azithromycin (500 mg once daily for 3 days), those who would receive clarithromycin (500 mg twice daily for 7 days), and two placebo groups. The two placebo groups imitated the two macrolide regimens to ensure complete masking of the volunteers and of the researchers involved in sample analyses; they were merged for most of the data analyses. The first dose of study treatment was administered under supervision when the volunteers were supplied with the remaining drug or placebo to be self-administered.

Samples of the oral streptococcal flora were obtained by means of a swab firmly pressed over the tonsils and the posterior pharyngeal wall. The jaws, teeth, and gingiva were avoided when the swab was withdrawn. The first sample was taken before treatment (day 0), and the second within 48 h of the end of treatment (day 4 for azithromycin and the first placebo group; day 8 for clarithromycin and the second placebo group). Additional samplings were taken at day 8 for the azithromycin and first placebo groups. Subsequent samples were obtained at days 14, 28, and 42, after which individuals were financially remunerated. A final sample was taken from 99 volunteers at day 180, after questioning about any antibiotic use during the interim period. Five volunteers (two in the azithromycin group, two in the clarithromycin group, and one receiving placebo) had been prescribed norfloxacin, augmentin, or nitrofurantoin for urinary tract infections, but these volunteers were also included in the day 180 analyses since the drugs they had been prescribed do not interact with macrolides. All swabs were placed in an aerobic medium containing skimmed milk, glucose, and glycerol adapted from that of Gibson and Khoury,[21] and stored at –80°C until further analyses.

The primary outcome of this study was change in the proportion of streptococci that were macrolide resistant. 1218 samples were thawed, vortexed, and inoculated on streptococcus selective medium (Oxoid, Basingstoke, UK) with and without erythromycin (2 µg/mL; Sigma Chemical Co, St Louis, MO, USA) with a spiral plater (Eddy Jet, IUL Instruments, Leerdam, Netherlands).[22] Plates were incubated overnight at 37°C in 5% carbon dioxide/95% air. Streptococcal densities were determined by counting two opposite octants of a grid superimposed on the spiral plate and normalised for the inoculated sample volume, as recommended by the manufacturer and described previously.[22] The proportion of macrolide-resistant streptococci was determined by division of the number of colonies on the erythromycin-containing plates by the number of colonies on plates without erythromycin.

Secondary outcomes were variation in the carriage of macrolide and tetracycline resistance genes caused by macrolide exposure, and the effect of antibiotic exposure on an increase in erythromycin MIC values in macrolide-resistant streptococci carrying the *mef* gene.

13 volunteers from both the azithromycin and clarithromycin groups, together with nine volunteers from the pooled placebo group, were randomly chosen and their samples from days 0, 8, 42, and if available, day 180,

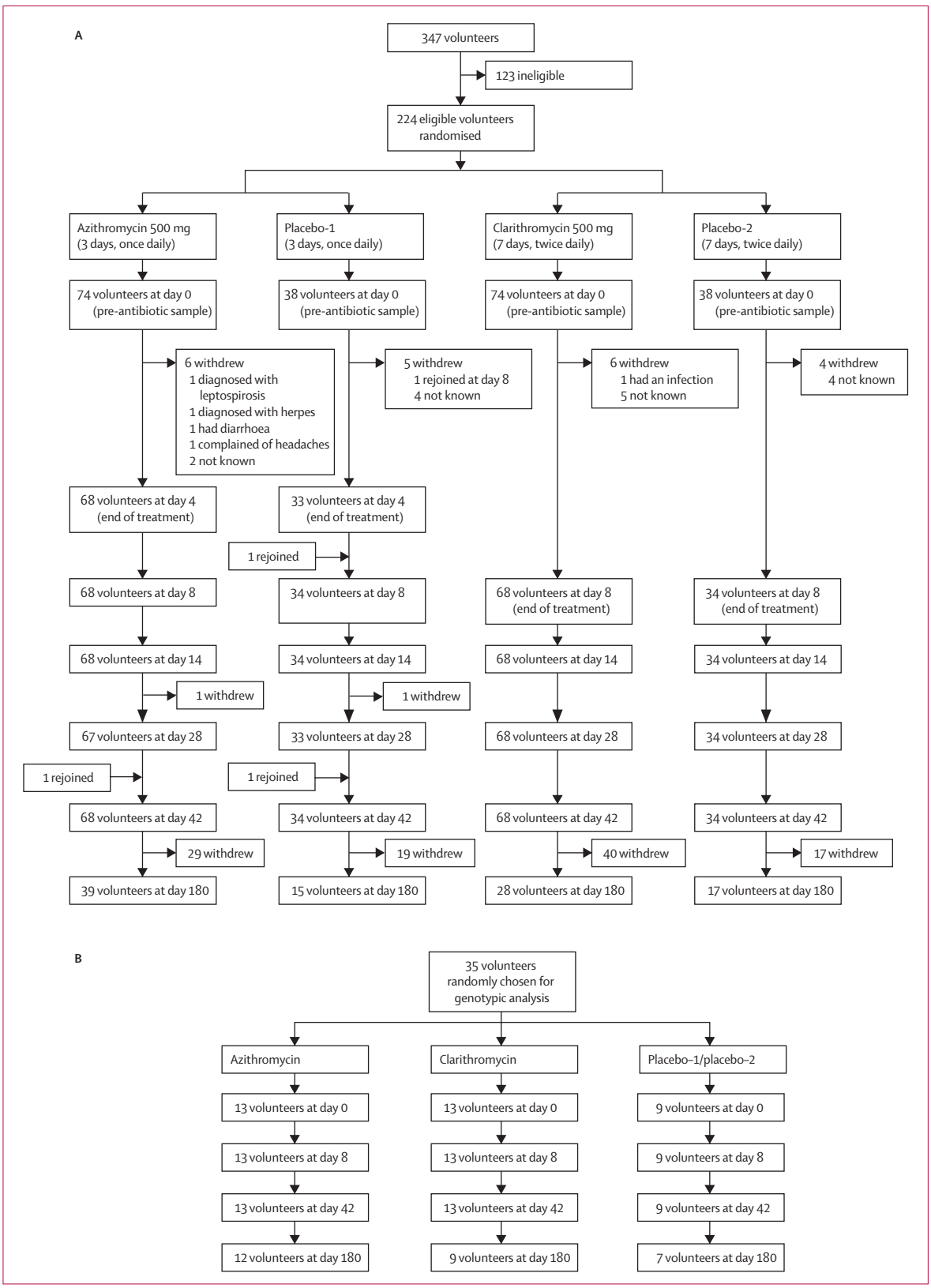

Figure 1: **Trial profile**
(A) For the carriage study (B) for the genotypic analysis.

	Clarithromycin group (n=68)	Azithromycin group (n=68)	Placebo group* (n=68)
Age (years)	24 (19–58)	24 (19–56)	24 (18–57)
Men	25 (37%)	31 (46%)	27 (40%)
Smokers	12 (18%)	13 (19%)	13 (19%)
Employed in hospital having contact with patients	22 (32%)	13 (19%)	12 (18%)
Previous antibiotic use in past 6 months†	6 (9%)	7 (10%)	10 (15%)
Proportion of macrolide-resistant streptococci at day 0	30·1% (24·2–36·0)	25·9% (21·8–30·1)	27·5% (22·0–32·9)

Data are median (range), number (%), or mean % (95% CI). *The two placebo groups combined. †Participants had not been given antibiotics in the previous 3 months.

Table 1: **Baseline characteristics of recruited individuals**

	Azithromycin group		Clarithromycin group		Placebo group*	
	Difference in proportion (95% CI)	p	Difference in proportion (95% CI)	p	Difference in proportion (95% CI)	p
Day 0	..	..	..	..	..	..
Day 4†	60·4% (53·9 to 67·0)	<0·0001	..	..	..	..
Day 8	56·8% (50·3 to 63·3)	<0·0001	51·9% (45·3 to 58·4)	<0·0001	3·8% (–2·7 to 10·4)	0·2520
Day 14	57·3% (50·7 to 63·8)	<0·0001	40·4% (33·9 to 46·9)	<0·0001	4·0% (–2·5 to 10·6)	0·2250
Day 28	54·0% (47·4 to 60·5)	<0·0001	33·2% (26·6 to 39·7)	<0·0001	2·4% (–4·2 to 8·9)	0·4790
Day 42	40·9% (34·4 to 47·5)	<0·0001	27·8% (21·3 to 34·3)	<0·0001	4·0% (–2·5 to 10·5)	0·2290
Day 180	14·5% (6·7 to 22·2)	0·0003	16·3% (7·5 to 25·1)	0·0003	–0·9% (–9·2 to 7·4)	0·8240

*Placebo groups were combined. †End-of-treatment data for azithromycin, data not available for clarithromycin or pooled placebo groups.

Table 2: **Change in mean proportion of macrolide-resistant streptococci from baseline**

underwent genotypic analysis. 20 isolated, dispersed, and average-sized colonies of macrolide-resistant streptococci were randomly selected from the erythromycin-containing streptococcus selective agar plates for all sampling time-points from the 35 volunteers and subcultured overnight on blood agar plates. Of these, 2134 colonies were successfully subcultured and purified, and underwent genotypic analysis for the macrolide-resistance genes *erm*(A), *erm*(B), and *mef* and for the tetracycline-resistance genes *tet*(M), *tet*(O), *tet*(K), and *tet*(L), by use of a multiplex PCR assay.[23]

To investigate whether macrolide use could also lead to the increased expression of macrolide-resistance genes, we studied specifically macrolide-resistant streptococci that carried the *mef* gene, since degrees of resistance in *erm*-carrying isolates are already very high. Any increase in expression of resistance genes can be detected in vitro by an increase in the MIC to erythromycin. 1146 macrolide-resistant streptococci carrying the *mef* gene that were isolated from the 26 volunteers given azithromycin or clarithromycin who had been analysed genotypically were studied for MIC to erythromycin (Sigma Co, St Louis, MO, USA) by agar dilution as a further secondary outcome, in accordance with guidelines from the Clinical and Laboratory Standards Institute.[24]

Statistical analysis

A sample size of 70 volunteers was needed to identify a 25% increase in the proportion of resistant bacteria[6] after antibiotic use with 80% power at a one-sided significance level of 2·5% (α=0·025), and also to identify a 25% difference in resistance selection potential between azithromycin and clarithromycin after antibiotic use with 80% power at a two-sided significance level of 5% (α=0·05).

Data analyses were done with SPSS version 12.0 and SAS version 9.1. After combination of the placebo groups, baseline (day 0) differences in sex, age (in years), smoking status, previous antibiotic use, and hospital employment with contact with patients were assessed in volunteers randomly assigned to the three study groups with a χ^2 test and one-way ANOVA.

Means and 95% CI were used to describe changes in the proportions of macrolide-resistant streptococci. The effect of macrolide use on mean proportion of macrolide-

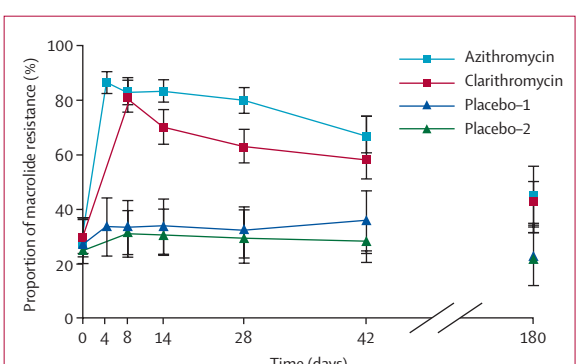

Figure 2: **Temporal changes in the proportion of macrolide-resistant streptococci after azithromycin and clarithromycin use**
Data shown are for all 204 volunteers followed through to day 42, and for 99 volunteers followed through to day 180. Error bars are 95% CI.

	Azithromycin *vs* placebo*		Clarithromycin *vs* placebo*		Clarithromycin *vs* azithromycin	
	Difference in proportion (95% CI)	p	Difference in proportion (95% CI)	p	Difference in proportion (95% CI)	p
Day 0	–1·5% (–9·6 to 6·7)	0·7230	1·9% (–6·3 to 10·2)	0·6439	3·4% (–4·8 to 11·6)	0·4150
Day 4†	53·4% (43·4 to 63·5)	<0·0001	..	..	..	..
Day 8	51·5% (43·3 to 59·7)	<0·0001	50·0% (41·7 to 58·2)	<0·0001	–1·6% (–9·7 to 6·7)	0·7120
Day 14	51·8% (43·6 to 59·9)	<0·0001	38·3% (30·1 to 46·5)	<0·0001	–13·5% (–21·7 to –5·3)	0·0010
Day 28	50·1% (41·9 to 58·3)	<0·0001	32·7% (24·5 to 41·0)	<0·0001	–17·4% (–25·6 to –9·2)	<0·0001
Day 42	35·5% (27·3 to 43·6)	<0·0001	25·7% (17·5 to 33·9)	<0·0001	–9·7% (–17·9 to –1·5)	0·0200
Day 180	13·9% (3·4 to 24·4)	0·0090	19·1% (7·8 to 30·5)	0·0010	5·2% (–5·7 to 16·2)	0·3490

*Placebo groups combined. †Compares azithromycin with placebo-1.

***Table 3*: Difference in mean proportion of macrolide-resistant streptococci between groups**

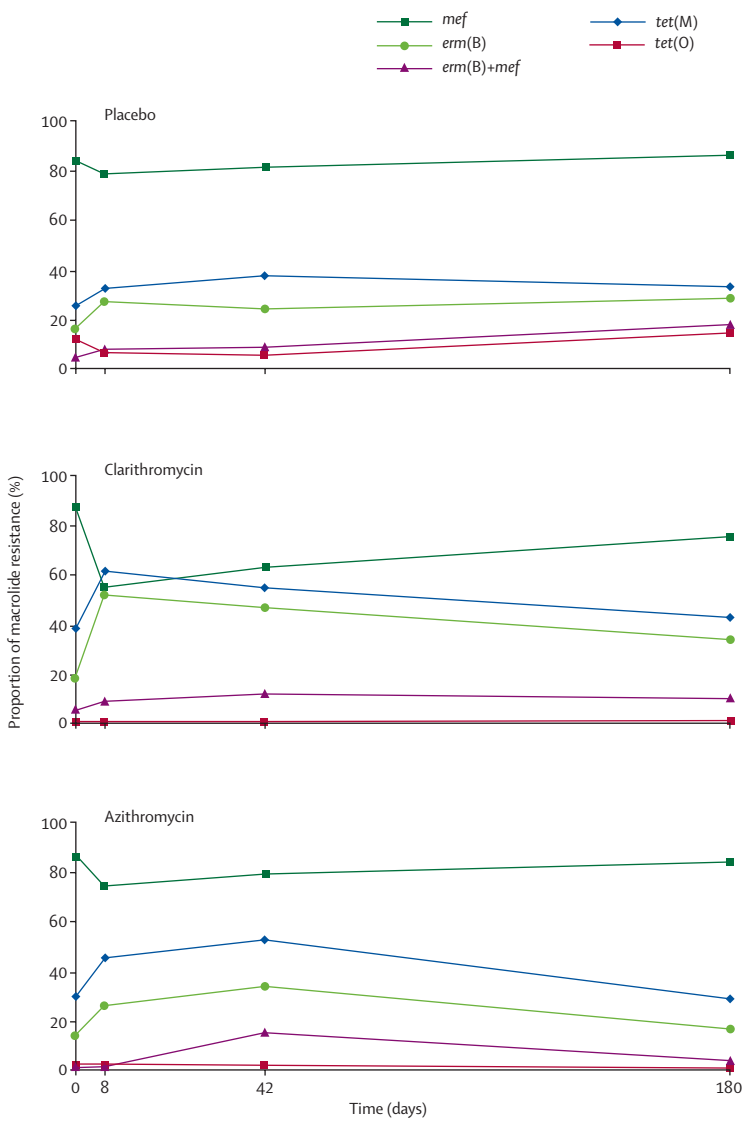

***Figure 3*: Temporal changes in the frequency of genes for macrolide resistance and tetracycline resistance in oral streptococci**
Includes data for 13 volunteers from both the azithromycin and clarithromycin groups and nine volunteers from the pooled placebo group.

resistant streptococci between and within study groups for different sampling time-points was analysed in a general linear mixed model in SAS, with the following covariates: sex, age in years (as decades), smoking status, previous antibiotic use, participation in the winter or summer phase of the study, and employment in hospital with contact with patients.

Variations in the carriage of macrolide-resistance genes within bacteria isolated from volunteers in the azithromycin and clarithromycin groups were normalised for the variations seen in the placebo group. The frequency of resistance genes at the level of the streptococcal colonies at three post-antibiotic sampling time-points (days 8, 42, and 180) was compared with that at the pre-antibiotic time-point (day 0) in a generalised linear mixed model in SAS. Erythromycin MIC values were $\log_e$ transformed and compared within azithromycin and clarithromycin groups with a general linear mixed model in SAS.

The use of a linear mixed model allows combination of regression methods while accounting for the repeated-measures nature of the data. Since model parameters are estimated using maximum likelihood, the resulting inferences and conclusions are valid under a wide variety of missing data mechanisms as well. Moreover, a person-specific random effect was introduced in the models to take into account the intra-class (intra-person) correlation. Analyses were done on an intent-to-treat basis.

This study is registered with ClinicalTrials.gov, number NCT00354952.

Role of the funding source

The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. 224 volunteers were randomly assigned a course of clarithromycin, azithromycin, or placebo. Age, sex, mean proportion of macrolide-resistant streptococci, and other characteristics

of the volunteers were much the same in all three groups at baseline (table 1).

20 volunteers did not continue with the study; of these individuals, reasons for 15 dropouts were not known, two experienced side-effects due to the antibiotics being administered, one was diagnosed with herpes, and two developed an infection for which they had to take another antibiotic course. Although the number of dropouts was small and much the same in the four groups until day 42, 105 volunteers were lost to follow-up on day 180.

Immediately after macrolide use, a large increase in the mean proportion of macrolide-resistant streptococci was noted in both the azithromycin and clarithromycin groups but not in the placebo group, with resistance peaking at day 4 in the azithromycin group and at day 8 in the clarithromycin group (table 2 and figure 2). These increases remained significantly higher in the antibiotic groups than in the placebo group until day 180 (table 2, table 3 and figure 2). Resistance in the placebo group remained stable over the 180 days studied, with variation of about 8% (table 2).

Although there was no difference in the selection of macrolide-resistant streptococci between the two macrolides immediately after therapy, the proportion of macrolide-resistant streptococci was significantly higher in the azithromycin group than in the clarithromycin group at days 14, 28, and 42 (table 3 and figure 2). The largest differences were seen at day 28: the mean proportion of macrolide-resistant streptococci was 17·4% higher in the azithromycin group than in the clarithromycin group (95% CI 9·2–25·6, p<0·0001). This difference decreased by day 42, and was no longer significant by day 180. Multivariate analysis showed that macrolide exposure was the strongest variable independently associated with the proportion of macrolide-resistant streptococci at different time-points (table 2 and table 3). This analysis also showed that age significantly affected the selection of macrolide-resistant streptococci; every extra decade of life was associated with a 3·25% decrease in the mean proportion of macrolide-resistant streptococci (p=0·0114).

Carriage of the macrolide-resistance genes *mef* and *erm*(B) by streptococci was studied in selected volunteers from the three groups at days 0, 8, 42, and 180. The baseline proportion of bacteria carrying the resistance genes was much the same in the three groups; of the 581 bacterial isolates (including those from volunteers in the placebo group) analysed at baseline, 492 (85%) carried the *mef* gene and 102 (18%) the *erm*(B) gene (figure 3 and table 4). Random fluctuation in the frequency of the resistance genes was seen in the placebo group. After correction for these fluctuations, azithromycin use had no significant effect on the frequency of either resistance gene compared with the baseline values (table 4). By contrast, use of clarithomycin was associated with a significantly decreased odds of *mef*-carrying macrolide-resistant streptococci immediately after therapy (odds ratio 0·12, 95% CI 0·04–0·32; p<0·0001 at day 8); this difference persisted through to day 180 (table 4). The decrease in *mef* carriage was paralleled by a significant increase in carriage of the higher resistance-conferring *erm*(B) gene by streptococci: odds of *erm*(B) carriage increased by 4·75 times (95% CI 1·99–11·30; p=0·0004) immediately after clarithromycin use and remained 2·46 times (0·96–6·30; p=0·0588) higher than baseline even at day 180 (table 4). Carriage of the tetracycline resistance gene *tet*(M) followed the same trend as *erm*(B), whereas the proportion of macrolide-resistant streptococci carrying *tet*(O) did not change with the use of either macrolide. Baseline carriage of *tet*(L), *tet*(K), and *erm*(A) was 0–0·94% in the three groups and remained stable throughout the study period (data not shown).

The MIC for erythromycin of macrolide-resistant streptococci that carried *mef* increased by one dilution (two-fold increase) after a course of azithromycin, but not of clarithromycin (webfigure). At day 0, the MIC below which 90% of the isolates tested were inhibited (MIC_{90}) of *mef*-carrying macrolide-resistant streptococci was 8 μg/mL in either group; it increased to 16 μg/mL at day 42 in the azithromycin group. MIC of *mef*-carrying macrolide-resistant streptococci isolated from volunteers given azithromycin were about 1·15 times (95% CI 1·01–1·31) higher at day 42 than those at day 0.

See Online for webfigure

	Proportion	Difference in proportion *vs* placebo (%)	Odds ratio of carriage (95% CI)	p
mef				
Azithromycin group				
Day 0	175/206 (85·0%)	0·04%	..	..
Day 8	163/217 (75·1%)	–4·8%	0·93 (0·39–2·23)	0·8765
Day 42	181/227 (79·7%)	–2·3%	1·40 (0·57–3·43)	0·4611
Day 180	170/203 (83·7%)	–3·9%	0·50 (0·18–1·36)	0·1774
Clarithromycin group				
Day 0	182/212 (85·9%)	0·94%	..	..
Day 8	103/181 (56·9%)	–23·%	0·12 (0·04–0·32)	<0·0001
Day 42	110/171 (64·3%)	–17·7%	0·23 (0·09–0·60)	0·0026
Day 180	88/116 (75·9%)	–11·7%	0·10 (0·03–0·32)	<0·0001
***erm*(B)**				
Azithromycin group				
Day 0	32/206 (15·5%)	–2·1%	..	..
Day 8	57/217 (26·3%)	–1·8%	0·75 (0·32–1·74)	0·5062
Day 42	77/227 (33·9%)	9·3%	1·48 (0·63–3·44)	0·3592
Day 180	33/203 (16·3%)	–12·7%	0·63 (0·25–1·57)	0·3274
Clarithromycin group				
Day 0	42/212 (19·8%)	2·2%	..	..
Day 8	96/181 (53·0%)	25·0%	4·75 (1·99–11·30)	0·0004
Day 42	81/171 (47·4%)	22·7%	3·60 (1·51–8·54)	0·0037
Day 180	41/116 (35·3%)	6·4%	2·46 (0·96–6·30)	0·0588

Within group comparisons were made between gene carriage before and after macrolide use, taking into account the variations in prevalence of macrolide-resistance genes in the placebo group. Data are n/N (%) or odds ratio (95% CI), unless specified otherwise.

Table 4: **Carriage of the macrolide-resistance genes, *mef* and *erm*(B), in streptococci isolated from volunteers treated with clarithromycin or azithromycin**

Discussion

By use of oral streptococci as model organisms, we have shown that macrolide use is the single most important driver of the emergence of macrolide resistance in human beings. Our results also show important differences in the outcome of selection of resistance by two antibiotics in the same class: azithromycin selected quantitatively more resistant organisms in the early post-therapy phases, whereas clarithromycin qualitatively selected for the higher resistance-conferring *erm*(B) gene. Finally, the effect of a single course of antibiotics on the oral commensal flora lasted for more than 180 days, which emphasises that the commensal flora could serve as a reservoir of resistance for potentially pathogenic bacteria.

Both azithromycin and clarithromycin achieve high extracellular concentrations in respiratory tissue (ie, nasal mucosa and tonsil) and are commonly used as first-line empirical treatment for community-acquired respiratory tract infections. However, they differ substantially in their plasma half-life and tissue persistence, factors that are thought to be very important in the selection of macrolide-resistant organisms. For instance, the plasma half-life of azithromycin is 68 h and because clearance of a drug or a decrease in concentration to below the MIC takes between five and seven half-lives, azithromycin might persist in vivo for at least 3–4 weeks after treatment.[25] By contrast, clarithromycin has a half-life of only 5–7 h and therefore exerts little post-treatment effect.[26] This difference explains why azithromycin use selected for significantly more macrolide-resistant streptococci until about 4 weeks (ie, for as long as the drug persists in tissue after the end of therapy) than did clarithromycin. The prolonged selection with azithromycin heightens the threat of increased dissemination of resistant organisms into the community.

We also studied the underlying genetic mechanism for differences in the effect of selection by azithromycin and clarithromycin on oral streptococci. Both macrolides eradicated the susceptible flora to comparable extents immediately after therapy. However, clarithromycin, but not azithromycin, also perturbed the distribution of macrolide-resistance genes in oral streptococci by decreasing the frequency of *mef* carriage and increasing that of *erm*(B), effects that persisted at least until 180 days after the start of therapy. These selection trends are explained by clarithromycin's greater efficacy against *mef*-carrying streptococci; clarithromycin in the recommended doses can eradicate *mef*-carrying *S pneumoniae* with MIC up to 8 μg/mL,[27] whereas azithromycin is far less potent and produces only a bacteriostatic effect against *mef* isolates with MIC up to 2 μg/mL.[28]

In-vitro pharmacodynamic studies also show that both macrolides fail to eradicate *erm*(B) strains, which generally have MIC of 32 μg/mL or more.[27,28] Thus, whereas azithromycin might also eradicate *mef*-carrying macrolide-resistant streptococci, the higher efficacy of clarithromycin against *mef* isolates translates into a steeper decrease in *mef*-carrying macrolide-resistant streptococci, which in turn allows an expansion of *erm*(B) isolates that are able to persist in higher numbers for at least 180 days. These data corroborate an earlier study that showed an increase in *erm*(B)-carrying isolates for 8 weeks after prophylaxis with a slow-release clarithromycin preparation in preoperative patients with coronary artery disease.[6]

The increased risk of *erm*(B) carriage in volunteers treated with clarithromycin for up to 180 days is important for several reasons. First, knowledge of macrolide use in the preceding 6 months can decrease macrolide treatment failures. Second, *erm*(B) methylase affords protection not only against macrolides but also against lincosamides and streptogramins B that share overlapping drug-binding sites on the bacterial ribosome. Furthermore, *erm*(B) and the tetracycline resistance determinant *tet*(M) are both present on the same mobile genetic element.[29] Thus, *erm*(B) acquisition after clarithromycin therapy might restrict the use of not only all macrolides, but also of the lincosamides, streptogramins B, and tetracyclines. Finally, from an evolutionary point of view, the persistence of *erm*(B)-carrying macrolide-resistant streptococci in high numbers for a long time after elimination of the drug from the system might be indicative of a low biological cost of *erm*(B) carriage.

Our results parallel the substantial differences in the prevalence of macrolide resistance mechanisms in *S pneumoniae* seen between Europe and the USA, the reasons for which have remained a matter of debate. The increased frequency of *erm*(B) after clarithromycin use supports the predominance of the *erm*(B)-mediated phenotype in macrolide-resistant *S pneumoniae* in most European countries, which also show a higher consumption of clarithromycin than of azithromycin.[2,30,31,32] By contrast, *mef* predominates in countries, such as the USA, where azithromycin use is higher.[32–34]

Our results also corroborate earlier data that indicate a shift towards higher erythromycin MIC in *mef* isolates paralleled by an increase in azithromycin use.[33] Thus, the emergence of *mef*-carrying streptococcal clones with higher MIC over the long term, related to azithromycin use, might be the cause of the observed community-wide increase in *mef* resistance levels that further heightens the risk of treatment failures during empirical macrolide therapy.

Finally, despite a large increase in the proportion of macrolide-resistant streptococci after macrolide use, at no point during the trial did the proportion of resistant bacteria reach 100%. In fact, even in the immediate post-therapy period (within 48 h of the end of therapy), about 18% of the streptococcal flora were susceptible to macrolides (figure 2). This observation might be due to phenotypic tolerance—an ingenious stress-survival adaptation, wherein a fraction of an antibiotic-susceptible bacterial population is able to survive antibiotic treatment.[35] These so-called persister cells exist in a state of dormancy, with metabolic activity reduced to a minimum and major drug targets (eg, protein synthesis)

shut down. As a consequence, these cells escape antibiotic action.[36,37] Such cells represent a third physiological state of bacteria, distinct from both the known exponential and stationary forms, and are indigenous to any normally distributed bacterial population.[36] Although the mechanisms that underlie phenotypic tolerance have only recently come to light, the process itself is well documented in relation to longstanding infections such as tuberculosis, in which antibiotic-susceptible bacteria persist in the form of a long-term asymptomatic infection (ie, latent tuberculosis) despite antibiotic treatment.[38,39] Commensal flora, like asymptomatic infections, is also longstanding and some dormancy would also be expected, although there might be other factors that facilitate the survival of macrolide-susceptible streptococci in vivo: for example, too high a streptococcal load in the pharynx to be cleared by antibiotics in therapeutic doses; secretion of chemicals or pheromones by resistant bacteria that inhibit antibiotic action; or the formation of biofilms that afford protection to sensitive streptococci.

Strengths of the study were that both volunteers and researchers were completely masked by means of the placebo imitating the two macrolide regimens that differ widely in dosage; furthermore, the rate of dropouts and loss to follow-up was small up to day 42 and, although it became substantial at day 180 (50% or more, probably because volunteers were fully remunerated at day 42), it did not affect our conclusions (data not shown). A longer study period would have enabled us to define the time needed for the resistant oral flora to revert to baseline levels. Nevertheless, our successful use of the oral streptococcal flora as model organisms paves the way for similar studies with other antibiotic classes. Such studies, carried out in different countries, would allow comparisons of baseline resistance as well as clarify the underlying reasons for the differences in resistance levels observed between different countries.

In conclusion, we have clearly defined, at the individual level, the direct effect of antibiotic use in selecting resistant organisms. Antibiotic use is an important driver of the emergence of antibiotic resistance in vivo. In view of the consequences of antibiotic use seen here, physicians should take into account the striking ecological side-effects of antibiotics when prescribing such drugs to their patients.

Contributors

S Malhotra-Kumar and H Goossens designed the study, analysed the data, and wrote the final draft of the manuscript. S Malhotra-Kumar and C Lammens did the sampling and experimental work. S Coenen and K Van Herck did the statistical analysis. All authors saw and approved the final version of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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