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INVESTIGATIVE REPORT

Bacterial Resistance and Therapeutic Outcome Following Three Months of Topical Acne Therapy with 2% Erythromycin Gel Versus Its Vehicle

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Two-hundred-and-eight acne vulgaris patients were enrolled in a 24-week study to determine the bacterial resistance issues associated with the use of a topical 2% erythromycin gel. It consisted of a 12-week randomized, double-blind, parallel-group treatment phase comparing the active gel versus its vehicle followed by a 12-week single-blind regression phase with gel vehicle only. Bacteriological samples were taken from the face, back and nares for quantification by species and antibiotic resistance characteristics. Acne efficacy was assessed through week 12. The prevalence of erythromycin-resistant coagulase-negative *Staphylococci* on the face was extremely high (87%) at baseline, increased to 98% by week 12 in the erythromycin-treated group and did not change during regression. The density of these resistant organisms also significantly increased with erythromycin treatment with no change during regression. Similar prevalence and density patterns were also observed on the untreated back and in the nares. Nearly all of the resistant isolates were highly resistant (minimal inhibitory concentrations $>128\ \mu g/ml$). Resistance development was confined to the macrolide class of antibiotics. No anti-acne efficacy was observed. *Key words: controlled clinical trial; dermatologic agents; drug resistance, microbial; macrolide; staphylococcus*.

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The indiscriminate use of antibiotics, whether topically or orally, has raised concerns globally about the development and spread of resistant organisms and fears about resulting failures to antibiotic therapy (1, 2). That bacterial resistance mechanisms are commonly effective against whole classes of antibiotics, and not just against the single antibiotic an organism was exposed to (3), has heightened concerns. The known propensity for resistance mechanisms to be transferable across large differences in microbial relatedness, i.e. across much larger genetic differences than separate species (4), has added to the concern. Prevalence studies on antibiotic resistance suggest that indiscriminate use of antibiotics leads to high levels of resistance in the community (5), and just such a situation typifies topical erythromycin use for acne in the United States.

Topical erythromycin has been a well-accepted and widely used therapy for the treatment of acne vulgaris (6, 7). This macrolide antibiotic reduces the population of *Propionibacterium acnes* (8), which are suspected to play a key role in inflamed lesion pathogenesis. Additionally, erythromycin has anti-inflammatory properties (9), suppresses the chemotaxis of inflammatory cells (10) and decreases the percentage of pro-inflammatory free fatty acids in sebum (11) by decreasing triglyceride hydrolysis through the suppression of *P. acnes* metabolism and/or extracellular lipase production (12). Development of resistant *P. acnes* flora has been shown to lead to therapeutic failure (13).

This study was conducted to determine if the topical use of erythromycin for the treatment of acne would result in the increased development, expansion and dissemination of antibiotic-resistant bacteria.

MATERIALS AND METHODS

Patients and study design

After giving written informed consent, 208 male and female patients with mild to moderate acne vulgaris were enrolled in February, 1994, into a 24-week, parallel-group comparison study at a single center in the New York City metropolitan area. The initial 12 weeks consisted of a randomized, double-blind portion comparing twice daily topical 2% erythromycin gel to its placebo. In order to study regression of any bacteriologic changes, at 12 weeks those patients on active treatment were switched over to placebo product, while those initially randomized to placebo continued placebo treatment for another 12 weeks. To ensure balance between the two treatment groups, patients were stratified by gender and inflamed lesion count (15–20, or >20) prior to randomization to treatment. All patients were given the same mild bar soap (Johnson's Baby Bar®) to standardize their facial cleansing product. Patients should not have taken or applied any antibiotics within 3 months prior to participating in the study.

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Assessment of acne

Acne severity was assessed at baseline and after 4, 8 and 12 weeks of therapy. Each type of acne lesion (open comedo, closed comedo, papule, pustule and nodule) was counted separately over the entire face. Open and closed comedone counts were summed to provide the non-inflamed lesion count. Since there were very few nodules in this patient population, papule and pustule counts were summed to give the inflamed lesion count. The sum of the inflamed and non-inflamed lesion counts was the total lesion count for each patient at each visit. A photograph of each patient's face was taken at the baseline visit and global change in the patient's acne graded by comparison to the baseline photograph. The following seven-point asymmetrical scale was used: total clearing (1), marked improvement (2), moderate improvement (3), mild improvement (4), slight improvement (5), no change (6) or exacerbation (7).

Bacteriologic sampling

Bacteriologic samples were taken from the center of the forehead, right scapula and anterior nares at baseline and at weeks 4, 12, 16 and 24, approximately 12 h after treatment. For the skin sites, a 5 cm^2 circular area was repeatedly swabbed with first one and then a second cotton swab containing Letheen broth, both of which were placed into a single tube containing 2 ml of sterile broth. The anterior nares were sampled using 4 swabs, two consecutively within each nare, each rotated 10 times. The total number of aerobic bacteria, coagulase-negative *Staphylococci*, *S. aureus*, gram-negative organisms, diphtheroids and *Propioibacterium* species were quantified using serial dilutions and selective media. All plating was done in triplicate. Initial erythromycin resistance was determined by aerobic plating on Mueller-Hinton II agar containing 8.0 μg/ml of erythromycin. Isolates for further work-up of erythromycin resistance were chosen on the basis of colony morphology. Every morphologic variant was tested. Aerobic plates were incubated at 37°C for 72 h and then stored at room temperature for 4 days to enhance pigment production and colony morphology. Colonies appearing to be staphylococci were coagulase-tested to confirm identification of *S. aureus*. Anaerobic plates were incubated at 36°C for 7 days. Initial erythromycin resistance was determined by anaerobic culture on Wilkins Chalgren agar containing erythromycin at 0.5 μg/ml. The minimal inhibitory concentrations to erythromycin were determined using erythromycin incorporation plates for the erythromycin-resistant *Staphylococcus* and *Propionibacterium* species isolated. Cross-resistance of the erythromycin-resistant staphylococci to 15 other antibiotics (penicillin, oxacillin, cephalothin, ampicillin, vancomycin, clindamycin, trimethoprim-sulfamethoxazole, neomycin, gentamicin, ciprofloxacin, chloramphenicol, tetracycline, minocycline, doxycycline and nitrofurantoin) was determined using the Bauer Kirby disk diffusion technique, with the antibiotic concentrations recommended by the National Committee of Laboratory Standards (NCCLS) document M2-A4, volume 10, no. 7.

An additional set of samples was taken from the left side of the forehead and both nares at the end of active treatment (week 12). These samples were used to determine the minimal inhibitory concentration (MIC) of the erythromycin-sensitive *Staphylococcus* species, and all streptococcal and gram-negative species to erythromycin, clarithromycin and azithromycin, as well as their susceptibility to the same 15 other antibiotics, except that ceftriaxone was used instead of clindamycin, using the microtiter dilution procedure and MIC breakpoints of the NCCLS document M7-A3. To isolate the erythromycin-sensitive strains, replica plating was used in conjunction with selective media and erythromycin incorporation plates at 8 μg/ml. Isolated bacteria were speciated based on standard morphological and physiological characteristics.

Treatment products

The erythromycin gel consisted of 2% erythromycin base dissolved in denatured ethanol (SDA 40B) that was gelled with 3% hydroxypropyl cellulose. The placebo gel was identical except for the erythromycin. Stability studies confirmed the potency of the active formulation throughout the study and analyses of product returned by the patients confirmed that all products had been correctly distributed and labeled according to the randomized and blinded treatment code.

Statistics

The baseline acne lesion counts and their various sums were subjected to an analysis of variance with factors for patient and treatment as the test for homogeneity. At weeks 4, 8 and 12, an analysis of covariance was performed using the baseline lesion count as the covariant for factors of patient and treatment. Global acne change grades were similarly analyzed using the rank transformed data. Therapeutic outcome was determined as a binomial variable of responder or non-responder at week 12. A treatment failure was defined based on a global acne change score of no change or exacerbation, whereas all other scores were classed as responders. Only two-sided *p*-values of 0.05 or less were considered significant.

For each anatomical site, the log of the bacterial counts (expressed as colony forming units/cm^2 or ml) and the various sums of counts were subjected to a one-way analysis of variance at baseline to check for homogeneity. A similar analysis of variance was performed at each subsequent visit. Frequency counts of organisms isolated (prevalence) were subjected to a chi-square test of association or in cases where numbers in cells were sparse, exact *p*-values were computed using Fisher's exact test.

RESULTS

In general, the bacteriologic results are reported in terms of the proportion of patients in each treatment group having a particular finding (prevalence) and the average number of recovered organisms per square centimeter (density) at each skin site sampled.

For the predominant flora of the general body surface, coagulase-negative staphylococci, it was found that 87% of the patients who entered the study at baseline were carrying erythromycin-resistant (MIC > 8 μg/ml) such bacteria on their faces. By the end of the study, the prevalence had increased to 98% in the erythromycin-treated group and decreased only slightly during the 12-week regression period (Fig. 1). Similarly, the average density of erythromycin-resistant coagulase-negative staphylococci increased approximately 0.6 log units relative to the vehicle-treated group and did not regress when the active product was removed during the regression phase. The expansion of erythromycin-resistant organisms was confirmed by the increased ratios of erythromycin-resistant to total recovered coagulase-negative staphylococci (Table I). Nearly all of the

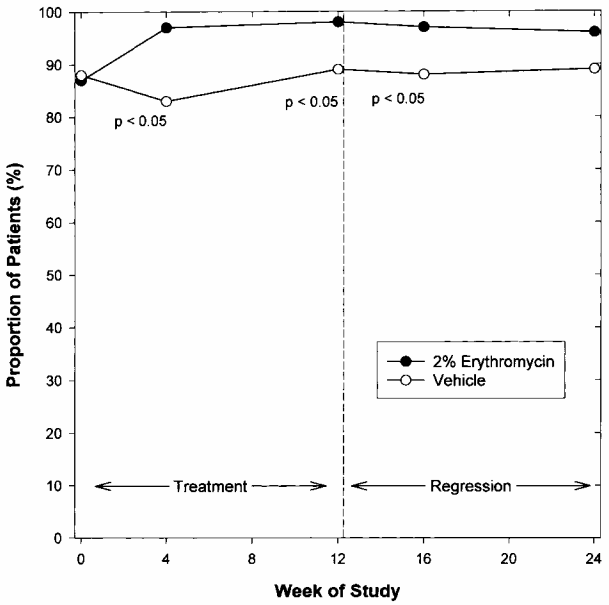

Fig. 1. Prevalence of erythromycin-resistant coagulase-negative staphylococci on the face.

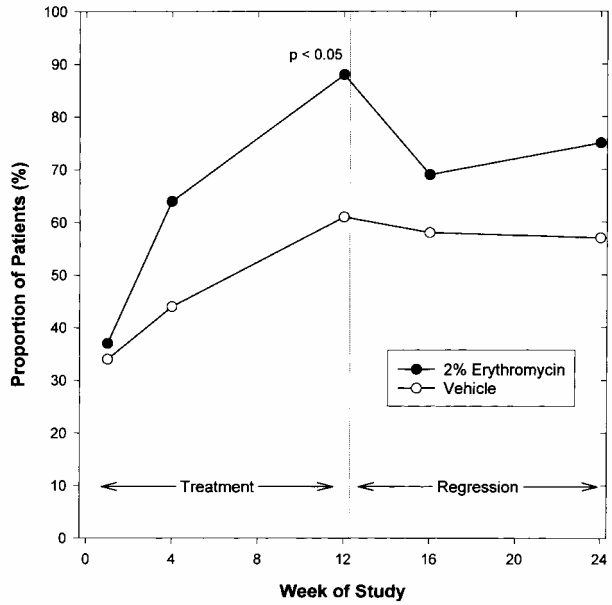

Fig. 2. Prevalence of erythromycin-resistant coagulase-negative staphylococci on the back.

Table I. *Proportion (%) of erythromycin-resistant (Er) coagulase-negative staphylococci to total recovered (T) coagulase-negative staphylococci on the face*

	Placebo (log Er/log T)		2% Erythromycin (log Er/log T)	
Baseline	15.5	(2.20/3.01)	12.6	(2.26/3.16)
Week 4	18.2	(2.64/3.38)	72.4	(3.03/3.17)
Week 12	12.9	(2.50/3.39)	79.4	(3.11/3.21)
Week 16	26.3	(2.67/3.25)	75.9	(3.23/3.35)
Week 24	13.8	(2.53/3.39)	55.0	(3.03/3.29)

Table II. *Proportion (%) of erythromycin-resistant (Er) coagulase-negative staphylococci to total recovered (T) coagulase-negative staphylococci on the back*

	Placebo (log Er/log T)		2% Erythromycin (log Er/log T)	
Baseline	26.3	(0.78/1.36)	93.3	(1.23/1.26)
Week 4	26.9	(0.90/1.47)	50.1	(1.08/1.38)
Week 12	18.6	(0.74/1.47)	75.9	(1.30/1.42)
Week 16	46.8	(1.42/1.75)	69.2	(1.45/1.61)
Week 24	17.4	(0.94/1.70)	60.3	(1.50/1.72)

resistant phenotypes were highly resistant to erythromycin with MICs > 128 μg/ml.

The situation on the untreated back was different. Here the baseline prevalence of erythromycin-resistant coagulase-negative staphylococci was 37%. This increased to 88% by the end of treatment with erythromycin and did not change appreciably during the regression period (Fig. 2). The density of erythromycin-resistant coagulase-negative staphylococci similarly increased with erythromycin treatment and did not change during the regression phase. The ratio of resistant organisms to total coagulase-negative staphylococci counts and levels of erythromycin resistance were similar in pattern to the face but lower overall (Table II).

The patients entering the study had a very high carriage rate of erythromycin-resistant coagulase-negative staphylococci (89%) in their anterior nares, which increased slightly to 93% during topical erythromycin therapy and failed to change during the regression phase. This increased prevalence over placebo treatment was significant at each post-treatment visit except for week 12 (Fig. 3). The density, in this case reported as

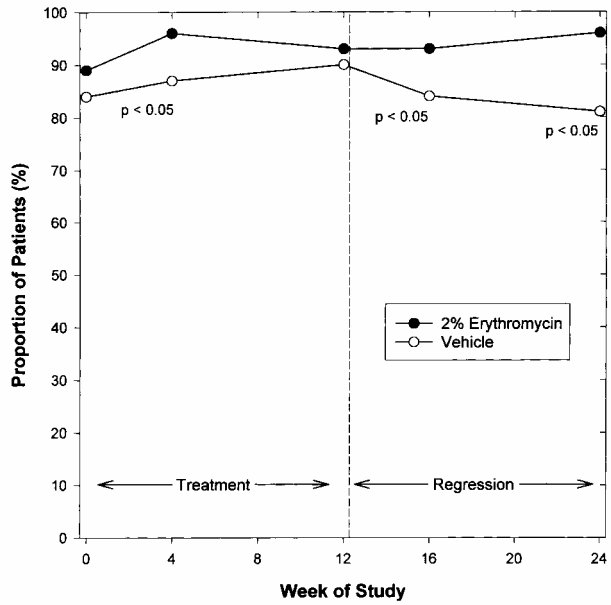

Fig. 3. Prevalence of erythromycin-resistant coagulase-negative staphylococci in the nares.

organisms/ml in the recovery fluid, of erythromycin-resistant coagulase-negative staphylococci increased by 0.77 log units, with a trend toward some reduction during the regression phase, which was still, however, significantly higher than in the placebo group. The ratio of resistant organisms to total coagulase-negative staphylococci counts and levels of erythromycin resistance were similar to the face (Table III).

At baseline the majority of the erythromycin-resistant coagulase-negative staphylococci from the face, back and nares also expressed resistance to penicillin (65.7%), ampicillin (65.7%) and clindamycin (60.1%), and 28.1% were resistant to tetracycline. These proportions did not change over the course of the study nor were there any significant differences between the treatment groups in this regard.

Erythromycin-resistant *S. aureus* carriage rates in the anterior nares increased from 15% to 40% during the course of therapy in the erythromycin-treated group, while the prevalence remained stable at about 20% in the placebo-treated group ($p = 0.09$ at week 12). This trend between treatment groups persisted through the first month of the regression phase ($p = 0.11$) and then disappeared. Additionally, the proportion of *S. aureus* isolates that were erythromycin-resistant at the end of therapy was significantly ($p < 0.01$) and substantially higher in the erythromycin-treated group (63%) versus the vehicle-treated group (37%).

At week 12, the erythromycin-resistant staphylococci recovered showed no differences in cross-resistance to any of the other antibiotics tested, including those of the macrolide class between the treatment groups.

Half of the patients entered the study with erythromycin-resistant *P. acnes* (MIC > 0.5 μg/ml) on their faces and the prevalence of this resistance did not increase appreciably over the course of treatment (Fig. 4a). During the regression period, however, significantly more of the patients who had been on active treatment continued to have erythromycin-resistant *P. acnes* than those who had been treated the entire time with placebo product. When adjusted for baseline differences, there were no significant differences between the treatment groups at any time in the density of erythromycin-resistant *P. acnes* recovered from the face (Fig. 4b).

Table III. *Proportion (%) of erythromycin-resistant (Er) coagulase-negative staphylococci to total recovered (T) coagulase-negative staphylococci in the nares*

	Placebo (log Er/log T)		2% Erythromycin (log Er/log T)	
Baseline	12.9	(3.01/3.90)	24.5	(3.09/3.70)
Week 4	8.9	(2.81/3.86)	64.6	(3.52/3.71)
Week 12	9.5	(2.63/3.65)	72.4	(3.48/3.62)
Week 16	13.2	(2.64/3.52)	85.1	(3.48/3.55)
Week 24	11.2	(2.77/3.72)	36.3	(3.27/3.71)

Erythromycin treatment was associated with a significant 0.5 log reduction in total *P. acnes* counts from the face that tended to persist during regression. The erythromycin-resistant *P. acnes* recovered showed almost complete cross-resistance to azithromycin and partial cross-resistance to clindamycin; however, no significant cross-resistance was seen to the other antibiotics tested against.

No significant differences were found between the treatment groups in the evaluations of acne at any time during the study, either in terms of acne lesion counts (Fig. 5) or global scores, which averaged 5.46, 5.39 and 5.56 in the erythromycin group versus 5.57, 5.40 and 5.64 in the vehicle group at weeks 4, 8 and 12, respectively. The study did provide evidence suggesting that this could have been a function of the high level of initial *P. acnes* resistance found in the study population, supporting prior findings using systemic erythromycin for treating acne (13, 14). If one considers only the 60 patients who had erythromycin-sensitive strains of *P. acnes* throughout the treatment phase of the study, there was a trend ($p = 0.066$) toward clinical improvement after 12 weeks of therapy in the global acne grades in the erythromycin-treated patients versus placebo. Fifty-two percent of the patients using the erythromycin gel were classed as responders (grades 3–5) compared to 29% of those using the placebo gel. Additionally, 36 patients in the erythromycin-treated group had resistance develop to the extent that more than 10% of the recovered *P. acnes* were classed as resistant. Of these 36 patients, 27 failed to respond to treatment.

DISCUSSION

The 2% erythromycin gel product used in this study had never previously been tested for anti-acne efficacy. It is, however, nearly identical in composition to another 2% erythromycin gel product that has been reported to be effective (15), albeit at a time when erythromycin resistance was not yet a problem. The particular formulation tested in this study contained 95% ethanol and was gelled with 3% hydroxypropyl cellulose, whereas the product with proven efficacy contains 92% ethanol and is also gelled with hydroxypropyl cellulose. It is difficult to explain any differences in efficacy based on this formulation difference.

The *S. aureus* results from the nares suggest that topical treatment with erythromycin may result in higher carriage rates and dissemination of erythromycin-resistant *S. aureus* from the nares and would seem to be a topic deserving additional study. The dramatic results obtained on the untreated back would seem to have clinical relevance as well. In the erythromycin-treated patients the prevalence of erythromycin-resistant coagulase-negative staphylococci increased from 37% to 88% over the course of treatment and failed to change during the regression phase. Additionally, the density of

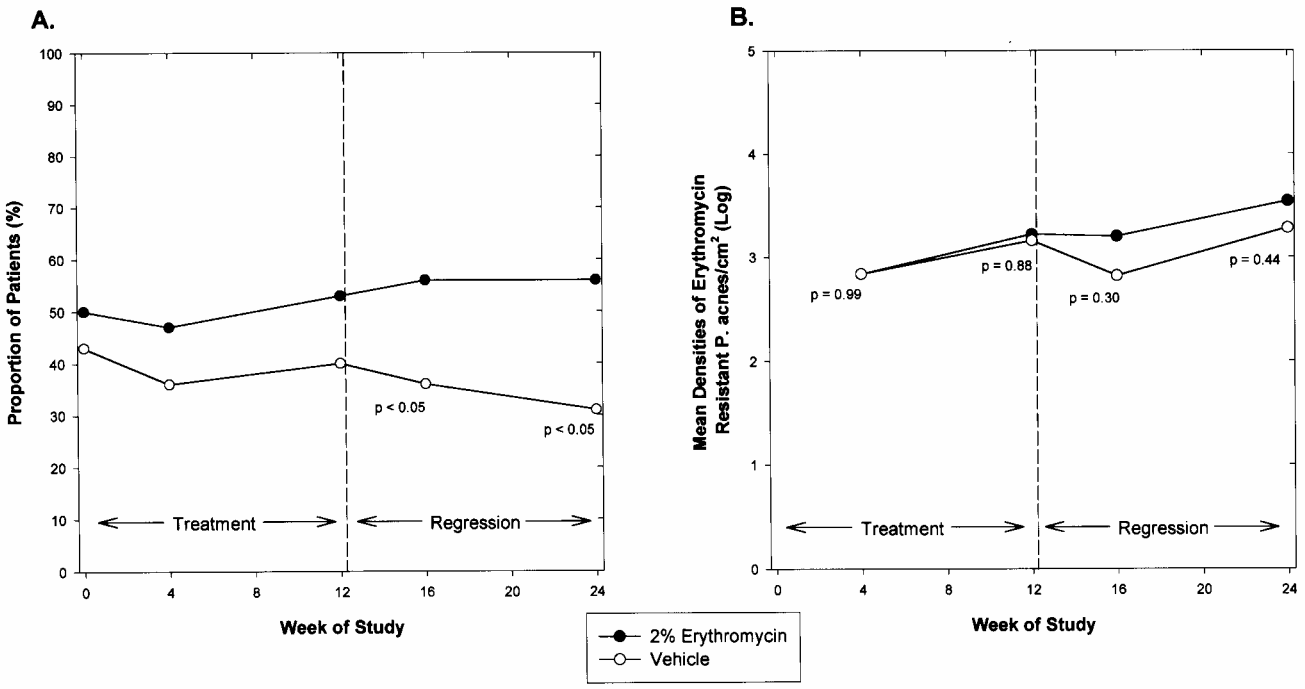

Fig. 4. Prevalence of erythromycin-resistant *P. acnes* (A) and mean density of erythromycin-resistant *P. acnes* (B) on the face after adjustment for baseline differences.

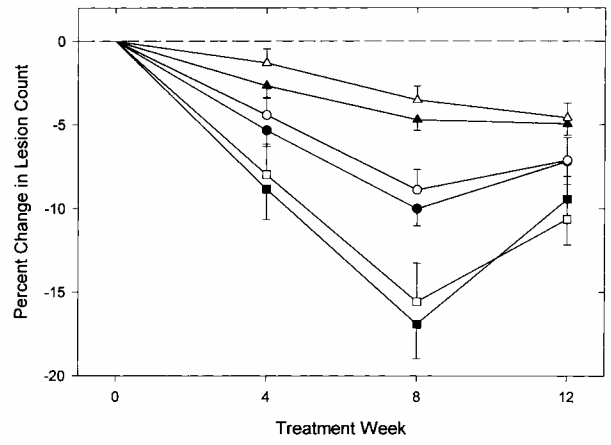

Fig. 5. Percentage changes in the numbers of acne lesions. Solid symbols represent 2% erythromycin while open symbols represent vehicle. Circles: total lesions, squares: inflamed lesions, triangles: non-inflamed lesions. There were no statistical differences between the study groups.

erythromycin-resistant organisms increased by approximately 0.75 log units and also failed to regress. This study cannot distinguish whether these results were due to the transfer of erythromycin to the untreated back, or to the transfer and persistence of resistant organisms. In either case, however, the results suggest substantial dissemination of resistance to an area not even directly treated with the topical antibiotic. These results raise the possibility of resistance spread to close contacts, which is indirectly supported by the prior work of Miller et al. (16).

One of the key conclusions from this study is that antibiotic resistance can persist for significant periods of time after exposure to the antibiotic has ceased. This finding puts into question several popular theories about the metabolic cost and survival disadvantages of carrying the resistant phenotype when antibiotic pressure does not exist (17). Others, however, have shown in *in vitro* model systems that there may often be no survival disadvantage in carrying the resistant phenotype constitutively (18). In either case, the persistence and transfer of resistance shown would argue that continued use of topical 2% erythromycin as monotherapy for the treatment of acne vulgaris is ill-advised from a public health standpoint, unless action is taken to prevent resistance development and transfer. Concomitant use of topical benzoyl peroxide, for example, has been shown to mitigate the bacterial resistance issues associated with topical erythromycin use (19).

Prior work using the same methods, except for a 6-week instead of a 12-week treatment period, gave similar results at baseline and during treatment (20); however, after treatment stopped, they differ. The prior study found erythromycin-resistant coagulase-negative staphylococci declined to baseline values within 6 weeks after cessation of 6 weeks of therapy. The present study found resistance levels to persist essentially unchanged for 12 weeks following cessation of 12 weeks of therapy. One possible explanation is that more than 6 weeks of therapy may be required to make the resistant phenotype more or less permanent.

One finding of concern to us was the often seen

increase in the prevalence and density of erythromycin-resistant organisms in the placebo-treated group over the course of the study. We have no ready explanation for this, although it was observed to some extent in the prior study (20). We retrieved the treatment products from all patients and had them analyzed for erythromycin and know that these results were not due to any mistakes in labeling the products or in their distribution to the patients. Perhaps study site personnel acted as resistance transfer vectors, as the work of Miller et al. (16) might suggest is possible, or there was some sort of seasonal effect or a general increase in erythromycin-resistance levels due to factors operating outside of the study. Since this study was conducted in 1994, it may not reflect the current situation in terms of erythromycin resistance in acne patients.

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