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# Effects of Topical Erythromycin on Ecology of Aerobic Cutaneous Bacterial Flora

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**We have demonstrated previously that application of topical erythromycin, an antibiotic commonly used for the treatment of acne, results in an increased density of cutaneous erythromycin-resistant ($Em^r$) coagulase-negative staphylococci; however, it is unknown if this increase results in an overall higher density of total cutaneous staphylococci or if upon cessation of erythromycin use, $Em^r$ coagulase-negative staphylococci remain at an increased density compared with the pretreatment density. To investigate this, 2% erythromycin or vehicle was applied to each subject's forehead ($n = 225$) twice a day by laboratory personnel for a period of 6 weeks. Samples were obtained for culture from the forehead, anterior nares, and back of the subjects at baseline and at weeks 6, 9, and 12 of the study. Cultures were performed on differential media. Plates into which erythromycin was incorporated (8 μg/ml) were used to identify $Em^r$ coagulase-negative staphylococci. The species of all $Em^r$ coagulase-negative staphylococci were determined, and an antibiogram for 16 antibiotics was obtained. The baseline prevalence of $Em^r$ coagulase-negative staphylococci on the forehead and nose was about 80% at the two study sites, whereas that on the back was 50%. The baseline density of $Em^r$ coagulase-negative staphylococci on the forehead, nose, and back was approximately 20% of the total flora. Following 6 weeks of erythromycin treatment, the prevalence of $Em^r$ coagulase-negative staphylococci on the forehead and nose was nearly 100% and the densities were 73 and 62%, respectively; the prevalence and density for the back were 78 and 42%, respectively. The most prevalent erythromycin resistance gene expressed by the $Em^r$ coagulase-negative staphylococci was *ermC*. There was no increase in the numbers of *Staphylococcus aureus*, gram-negative rods, or yeasts, nor was there increased resistance to any other antibiotic except clindamycin. The density of total aerobic organisms also remained static. There were no changes in the prevalence or density of $Em^r$ coagulase-negative staphylococci in the vehicle group. A statistically significant decrease in the prevalence and density of $Em^r$ coagulase-negative staphylococci in the erythromycin group was observed within 3 weeks posttreatment and by 6 weeks posttreatment, the prevalence and density returned to baseline values. These data demonstrate that the increased prevalence and density of $Em^r$ coagulase-negative staphylococci as a result of topical 2% erythromycin use are transient on both population and individual levels.**

Antibiotics have successfully been used for several decades to suppress infectious organisms. However, the widespread use of antibiotics has raised fears that increased numbers of bacteria will become resistant, thereby diminishing the overall ability of antibiotics to control infection. These concerns are not without basis, since antibiotics such as penicillin are largely ineffective against a wide range of previously susceptible organisms (15, 16). Compounding this problem is the possibility of transfer of the resistance gene, via plasmids or transposons, to different strains, species, or even genera of susceptible bacteria which occupy the same environmental niche (14, 19).

While many investigations have indicated that resistance to a particular antibiotic increases during the time that the antibiotic is being used (9, 13), few studies have examined if the increased numbers of antibiotic-resistant bacteria persist subsequent to the cessation of antibiotic use. If use of an antibiotic results not only in an increased density of resistant bacteria but also in the persistence of the increased numbers of resistant bacteria, the possibility of translocation of the resistant bacteria to other body sites on the same individual or even to other individuals increases. Moreover, any subsequent use of the same antibiotic in the patient will likely result in decreased efficacy.

Topical erythromycin (2%) is widely prescribed, and its prolonged use is normative in the treatment of acne vulgaris. We have demonstrated previously in a small group of patients ($n = 20$) that application of topical erythromycin does result in an increased density of cutaneous erythromycin-resistant ($Em^r$) coagulase-negative staphylococci (6). In the present, larger study ($n = 225$), we sought to determine if the $Em^r$ staphylococci that emerge subsequent to application of topical erythromycin are localized only at the site of application, if upon cessation of erythromycin use, $Em^r$ staphylococci remain at an increased density compared with the baseline density, and if multidrug resistance develops as a result of prolonged erythromycin treatment.

## MATERIALS AND METHODS

**Subjects.** Healthy male and nonpregnant female subjects between the ages of 18 and 45 years who met the study criteria were randomized into treatment and placebo groups. Criteria included no history of oral or topical erythromycin intolerance, no history of recurrent infections, and a willingness to participate in the study for 13 weeks. Our intent was to recruit individuals who had not been receiving either chronic or multiple courses of antibiotics. Written, informed consent was obtained from each subject. Subjects were to discontinue the use of

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all antimicrobial soaps and medicated shampoos beginning 2 weeks prior to commencing the study.

**Study design.** The study was designed as a blinded, multidose, within-patient clinical comparison with an attendant placebo reference group and was conducted at two study sites (Philadelphia and Boston). Subjects were treated twice daily with topical 2% erythromycin (EryGel) or placebo for a period of 6 weeks. Subjects were required to report to the clinic twice daily, in the early morning and late afternoon (5 days per week), for the duration of the 6-week study. At each visit, 0.2 g of the assigned test product was applied by a technician to the forehead between the eyebrows and hairline and from eye corner to eye corner by using a glass policeman. On weekends, subjects were provided with test material and specific instructions for home application.

Microbiological samples were collected from the forehead, scapular back, and anterior nares at the baseline, after 6 weeks of therapy, 3 weeks posttherapy, and 6 weeks posttherapy. During the treatment phase, samples were collected immediately prior to the morning application of the test material. All microbiological analyses were conducted at one laboratory (Cutaneous Microbiology Laboratory, University of Pennsylvania).

**Microbiological sample collection.** A sterile Teflon template with an open circular area of 4.0 $cm^2$ was placed in the center of the forehead slightly above the bridge of the nose or on the scapular region of the upper back. A sterile cotton-tipped swab was dipped into a sterile test tube containing 2.0 ml of Letheen broth, and the excess broth was removed from the swab by wringing it out on the inside of the culture tube. The circular area delineated by the template was swabbed in a clockwise direction for 10 s and in a counterclockwise direction for another 10 s. The swab was placed into the tube of broth and rinsed and wrung out again, and the swabbing procedure was repeated. The swab was returned to the same sample tube and the end was broken off. Both swabbings were pooled in the same tube of broth. The anterior nares were swabbed by rotating the swab around the inner perimeter of the right naris for 10 rotations in one direction. The swab was placed into the tube of broth to be rinsed and wrung out again, and the swabbing procedure was repeated in the opposite direction in the same naris. The same procedure was followed for the left naris. The same sample tube was used for all four swabbings for both nares. Samples were immediately processed (within 0.5 h).

**Microbiological analysis.** Samples were diluted (four 10-fold dilutions) in 0.1% Tween 80, and 50 μl of each dilution was plated onto tryptic soy agar containing 5% sheep blood, 0.1% Tween 80, and metallic salts. Samples were also plated onto Mueller-Hinton II (MHII) agar containing 8 μg of erythromycin (Sigma) per ml. The plates were incubated aerobically at 37°C for 72 h and were then stored at room temperature for 4 days to enhance pigment production and colonial morphology. Five representative colonies were selected from the erythromycin incorporation plate, Gram stained, and identified by using the API Staph Ident system (Analytab Products, Plainview, N.Y.). Antibiotic susceptibility was determined by the Bauer-Kirby technique with MHII agar or MHII agar with 4% sodium chloride and standard antibiotic disks. The concentrations of the disks were as follows: erythromycin, 15 μg; clindamycin, 2 μg; tetracycline, 20 μg; minocycline, 30 μg; gentamicin, 10 μg; neomycin, 2 μg; chloramphenicol, 30 μg; penicillin, 10 U; ampicillin, 10 μg; oxacillin, 1 μg; methicillin, 5 μg; cephalothin, 30 μg; and cefoxitin, 30 μg. Resistance was determined by standard zone inhibition analysis.

**Analysis of erythromycin resistance gene usage.** Oligonucleotide probes for the erythromycin resistance genes *ermA*, *ermC*, and *msrA* were designed on the basis of published sequences by using an oligonucleotide selection program (7). The sequences of the probes were as follows: for *ermA*, GTCACTTGACATA AGCCTCCATC; for *ermC*, CAATGGCAGTTACGAAA TTACACCTC; and for *msrA*, TATTGTGCCATCTTCTTGTATATCCC.

Isolates of erythromycin-resistant coagulase-negative staphylococci were grown overnight in 2 ml of brain heart infusion broth containing 0.5% glycine. The culture was then transferred to a microcentrifuge tube and pelleted at 12,000 rpm (13,200 × *g*) for 2 min. The supernatant was discarded, the pellet was suspended in 50 μl of 10 mM Tris-HCl–10 mM EDTA (pH 8), and 5 μl of lysostaphin (5 mg/ml; 15,000 U; Sigma) and 5 μl of lysozyme (20 mg/ml; 80,000 U; turkey egg; Sigma) were added. The mixture was incubated for a minimum of 20 min at 37°C, which was followed by the addition of 100 μl of 0.5 M NaOH containing 0.2% sodium dodecyl sulfate. After mixing, 200 μl of 3 M sodium acetate (pH 6) was added and the contents were mixed. The sample was centrifuged for 3 min. The supernatant was removed and transferred to a new tube. One milliliter of 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was added to the DNA extract, and 400 μl of the sample was transferred to a slot blotter. Samples were deposited on a nylon filter by vacuum and were then cross-linked to the filter with UV light.

The oligonucleotide probes were tailed with [α-$^{32}$P]dATP by using terminal deoxynucleotide transferase (Boehringer Mannheim) and were added to prehybridized filters. The filter was hybridized for 4 h at temperatures of 56 to 62°C, depending on the oligonucleotide. The filter was then subjected to a series of posthybridization washes, with the initial wash being in 2× SSC at 7°C below the hybridization temperature. Following these washes, the filter was autoradiographed at −80°C for 2 to 24 h and then developed. The controls used to verify a positive reaction were plasmid-containing staphylococci which were extracted in parallel with the unknown strains. They consisted of *Staphylococcus aureus* 1206 (*ermA*), *S. aureus* RN2442 (*ermC*), and an isolate from our laboratory, *Staphylococcus epidermidis* 87-1213b (*msrA*).

**Statistical analysis.** Patients were considered for evaluation if they had a total of 80% compliance and complete microbiological data were available for all visits and anatomical sites. Three statistical measures were used to assess the results and were scored for each of three anatomical sites, for each individual subject, on each follow-up visit. First, we assessed the total logarithmic count of resistant organisms. Second, we evaluated the percentage of a subject's total organisms which were resistant. Finally, we analyzed the prevalence of subjects with resistant organisms or whether an individual subject harbored any resistant organisms at a particular site.

A repeated-measures analysis of variance by using a linear model with one between-subject term (study site) and one within-subject term (follow-up visit) was used to evaluate both the logarithmic count of resistant organisms and the percentage of all organisms that were resistant. Models were fit for each anatomical site and each treatment group. Subsequent estimates and hypothesis tests, directed at specific questions, were then tabulated for review.

The prevalence of resistance was assessed by a general linear model based on the logarithm link function and Poisson distribution. The standard model was fit with terms for study site, treatment group, and the interaction of these factors. The method is specifically intended for binary outcomes such as prevalence and is widely used in medical and epidemiologic research. The method provides estimates of relative risk rather than simple differences, as in more standard analysis of variance techniques. A relative risk of 2.0 suggests, for example, that the risk of resistance is two times greater for one group compared with that for another reference group.

Statistical analyses were performed by using SAS on a VAX computer and a personal computer and the GLIM package on a personal computer.

## RESULTS

**Baseline prevalence and density of Em$^r$ staphylococci.** Subsequent to dividing the subjects into treatment and vehicle groups, microbiological samples were obtained from the forehead, back, and anterior nares in order to assess the prevalence and density of Em$^r$ staphylococci. The prevalence, which indicates the percentage of individuals carrying Em$^r$ staphylococci, at both venues was quite high, with a 79.3% rate on the forehead, 51.9% on the back, and 88.0% in the nose (Fig. 1). While a slight difference in the prevalence was present between the back and the forehead and nares, the density (i.e., the proportion of Em$^r$ staphylococci among the total staphylococci population) was similar, ranging from 14 to 26% at all three body sites (Fig. 2). The exception to this was the vehicle group at the Boston venue, which had a density range of 32 to 41%. The reasons for this difference are unclear.

**Prevalence and density of Em$^r$ staphylococci increase with erythromycin therapy.** To determine the effect of prolonged application of 2% erythromycin to the forehead, samples were obtained from the subjects after 6 weeks of daily application of erythromycin or vehicle. In subjects treated with 2% erythromycin, the prevalence of Em$^r$ staphylococci at the site of application was nearly 100%. Interestingly, the prevalence of Em$^r$ staphylococci on the back, a site distal to the application, also increased by roughly 25%, suggesting drug and/or bacterial translocation. No relevant differences in prevalence were observed in the vehicle group (Fig. 1).

In parallel with the increased prevalence of Em$^r$ staphylococci, the density of Em$^r$ staphylococci increased significantly at all three body sites (Fig. 2). The greatest increase was observed on the forehead (~50% increase); however, the increased density of Em$^r$ staphylococci in the nares was nearly as great. An increased density of Em$^r$ staphylococci was also observed on the back (~20% increase).

While both the prevalence and density of Em$^r$ staphylococci increased in the group treated with erythromycin, there was no significant increase in the density of total staphylococci (Table 1). The maximum increase observed in the erythromycin treatment group was 0.69 $\log_{10}$ CFU/ml; however, an increase in the total density of staphylococci of 0.76 $\log_{10}$ CFU/ml was also observed in the vehicle group, suggesting that the change was

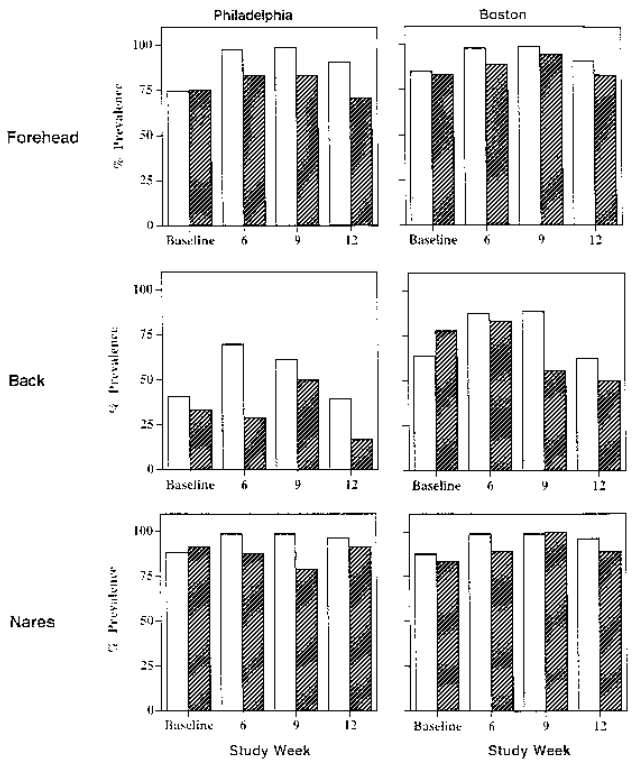


FIG. 1. Prevalence of Em$^r$ coagulase-negative staphylococci at the sampling time points. Subjects from both the Philadelphia and Boston venues were divided into unbalanced treatment ($n$ = 179) and control ($n$ = 46) groups, and samples were obtained at the baseline, after 6 weeks of treatment, at 3 weeks posttreatment, and at 6 weeks posttreatment. Prevalence was determined by growth on MHII agar containing 8.0 μg of erythromycin per ml. The prevalence is expressed as the percentage of total individuals that carried at least 1 CFU of Em$^r$ coagulase-negative staphylococci. ▨, 2% erythromycin; ▨, vehicle.

not due to erythromycin therapy. These data indicate that erythromycin-susceptible (Em$^s$) staphylococci are replaced by Em$^r$ staphylococci either by the acquisition of erythromycin resistance by the Em$^s$ strains or, more likely, by the death of the Em$^s$ strains with the subsequent outgrowth of the Em$^r$ strains.

**Prevalence and density of Em$^r$ staphylococci return to baseline levels with cessation of erythromycin therapy.** Three and 6 weeks after ending treatment, samples were again obtained from the three sites of each subject to determine if changes in the prevalence and density of Em$^r$ staphylococci had occurred. At 3 weeks posttreatment (week 9 of the study), there was no significant change in the prevalence of Em$^r$ staphylococci at any of the body sites (Fig. 1). However, a decrease in the density of Em$^r$ staphylococci was observed at all three body sites (Fig. 2).

Six weeks after ending treatment (week 12 of the study), both the prevalence and density of Em$^r$ staphylococci were not significantly different from those obtained prior to the initiation of the study. However, there was a slight increase in the density of resistant staphylococci at both the forehead and anterior naris sites.

**Erythromycin therapy does not result in outgrowth of multidrug-resistant coagulase-negative staphylococci.** To determine if erythromycin therapy evokes resistance to other antibiotics as well as to erythromycin, Em$^r$ staphylococci were tested for their resistance to 15 antibiotics at each sampling time point. Em$^r$ staphylococci were selected from the plate into which erythromycin was incorporated on the basis of colonial morphology and were tested for resistance by the disk diffusion method. Over the course of the study, no significant changes in the prevalence of resistance to any particular antibiotic was observed in conjunction with Em$^r$ (Table 2). The most prevalent resistance to other antibiotics in both the erythromycin-treated and the vehicle-treated groups was to penicillin, ampicillin, and clindamycin.

The development of multiply drug-resistant (MDR) isolates in the Em$^r$ staphylococcal population, defined as those isolates resistant to three or more antibiotics including erythromycin, was monitored throughout the study. Prior to treatment, 64.8% of the Em$^r$ isolates from the erythromycin treatment group were MDR, and after 6 weeks of daily treatment, the percentage of MDR isolates remained largely unchanged at 68%. Moreover, there was no significant change in the number of antibiotics to which the isolates were MDR. While some isolates were resistant to as many as 10 of the 15 antibiotics tested, the majority of strains were resistant to four or fewer antibiotics, including erythromycin (Fig. 3).

**Distribution of Em$^r$ staphylococci species does not change with erythromycin treatment.** The predominant organisms isolated from the skin are coagulase-negative staphylococci, particularly *S. epidermidis*. In the present study, we examined the species distribution of Em$^r$ staphylococci and similarly observed that the most prevalent species was *S. epidermidis*, com-

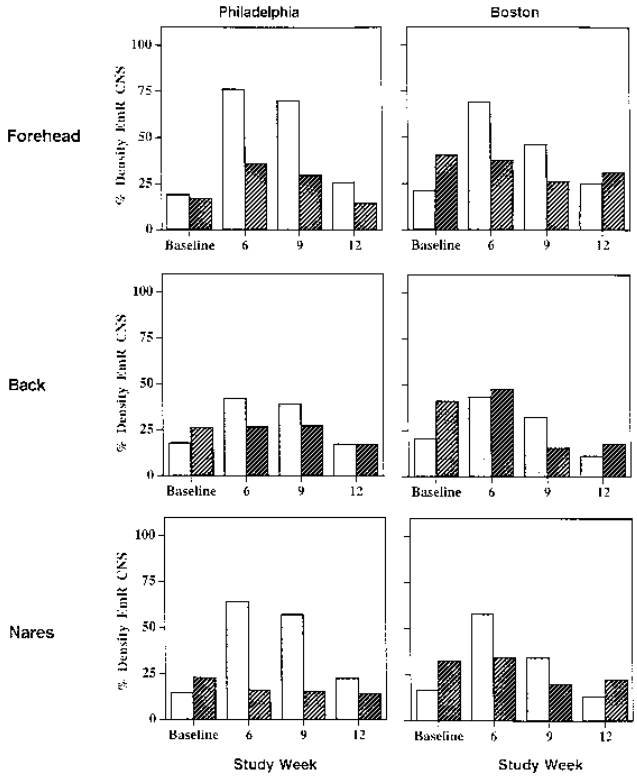


FIG. 2. Density of Em$^r$ coagulase-negative staphylococci at the sampling time points. Subjects from both the Philadelphia and Boston venues were divided into unbalanced treatment ($n$ = 179) and control ($n$ = 46) groups, and samples were obtained at the baseline, after 6 weeks of treatment, at 3 weeks posttreatment, and at 6 weeks posttreatment. The density of Em$^r$ coagulase-negative staphylococci was determined by quantifying growth on MHII agar containing 8.0 μg of erythromycin per ml and dividing the geometric count of Em$^r$ coagulase-negative staphylococci by the total count of coagulase-negative staphylococci to obtain the percentage of total organisms that were Em$^r$. ▨, 2% erythromycin; ▨, vehicle.

TABLE 1. Total numbers of coagulase-negative staphylococci at various sampling times

| Anatomical site | Treatment | Study site | Mean ± SD $\log_{10}$ CFU/ml at: | | | |
|---|---|---|---|---|---|---|
| | | | Baseline | 6 wk of treatment | 3 wk posttreatment | 6 wk posttreatment |
| Forehead | Erythromycin[a] | Philadelphia | 4.46 ± 1.26 | 4.55 ± 1.14 | 4.89 ± 1.19 | 5.15 ± 1.22 |
| | | Boston | 4.39 ± 1.29 | 4.21 ± 1.36 | 4.29 ± 1.22 | 4.17 ± 1.34 |
| | Placebo | Philadelphia | 3.95 ± 1.41 | 4.38 ± 1.32 | 4.75 ± 1.58 | 4.67 ± 1.17 |
| | | Boston | 4.54 ± 1.11 | 4.65 ± 1.30 | 4.63 ± 1.29 | 4.57 ± 0.93 |
| Back | Erythromycin | Philadelphia | 2.99 ± 1.24 | 2.97 ± 1.49 | 2.96 ± 1.48 | 3.06 ± 1.43 |
| | | Boston | 3.12 ± 1.20 | 3.20 ± 1.31 | 3.52 ± 0.99 | 3.23 ± 0.98 |
| | Placebo | Philadelphia | 2.31 ± 1.15 | 2.64 ± 1.24 | 2.62 ± 1.56 | 2.47 ± 1.39 |
| | | Boston | 2.70 ± 1.16 | 2.92 ± 1.23 | 3.08 ± 0.91 | 3.46 ± 1.11 |
| Nose | Erythromycin | Philadelphia | 5.88 ± 0.79 | 5.98 ± 0.68 | 5.90 ± 0.70 | 6.09 ± 0.62 |
| | | Boston | 5.62 ± 1.02 | 5.84 ± 0.88 | 5.81 ± 1.04 | 5.76 ± 0.88 |
| | Placebo | Philadelphia | 5.90 ± 0.79 | 5.64 ± 0.73 | 5.54 ± 0.81 | 5.62 ± 0.88 |
| | | Boston | 5.75 ± 0.79 | 5.85 ± 0.94 | 5.87 ± 0.71 | 5.48 ± 1.52 |

[a] Erythromycin as EryGel (2% topical erythromycin).

prising 76.4 to 87.5% of all $Em^r$ staphylococci isolates from the forehead (Table 3). No significant alterations in the distribution were observed over the course of the study.

**Prevalence of specific erythromycin resistance gene is similar before and after erythromycin therapy.** Resistance to erythromycin by cutaneous coagulase-negative staphylococci is mediated primarily by one of three different genes, *ermA*, *ermC*, or *msrA* (3). The presence of *ermA* or *ermC* confers resistance to the macrolide-lincosamide and streptogramin B group of antibiotics, whereas *msrA* confers resistance to the macrolide-streptogramin B group. In the present study, we examined the prevalence of specific erythromycin resistance genes prior and subsequent to erythromycin therapy. At both sampling times, the predominant erythromycin resistance gene carried was *ermC* (Table 4). Prior to treatment, 46% of the $Em^r$ isolates carried *ermC*, whereas after treatment, 63% of the isolates carried *ermC*. The erythromycin resistance gene usage was untypeable or uninterpretable for less than 10% of the isolates, which may be the result of a technical error or the mediation of $Em^r$ by a different gene.

**Erythromycin therapy does not increase the prevalence or density of GNRs or fungi.** Disruption of the normal cutaneous ecological balance via the application of a selective pressure can result in the outgrowth of certain organisms that are either normally not found on the skin or present at low levels. Because prolonged erythromycin therapy represents a selective pressure, we examined the serial samples for the outgrowth of gram-negative rods (GNRs) and fungi using selective media. Over the course of the study, no significant changes in either the prevalence or density of GNRs were observed. As expected, the highest prevalence in both groups at all four sampling times was found in the anterior nares. Initially, 27.5% of the erythromycin-treated subjects had naris GNR isolates which remained largely unchanged (35.0%) after 6 weeks of

TABLE 2. Antibiotic resistance patterns of erythromycin-resistant staphylococci

| Antibiotic | % Prevalence (95% bounds) | | | | | |
|---|---|---|---|---|---|---|
| | Erythromycin-treated group | | | Vehicle-treated group | | |
| | Baseline | 6 wk of treatment | 6 wk posttreatment | Baseline | 6 wk of treatment | 6 wk posttreatment |
| Ampicillin | 63.6 (55.9–72.4) | 65.8 (58.7–73.3) | 70.3 (63.3–78.2) | 65.6 (51.1–84.3) | 47.1 (32.9–67.2) | 70.6 (56.8–87.7) |
| Amoxicillin | 1.5 (0.4–6.0) | 0.6 (0.1–4.6) | 0.7 (0.1–4.9) | 0 | 2.9 (0.4–20.3) | 2.9 (0.4–20.3) |
| Chloramphenicol | 6.1 (3.1–11.9) | 2.6 (1.0–6.8) | 5.5 (2.8–10.8) | 3.1 (0.5–21.5) | 8.8 (30.0–26.0) | 5.9 (1.5–22.6) |
| Clindamycin | 72.7 (65.5–80.7) | 78.7 (72.5–85.4) | 81.4 (75.3–88.0) | 62.5 (47.8–81.7) | 67.6 (53.6–85.4) | 76.5 (63.5–92.1) |
| Cephalothin | 0.8 (0.1–5.3) | 0.6 (0.1–4.6) | 0 | 0 | 0 | 0 |
| Methicillin | 14.4 (9.5–21.8) | 17.4 (12.4–24.5) | 11.7 (7.5–18.3) | 12.5 (5.0–31.3) | 11.8 (4.7–29.5) | 11.8 (4.7–29.5) |
| Erythromycin | 100 | 100 | 100 | 100 | 100 | 100 |
| Nitrofurantoin | 0 | 1.9 (0.6–5.9) | 2.1 (0.7–6.3) | 0 | 0 | 2.9 (0.4–20.3) |
| Cefoxitin | 9.8 (5.9–16.5) | 12.3 (8.0–18.7) | 5.5 (2.8–10.8) | 3.1 (0.5–21.5) | 5.9 (1.5–22.6) | 8.8 (3.0–26.0) |
| Gentamicin | 2.3 (0.7–7.0) | 0.6 (0.1–4.6) | 1.4 (0.3–5.5) | 3.1 (0.5–21.5) | 5.9 (1.5–22.6) | 0 |
| Oxacillin | 15.2 (10.1–22.7) | 9.4 (14.0–26.7) | 1.4 (8.1–19.1) | 18.8 (9.1–38.6) | 8.8 (3.0–26.0) | 11.8 (4.7–29.5) |
| Penicillin | 68.9 (61.5–77.3) | 74.2 (67.6–81.4) | 72.4 (65.5–80.1) | 71.9 (57.9–89.3) | 58.8 (44.4–77.9) | 73.5 (60.1–90.0) |
| Sulfamethoxazole | 11.4 (7.1–18.3) | 12.3 (8.0–18.7) | 11.7 (7.5–18.3) | 3.1 (0.5–21.5) | 20.6 (10.6–39.8) | 5.9 (1.5–22.6) |
| Tetracycline | 31.1 (24.1–40.0) | 18.7 (13.5–26.0) | 20.7 (15.0–28.5) | 37.5 (24.0–58.7) | 2.9 (0.4–20.3) | 23.5 (12.8–43.1) |
| Vancomycin | 0 | 0 | 0.7 (0.1–4.9) | 0 | 0 | 0 |

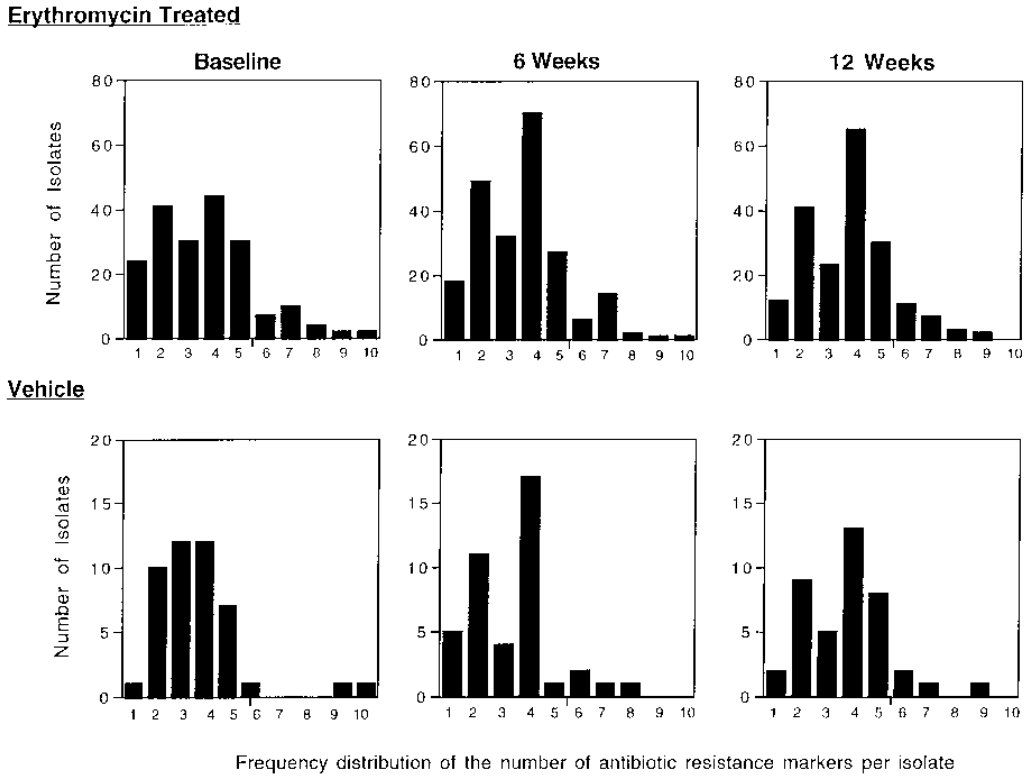


FIG. 3. Frequency distribution of antibiotic resistance markers of Em$^r$ coagulase-negative staphylococci. Isolates from erythromycin-treated and vehicle-treated subjects were examined for multiple drug resistance by disk diffusion at the specified time points. Resistance was determined by using the guidelines established by the National Committee for Clinical Laboratory Standards. Em$^r$ was counted as a single resistance, and resistance to erythromycin and clindamycin was counted as a double resistance.

erythromycin therapy. The density of the nares GNR, when found, remained constant over the 6 weeks of erythromycin therapy (Table 5).

The isolation of various fungi over the course of the study was infrequent, even with the use of selective medium, indicating that erythromycin therapy does not allow for the colonization of these organisms (data not shown).

## DISCUSSION

Coagulase-negative staphylococci have increasingly been recognized as potential human pathogens, particularly in the nosocomial setting (17, 18). The major reservoir of these organisms is the human, with some areas of the human body being colonized with upward of $10^7$ $\log_{10}$ CFU/cm$^2$. Many studies have examined clinically relevant isolates of coagulase-negative staphylococci to determine their antibiotic resistance patterns or have tracked the emergence of antibiotic-resistant organisms concurrently with the use of either an oral or a topically applied antibiotic (6, 10, 12, 15). In contrast, in the present study we assessed the prevalence and density of antibiotic-resistant organisms at multiple time points including 6 weeks subsequent to the cessation of antibiotic use in order to determine the ecological effects of long-term antibiotic use.

Of initial interest in the present study was the observation that >90% of the individuals sampled at the baseline carried Em$^r$ coagulase-negative staphylococci on at least one of the sampling sites, with >75% prevalence on the face and nares. These observations of the substantial prevalence and density of

TABLE 3. Distribution of Erythromycin-resistant staphylococcal species

| Staphylococcal species | Distribution (%) | | | | | |
|---|---|---|---|---|---|---|
| | Erythromycin-treated group | | | Vehicle-treated group | | |
| | Wk 0 ($n$ = 194) | Wk 6 ($n$ = 220) | Wk 12 ($n$ = 194) | Wk 0 ($n$ = 45) | Wk 6 ($n$ = 42) | Wk 12 ($n$ = 41) |
| *S. aureus* | 1.6 | 1.2 | 1.8 | 2.0 | 2.0 | 3.9 |
| *S. capitis* | 0.8 | 0.4 | 0.4 | 2.0 | 5.9 | 0.0 |
| *S. cohnii* | 5.5 | 3.1 | 3.6 | 5.9 | 0.0 | 3.9 |
| *S. epidermidis* | 76.4 | 86.7 | 87.5 | 82.4 | 86.3 | 84.3 |
| *S. haemolyticus* | 2.0 | 0.0 | 1.3 | 0.0 | 0.0 | 0.0 |
| *S. hominis* | 7.1 | 6.3 | 3.6 | 3.9 | 2.0 | 3.9 |
| *S. saphrophyticus* | 0.8 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| *S. simulans* | 0.8 | 1.2 | 0.4 | 2.0 | 2.0 | 0.0 |
| *S. warneri* | 3.9 | 0.8 | 0.9 | 2.0 | 2.0 | 3.9 |
| *S. xylosus* | 0.4 | 0.0 | 0.4 | 0.0 | 0.0 | 0.0 |

TABLE 4. Erythromycin resistance gene usage by coagulase-negative staphylococci prior and subsequent to erythromycin treatment

| Erythromycin resistance gene | Gene usage (%) at: | |
|---|---|---|
| | Baseline ($n$ = 122) | 6 wk of treatment ($n$ = 176) |
| *ermC* | 46 | 63 |
| *ermA* | 29 | 18 |
| *msrA* | 18 | 11 |
| Other | 7 | 8 |

TABLE 5. Effect of erythromycin treatment on prevalence and density of GNRs[a]

| Treatment group and anatomical site | Baseline | | 6 wk of treatment | | 3 wk posttreatment | | 6 wk posttreatment | |
|---|---|---|---|---|---|---|---|---|
| | Prevalence (%) | Density[b] | Prevalence (%) | Density | Prevalence (%) | Density | Prevalence (%) | Density |
| Erythromycin | | | | | | | | |
| Forehead | 5.9 | 2.88 ± 0.78 | 3.0 | 3.04 ± 0.98 | 12.0 | 3.04 ± 0.86 | 17.7 | 3.41 ± 1.55 |
| Back | 3.9 | 2.80 ± 1.46 | 3.0 | 2.70 ± 1.06 | 4.0 | 2.54 ± 0.55 | 12.5 | 3.38 ± 1.42 |
| Nose | 27.5 | 4.20 ± 1.27 | 35.0 | 4.21 ± 1.64 | 32.0 | 3.79 ± 1.05 | 30.2 | 3.85 ± 1.13 |
| Vehicle | | | | | | | | |
| Forehead | 3.8 | 1.6 | 8.0 | 2.49 ± 0.58 | 8.0 | 2.91 ± 0.75 | 26.0 | 3.13 ± 1.12 |
| Back | 11.5 | 1.90 ± 0.30 | 0.0 | 0 | 0.0 | 0 | 13.0 | 3.77 ± 2.65 |
| Nose | 46 | 3.81 ± 1.55 | 40.0 | 3.71 ± 1.30 | 40.0 | 3.95 ± 0.78 | 39.1 | 3.58 ± 1.05 |

[a] For the erythromycin treatment group, $n = 102$, 100, 100, and 96 subjects tested at the baseline, 6 weeks of treatment, 3 weeks posttreatment, and 6 weeks posttreatment, respectively. For the vehicle treatment group, $n = 26$, 25, 25, and 23 subjects tested at the baseline, 6 weeks of treatment, 3 weeks posttreatment, and 6 weeks posttreatment, respectively.

[b] Density is expressed as the mean ± standard deviation $\log_{10}$ CFU per square centimeter (when organisms were found).

$Em^r$ coagulase-negative staphylococci are not unique to the United States, because Cove et al. (2) found in Leeds, United Kingdom, that nearly 70% of untreated acne subjects carried $Em^r$ coagulase-negative staphylococci on their faces. By the 6th week of the present study, those individuals from whom no $Em^r$ coagulase-negative staphylococci had been isolated at baseline, including those in the vehicle group, carried $Em^r$ coagulase-negative staphylococci on at least one of the sampling sites. Accompanying this observation was the inability to isolate $Em^r$ coagulase-negative staphylococci from a small number of those individuals (<10%) from whom $Em^r$ had previously been isolated. These data may suggest that $Em^r$ coagulase-negative staphylococci are present ubiquitously, albeit in some cases in very small numbers. Alternatively or concomitantly, the colonization of individuals with various strains of coagulase-negative staphylococci may be a dynamic process in which new strains are acquired and lost at various intervals. It is apparent that treatment with erythromycin results in an increased prevalence of $Em^r$ coagulase-negative staphylococci at all three sampling sites; however, as stated previously, this may be due to the increased density of $Em^r$ coagulase-negative staphylococci, which allows for detection. This variation can be most easily observed by examining the prevalence of $Em^r$ coagulase-negative staphylococci on the back, which normally carries only small numbers of coagulase-negative staphylococci compared with the numbers on the face and nares (>100-fold). While the prevalence of $Em^r$ coagulase-negative staphylococci in the treated group increased after 6 weeks of erythromycin treatment and waned after the cessation of the erythromycin treatment, equally large changes occurred in the vehicle group at different time points.

There was a high degree of variability in the density of $Em^r$ coagulase-negative staphylococci among individuals prior to testing, with the total coagulase-negative staphylococci on untreated individuals comprising anywhere from <0.001 to 100% $Em^r$ coagulase-negative staphylococci. While it is likely that most of the individuals in the study had been exposed to a macrolide at some point in their life, none of them had used any antibiotic for at least 12 weeks prior to enrollment in the study. In contrast to the prevalence data, significant changes in the density of $Em^r$ coagulase-negative staphylococci could readily be observed. As expected, erythromycin treatment resulted in an expansion of the density of $Em^r$ coagulase-negative staphylococci on the forehead where the antibiotic had been applied. It is not surprising that the $Em^r$ coagulase-negative staphylococci also increased in the nares considering its proximity to the drug application site and the ease with which the drug and/or $Em^r$ coagulase-negative staphylococci could be translocated given the propensity to touch the forehead and nose in quick succession throughout the day, e.g., during washing and drying the face. Less expected was the increased density of $Em^r$ coagulase-negative staphylococci on the back; however, this is also likely due to the translocation of drug and/or $Em^r$ coagulase-negative staphylococci from the face during washing and drying after bathing. Also of interest was the increase in $Em^r$ coagulase-negative staphylococci on the forehead in the vehicle-treated group. While this increase was not statistically significant, there was a trend for increased numbers of $Em^r$ coagulase-negative staphylococci. The reason for this increase is not clear, but it may reflect the high concentration of alcohol in the vehicle, which may have had a greater suppressive effect on $Em^s$ coagulase-negative staphylococci. The return of the density of $Em^r$ coagulase-negative staphylococci to the baseline density at all three sites indicates that withdrawal of the selective pressure of erythromycin results in a decrease in the number of $Em^r$ coagulase-negative staphylococci. This may again suggest that the colonization of skin by coagulase-negative staphylococci is a dynamic process, with new strains being acquired on a continuing basis. Because the pool of $Em^s$ coagulase-negative staphylococcal strains is at present larger than that of $Em^r$ strains, probability dictates that the predominant cutaneous population of coagulase-negative staphylococci will be susceptible to erythromycin unless the selective pressure of erythromycin is present. This explanation is borne out by the observation that the initial density of $Em^r$ coagulase-negative staphylococci was similar at all three body sites, even though the total $\log_{10}$ number of CFU per square centimeter on the back was 100-fold less than that on the forehead and 1,000-fold less than that in the nares and the detectable prevalence of $Em^r$ coagulase-negative staphylococci on the back was 20 to 40% less than that on the forehead and in the nares. These data therefore indicate that approximately 20% of the pool of human coagulase-negative staphylococci are resistant to erythromycin.

The introduction of a selective pressure to an ecologically stable environment, in this case, the addition of an antibiotic to the cutaneous bacterial ecosystem, can result in the loss of organisms which cannot survive in the presence of that selective pressure. Such a loss can lead to a destabilization of that environment and allow organisms that would otherwise be unable to survive an opportunity to occupy that ecological niche. Of concern in the present study was the possible colonization of the skin by recognized pathogens such as *S. aureus*, GNRs, or fungi. However, none of these organisms was found

to be present in the erythromycin-treated group at a prevalence or density higher than those in the vehicle-treated group. Interestingly, the density of organisms at the various sites remained consistent at the various sampling times, suggesting that the loss of susceptible strains of coagulase-negative staphylococci was accompanied by an increase in the numbers of resistant strains either via the expansion of indigenous flora or by the acquisition of new strains. It is also possible that in the interval between the baseline and the first sampling point (6 weeks), there was a transition period in which the cutaneous niche was predominantly occupied by organisms other than coagulase-negative staphylococci.

Isolates of clinical relevance are often multiply antibiotic resistant (16), which can make therapeutic intervention difficult. In the present study, the majority of the $Em^r$ coagulase-negative staphylococci were also found to be resistant to penicillin, ampicillin, and clindamycin. The high prevalence of resistance to these three antibiotics is not surprising considering that previous studies have described a very high prevalence (>95%) of penicillin- and ampicillin-resistant coagulase-negative staphylococci in the general population (2). Moreover, the association of clindamycin resistance in conjunction with erythromycin is well documented (21) and is the result of the presence of a resistance element conferring resistance to the macrolide-lincosamide-streptogramin B group of antibiotics. The two predominant genes mediating macrolide-lincosamide-streptogramin B resistance in human coagulase-negative staphylococci are *ermC* and *ermA* (3). This observation is confirmed by the excellent correlation between the prevalence of *ermC* and *ermA* genes (75%) and clindamycin (a lincosamide) resistance (72.7%) at the baseline. Although there was some variance in the individual prevalence of *ermC* and *ermA* after 6 weeks of erythromycin treatment, their combined prevalence (81%) again correlated with the prevalence of clindamycin resistance in $Em^r$ coagulase-negative staphylococci (78.7%). The increased prevalence of *ermC* with a concomitant decrease in the prevalence of both *ermA* and *msrA* after 6 weeks of erythromycin treatment may again suggest a dynamic process of colonization with coagulase-negative staphylococci on human skin.

Increasing the density of antibiotic-resistant bacteria suggests the increased likelihood of transfer of the resistance via extrachromosomal genetic elements, such as plasmids and transposons, to other cutaneous bacteria. This is of particular concern with erythromycin resistance because the majority of $Em^r$ genes in human coagulase-negative staphylococci are contained in mobile genetic elements (1, 20) and the coagulase-negative staphylococci are likely to be in contact with *S. aureus*. Whether cutaneous coagulase-negative staphylococci are actually likely to transfer these erythromycin resistance elements is unclear. While several studies have reported the transfer of plasmid-borne antibiotic resistance genes from coagulase-negative staphylococci to *S. aureus* (5, 8, 11), a study by Fawcett et al. (4) which used 153 clinical coagulase-negative staphylococcal isolates failed to show transfer of resistance to any of five antimicrobial agents, including erythromycin from the coagulase-negative staphylococcal isolates to any of 11 strains of *S. aureus*. Their data suggest that the transfer of resistance genes from coagulase-negative staphylococci to *S. aureus* in vivo is an extremely rare event.

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