

Sequential antibiotic therapy for acne promotes the carriage of resistant staphylococci on the skin of contacts

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The selection of a predominantly resistant staphylococcal skin flora in acne patients during antibiotic treatment has been extensively documented. This study sought to determine whether antibiotic therapy for acne had any effect on skin carriage of resistant coagulase-negative staphylococci (CNS) by close contacts of treated patients. Bacterial samples were obtained using a scrub wash technique from facial skin of 41 contacts (parents, siblings or partners) of patients who had been treated with at least three different antibiotics over a minimum period of 2 years. Samples were also obtained from 41 control subjects who had no known contact with any antibiotic treated acne patient. None of the contacts or controls had received any antibiotic therapy in the preceding two years. The number, percentage and prevalence of CNS resistant to each of seven antibiotics was estimated by plating serial ten-fold dilutions of wash fluid directly onto antibiotic-containing and antibiotic-free medium. Significantly more contacts than controls carried strains resistant to erythromycin, clindamycin, fusidic acid, trimethoprim and chloramphenicol as well as more multiply resistant strains ($P < 0.05$, χ^2). The number and percentage of staphylococci resistant to tetracycline, erythromycin, clindamycin, fusidic acid and chloramphenicol were also significantly raised ($P < 0.05$, Mann-Whitney U-test) in contacts. Only aminoglycoside resistance was not increased by any of the above criteria. These observations provide evidence that sequential antibiotic therapy for acne exerts selective pressure for increased skin carriage of resistant CNS not only in patients but also in their close contacts.

Introduction

Coagulase-negative staphylococci (CNS) are well recognised nosocomial pathogens, particularly associated with infections of compromised hosts (Gemmell & McCartney, 1990; Patrick, 1990). The infecting strains are frequently multiply antibiotic resistant so that therapeutic options are limited (Archer, 1988). The skin is the largest reservoir of CNS and commensal staphylococci are significant not only because of their role in disease but also because they represent a continuously evolving store of resistance genes

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which can be transferred to *Staphylococcus aureus* (Archer, 1988). Cove, Eady & Cunliffe (1990) examined the carriage rates and population densities of CNS resistant to various antibiotics on facial skin of 64 untreated young adults and found that a majority (>50%) of individuals carried strains resistant to penicillin, tetracycline, erythromycin and/or fusidic acid as well as multiply resistant strains i.e. those resistant to three or more antibiotics. Furthermore, one in four individuals harboured $>10^2$ cfu/cm² of multiply resistant staphylococci.

It is widely recognised that the skin of patients entering hospital rapidly becomes colonized with multiply resistant CNS and that resistance patterns commonly reflect antibiotic usage policies on the ward/unit (Oppenheim *et al.*, 1989; Kotilainen, Nikoskelainen & Huovinen, 1990; Archer, 1991; Hedin & Hambræus, 1991). Resistances to methicillin, aminoglycosides and/or fluoroquinolones have followed acquisition of resistance to penicillin, tetracycline and erythromycin. There is, and has been for many years, widespread concern about lavish use of antibiotics in the hospital environment but there is generally less concern about use of antibiotics in the community. This is probably explained by the fact that treatment failures in general practice are not usually monitored.

Acne is one of the commonest skin diseases and, except for mild cases, is routinely treated with long courses of oral and/or topical antibiotics (Eady, Holland & Cunliffe, 1982). Most patients with acne will need therapy from adolescence until their early twenties when the disease naturally regresses in most but not all patients. Therefore, sequential use of several different antibiotics to treat acne is common practice. The conversion of the cutaneous staphylococcal flora of acne patients from predominantly antibiotic sensitive to predominantly resistant during long term antibiotic therapy has been well documented (Marples & Kligman, 1971; Leyden *et al.*, 1974; Mills, Kligman & Stewart, 1975; Bernstein & Shalita, 1980; Eady *et al.*, 1990; Harkaway *et al.*, 1992) but the possibility that more remote effects might occur has not previously been examined.

The skin of antibiotic treated acne patients in the community acts as a large reservoir of antibiotic resistant staphylococci which have the potential to be transferred to, and colonise, untreated contacts. Additionally, patients treated with topical therapy may unintentionally contaminate close contacts with significant quantities of active drug. The objective of this study was to determine whether sequential antibiotic therapy for acne alters the carriage rates and population densities of resistant staphylococci on the skin of untreated close contacts.

Materials and methods

Subjects

Forty-one contacts (parents 31, partners 8, siblings 2) of acne patients who had received at least three different anti-acne antibiotic preparations over a minimum period of 2 years were identified from records held at the Dermatology Department, Leeds General Infirmary. Between them the patients had been treated with 168 courses of antibiotic therapy: oral tetracycline or oxytetracycline 26, oral erythromycin 30, minocycline 27, doxycycline 7, trimethoprim 12, topical tetracycline 17, topical erythromycin (with or without zinc) 28, topical clindamycin 21, and they were still on antibiotic therapy at the time the contact was sampled. Twenty-one patients were receiving oral or topical

tetracycline, 12 were receiving oral or topical erythromycin and eight were receiving trimethoprim (three alone, three in combination with topical erythromycin and two in combination with topical clindamycin). Contacts and patients lived in the same household. Contacts were identified by each patient as the individual with whom they had the closest and/or most frequent regular contact. There were 15 males and 26 females (age range 12–56 years, mean 39.9). Direct questioning prior to sample collection revealed that none of the contacts had received any oral or topical antibiotic therapy in the preceding two years. Age and sex are common confounding variables in studies such as this and are known to affect microbial population densities on skin. Therefore, an equal number of control subjects with the same age and sex distribution as contacts (15 males and 26 females, age range 12–56 years, mean age 39.7) were identified either amongst colleagues at the University of Leeds who did not work in biomedical/bioscience departments (and hence with no exposure to antibiotics in the workplace, $n = 11$) or amongst patients attending their family doctor for a complaint other than acne or an infectious disease ($n = 30$). Each prospective control was asked to complete a questionnaire before inclusion in the study to verify that they had not had received any antibiotics in the preceding two years and had no known contact (within the home or elsewhere) with any antibiotic treated acne patients. The study was approved by the local Ethics Committee.

Sample collection and analysis of staphylococcal isolates

Skin bacteria were obtained from the surface of the right cheek using a scrub wash technique (Williamson & Kligman, 1965). Total viable staphylococcal counts were obtained by plating decimal dilutions of wash fluid onto heated blood agar and incubating for 48 h at 37°C. Antibiotic resistance in primary isolates was determined by plating wash fluid directly onto selective media containing one of seven different antibiotics at the following concentrations (mg/L): trimethoprim 20, tetracycline 10, chloramphenicol 10, erythromycin 5, clindamycin 5, kanamycin 5, fusidic acid 2. These concentrations have previously been shown to discriminate between sensitive and resistant strains (Cove *et al.*, 1990). Kanamycin was used to detect both of the commonest phenotypes of aminoglycoside resistance in skin staphylococci, namely co-resistance to gentamicin, kanamycin and tobramycin or co-resistance to neomycin, kanamycin and streptomycin (E. A. Eady, unpublished observations). The lower limit of detection was 2 cfu/cm² skin.

Each different colony type from the selective and the non-selective media (a minimum of six isolates per individual) was tested for multiple resistance (i.e. resistance to three or more antibiotics) by a combination of agar incorporation (the seven antibiotics in the primary screen and additional aminoglycosides where relevant in individual plates) and disc testing (erythromycin, clindamycin and penicillin G). Macrolide-lincosamide-streptogramin type B (MLS) resistance was differentiated from macrolide-streptogramin type B resistance (MS) in disc tests with clindamycin and erythromycin (Jenssen *et al.*, 1987). Resistances were recorded after overnight incubation at 37°C. *S. aureus* strain Oxford was used as a control. All isolates with different colony types and/or resistotypes were identified to species level using the method of Kloos & Schleifer (1975) and, if necessary, API Staph strips (bioMérieux *sa*, Marcy l'Etoile, France).

Data analysis

The data were analysed in three different ways. The number of staphylococci resistant to each antibiotic and the number of multiply resistant strains were expressed as \log_{10} cfu/cm² skin. The proportion of staphylococci resistant to each antibiotic and the proportion of multiply resistant strains were expressed as a percentage of the total staphylococcal count. Significances of the differences in population median values of both variables for contacts and controls were calculated using the Mann-Whitney U-test; 95% confidence limits were derived using the sign interval. All analyses were carried out using release 8.0 of Minitab⁸. Thirdly, the prevalence of carriage of staphylococci resistant to each antibiotic and of multiply resistant strains were expressed as a fraction of the total number of subjects sampled ($n/41$) and also as the ratio of the odds of a contact carrying resistant strains to the odds of a control carrying such strains. Significances of the odds ratios were determined using the χ^2 test with Yates's correction.

Results

The figure shows the frequency distributions for the \log_{10} counts per cm² skin of total viable staphylococci and of staphylococci resistant to each of the seven antibiotics as well as of multiply resistant strains. Extensive intra-individual variation was apparent in both the prevalence and population densities of resistant organisms as well as in total

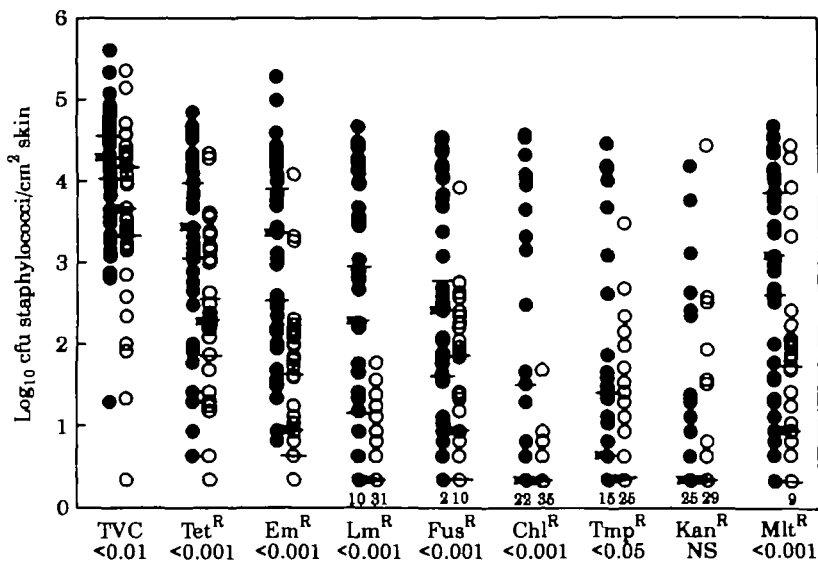


Figure Distributions of viable counts of total staphylococci, of staphylococci resistant to each of seven antibiotics and of multiply resistant strains. Each point represents the count expressed as \log_{10} cfu/cm² skin for individual contacts (●) and controls (○). The sample median (▶) and 95% confidence limits (—) are shown for each set of data. Where no confidence limit is shown it coincided with the median. Small numbers at the base of some columns indicate the number of individuals in whom strains resistant to those antibiotics were not detected. *P* values were calculated using the Mann-Whitney U-test and relate to differences between estimated population medians. TVC, Total viable count; Tet, tetracycline; Em, erythromycin; Lm, clindamycin; Fus, fusidic acid; Chl, chloramphenicol; Tmp, trimethoprim; Kan, kanamycin; Mlt, multiply resistant.

Table I. Median percentage of the staphylococcal flora resistant to each of seven antibiotics and to three or more antibiotics in contacts and controls

Staphylococci	Median percentage (95% confidence limits) of flora resistant in		P value*
	contacts	controls	
Tet ^R	18 (8.8 – 34.5)	4 (1.8 – 9.2)	<0.01
Em ^R	13 (4.0 – 33)	0.4 (0.1 – 0.7)	<0.001
Lm ^R	1.0 (0.19 – 6.7)	1 × 10 ³	<0.001
Fus ^R	3.0 (0.28 – 5.0)	0.2 (7.6 × 10 ⁻² – 0.6)	<0.01
Chl ^R	1 × 10 ⁻³ (10 ⁻³ – 0.13)	1 × 10 ⁻³	<0.01
Tmp ^R	3 × 10 ⁻² (10 ⁻³ – 0.2)	1 × 10 ⁻³ (1.7 × 10 ⁻³)	NS
Kan ^R	1 × 10 ⁻³ (10 ⁻³ – 1.2 × 10 ⁻³)	1 × 10 ⁻³	NS
Mlt ^R	11 (4 – 26.5)	0.4 (7.5 × 10 ⁻² – 0.75)	0.001

For abbreviations, see the Figure.

*Calculated from the differences in estimated population medians using the Mann-Whitney U-test.

staphylococcal counts (ranging from below the detection limit of 2 to >10⁵ cfu/cm² skin). It reveals that, compared with the controls, contacts carried significantly more viable staphylococci, significantly more strains independently resistant to six of the seven antibiotics tested and significantly more multiply resistant strains. The median percentages of the total viable staphylococcal count resistant to each of five of the seven antibiotics were also increased in contacts compared to controls as was the median percentage of multiply resistant strains (Table I). Presentation of data in this way eliminates the effect of the difference in total viable staphylococcal count between contacts and controls.

All 41 contacts carried some tetracycline resistant, some erythromycin resistant and some multiply resistant staphylococci (Table II). Tetracycline resistant strains were also present on the skin of 39/41 controls. The least common resistances in contacts and controls were to aminoglycosides and chloramphenicol respectively. The prevalence of carriage of staphylococci independently expressing resistance to erythromycin, clindamycin, fusidic acid, chloramphenicol and trimethoprim was significantly increased

Table II. Prevalance of carriage of staphylococci resistant to each of seven antibiotics and to three or more antibiotics in contacts and controls

Staphylococci	Prevalance (n/41) of carriage in		Odds ratio: contacts versus controls	P value*
	contacts	controls		
Tet ^R	41	39	∞ ^b	NS
Em ^R	41	29	∞ ^b	<0.01
Lm ^R	31	10	8.45	<0.001
Fus ^R	39	31	6.29	<0.05
Chl ^R	19	6	4.56	<0.01
Tmp ^R	26	16	2.71	<0.05
Kan ^R	16	12	1.38	NS
Mlt ^R	41	32	∞ ^b	<0.01

For abbreviations, see the Figure.

*P values were calculated using the χ^2 test with Yates's correction.

^bOdds ratio of ∞ were obtained for these resistances because the prevalence of carriage in contacts was 100%.

Table III. Skin carriage of MS^R, MLS^{IR} and MLS^{CR} staphylococci by contacts and controls

Subjects	Number of subjects (<i>n</i> /41) carrying staphylococci expressing		
	MS ^R	MLS ^{IR}	MLS ^{CR}
Contacts	31	20	31
Controls	21 ^a	17	7 ^b

MS^R, Macrolide-streptogramin B resistance; MLS^{IR}, inducible macrolide-lincosamide-streptogramin B resistance; MLS^{CR}, constitutive macrolide-lincosamide-streptogramin B resistance.

Differences in carriage rates between contacts and controls were analysed using the χ^2 test with Yates's correction: ^a*P* < 0.05; ^b*P* < 0.001.

(*P* < 0.05) in contacts compared to controls as was the prevalence of multiply resistant strains. Only aminoglycoside resistance was not found to be raised in contacts by any of the three criteria (prevalence, number or percentage). Calculation of odds ratios revealed that contacts were several times (2.71–8.45 fold, depending on the resistance) more likely to carry strains resistant to clindamycin, fusidic acid, chloramphenicol and trimethoprim than controls (Table II).

The incidence of carriage of MS^R and MLS^R staphylococci also differed in the two groups (Table III). Contacts carried significantly more MS and constitutively MLS resistant staphylococci than controls. The numbers of inducibly MLS resistant strains were similar in both groups.

Discussion

The possibility of remote effects of antibiotic therapy for acne has not previously been investigated. Acne patients are frequently treated with a variety of antibiotics because of the long duration of the disease (8–12 years for most subjects) and because single courses of therapy are palliative rather than curative. It thus seemed to us very likely that parents, siblings or partners might show changes in their commensal staphylococcal skin flora as a result of their regular contact with antibiotic-treated acne patients. The subjects we have chosen to study represent the worst case scenario in that these are the individuals who had the closest contact with patients treated with three or more chemically dissimilar antibiotics over a long period of time (≥ 2 years). The interpretation of the results of this study relies heavily on the statistical significances of the differences found between carriage rates in contacts and controls. This is why we have analysed the data in a number of different ways. If antibiotic therapy had no effect on skin carriage of resistant staphylococci by untreated contacts, then we would predict that either no significant differences would be found between the contacts and controls or that any significant changes would be randomly distributed between the two groups. The observations reported here clearly show that, whichever criterion is used, prevalence, number or percentage, all the significant changes are in one direction, namely an increase in contacts compared to controls. Particularly striking increases were observed in the population densities of erythromycin/clindamycin resistant staphylococci and in the number of multiply resistant strains. At the time of sample collection, only a minority (17/41, 41.5%) of patients were receiving therapy with either of these antibiotics although 40/41 (97.6%) patients had been treated with one or both drugs in the past.

In keeping with the observations of Cove *et al.* (1990) in untreated subjects, a majority of our controls carried staphylococcal strains resistant to tetracycline (88% *vs* 95% in this study), erythromycin (60% *vs* 70%) and fusidic acid (56% *vs* 76%) as well as multiply resistant strains (63% *vs* 78%). The very high carriage rate of tetracycline resistant strains in the control group explains why, using prevalence as a criterion, no difference was found between contacts and controls for this resistance. The almost universal carriage of tetracycline resistant strains by untreated subjects may reflect the extensive use of the tetracyclines in dermatology and general medicine.

The finding that contacts carried significantly more viable staphylococci than controls was unexpected. The \log_{10} counts for the contacts were similar to those previously observed by us for total staphylococcal counts in untreated acne patients (Eady *et al.*, 1990) indicating that it was the counts for the controls which were unpredictably low. The difference in median total viable count was accounted for by the inclusion of six controls but only one contact with staphylococcal population densities outside the lower limit of the range for the remaining 75 individuals (i.e. $< 2.88 \log_{10}$ cfu/cm² skin). These subjects may reflect a sub-group of people whose skin, for genetic reasons, cannot support the growth of large numbers of staphylococci. For example, they may produce very little sebum and/or sweat, both of which provide a range of nutrients for the resident microflora.

The question arises as to whether the higher median total staphylococcal count in contacts could be the single explanation of why contacts also carried more resistant staphylococcal strains. Although this is unlikely (see preceding paragraph), it cannot be ruled out completely. However, calculation of the percentage of the staphylococcal flora resistant to each antibiotic takes into account the difference in total viable count between the two sample groups. When percentage instead of actual numbers was substituted as a criterion, the significant increases in carriage of resistant staphylococci were preserved in all cases except trimethoprim resistance.

The observation that substantially more contacts than controls carried constitutively MLS resistant staphylococci and yet both groups carried similar numbers of inducibly MLS resistant strains (Table III) provides the strongest evidence that the staphylococcal flora of contacts was altered. In untreated subjects, skin carriage of constitutively MLS resistant staphylococci has been shown to be infrequent (9.4% of 64 individuals; Cove *et al.*, 1990). In the present study, such strains were detected on the skin of a minority of controls but a majority of contacts (Table III) suggesting that the contacts had either been directly exposed to erythromycin or clindamycin or had acquired constitutively MLS resistant strains from the treated patients. Erythromycin and clindamycin are each used extensively in the topical therapy of acne and it is conceivable that active drug is transferred from the skin of patients to the contacts. Most (34/41, 83%) of the acne patients whose contacts were sampled had been treated with topical erythromycin, clindamycin or both. One topical erythromycin preparation commonly prescribed in the UK contains 4% w/v of the drug and this had been used by 17/41 patients (41.5%).

In summary, this study has shown that sequential antibiotic therapy for acne apparently leads to increased carriage of singly and multiply resistant CNS on the skin of close contacts of acne patients. The extensive use of antibiotics in the management of acne patients thus appears to be exerting selective pressure for the acquisition of resistant organisms not only within treated individuals but also within persons who come into close or frequent contact with them. This mechanism might account, in part, for the high incidence of carriage of resistant CNS on the skin of untreated subjects.

However, several important questions remain unanswered. Are some antibiotics more prone to promote the acquisition of resistant staphylococci than others? Can a similar effect be observed in individuals who have occasional rather than regular contact with treated patients and by what means is resistance acquired? Two mechanisms are possible: transfer of resistant strains from patient to contact and/or transfer of active drug, especially following topical application. We hope this study stimulates others to help us find the answers to these important questions. It is almost 20 years since Richmond and co-workers (Petrocheilou, Richmond & Bennett, 1977) demonstrated the transfer of a tetracycline resistant *Escherichia coli* strain from the intestine of a female patient to her husband during prolonged tetracycline therapy for acne. Similar research on the much more accessible CNS flora of skin has lagged far behind.

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References

- Archer, G. L. (1988). Molecular epidemiology of multiresistant *Staphylococcus epidermidis*. *Journal of Antimicrobial Chemotherapy* **21**, Suppl. C, 133–8.
- Archer, G. L. (1991). Alteration of cutaneous staphylococcal flora as a consequence of antimicrobial prophylaxis. *Reviews of Infectious Diseases* **13**, Suppl. 10, 805–9.
- Bernstein, J. E. & Shalita, A. R. (1980). Effects of topical erythromycin on aerobic and anaerobic surface flora. *Acta Dermato-Venereologica (Stockholm)* **60**, 537–9.
- Cove, J. H., Eady, E. A. & Cunliffe, W. J. (1990). Skin carriage of antibiotic-resistant coagulase-negative staphylococci in untreated subjects. *Journal of Antimicrobial Chemotherapy* **25**, 459–69.
- Eady, E. A., Cove, J. H., Holland, K. T. & Cunliffe, W. J. (1990). Superior antibacterial action and reduced incidence of bacterial resistance in minocycline compared to tetracycline-treated acne patients. *British Journal of Dermatology* **122**, 233–44.
- Eady, E. A., Holland, K. T. & Cunliffe, W. J. (1982). The use of antibiotics in acne therapy: oral or topical administration? *Journal of Antimicrobial Chemotherapy* **10**, 89–115.
- Gemmell, C. G. & McCartney, A. C. (1990). Coagulase-negative staphylococci within the hospital environment. *Reviews in Medical Microbiology* **1**, 213–8.
- Harkaway, K. S., McGinley, K. J., Foglia, A. N., Lee, W.-L., Fried, F., Shalita, A. R. *et al.* (1992). Antibiotic resistance patterns in coagulase-negative staphylococci after treatment with topical erythromycin, benzoyl peroxide and combination therapy. *British Journal of Dermatology* **126**, 586–90.
- Hedin, G. & Hambræus, A. (1991). Multiply antibiotic resistant *Staphylococcus epidermidis* in patients, staff and environment—a one-week survey in a bone marrow transplant unit. *Journal of Hospital Infection* **17**, 95–106.
- Jenssen, W. D., Thakker-Varia, S., Dubin, D. T. & Weinstein, M. P. (1987). Prevalence of macrolides-lincosamides-streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. *Antimicrobial Agents and Chemotherapy* **31**, 883–8.
- Kloos, W. E. & Schleifer, K. H. (1975). Simplified scheme for routine identification of human *Staphylococcus* species. *Journal of Clinical Microbiology* **1**, 82–8.
- Kotilainen, P., Nikoskelainen, J. & Huovinen, P. (1990). Emergence of ciprofloxacin-resistant coagulase-negative staphylococcal skin flora in immunocompromised patients receiving ciprofloxacin. *Journal of Infectious Diseases* **161**, 41–4.
- Leyden, J. J., Marples, R. R., Mills, O. H. & Kligman, A. M. (1974). Tretinoin and antibiotic therapy in acne vulgaris. *Southern Medical Journal* **67**, 20–5.

- Marples, R. R. & Kligman, A. M. (1971). Ecological effects of oral antibiotics on the microflora of human skin. *Archives of Dermatology* **103**, 148–53.
- Mills, O. H., Kligman, A. M. & Stewart, R. (1975). The clinical effectiveness of topical erythromycin in acne vulgaris. *Cutis* **15**, 93–6.
- Oppenheim, B. A., Hartley, J. W., Lee, W. & Burnie, J. P. (1989). Outbreak of coagulase negative staphylococcus highly resistant to ciprofloxacin in a leukaemia unit. *British Medical Journal* **299**, 294–7.
- Patrick, C. C. (1990). Coagulase-negative staphylococci: pathogens with increasing clinical significance. *Journal of Pediatrics* **116**, 497–507.
- Petrocheilou, V., Richmond, M. H. & Bennett, P. M. (1977). Spread of a single plasmid clone to an untreated individual from a person receiving prolonged tetracycline therapy. *Antimicrobial Agents and Chemotherapy* **12**, 219–25.
- Williamson, P. & Kligman, A. M. (1965). A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology* **45**, 498–503.

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