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# Antibiotic resistance patterns of aerobic coryneforms and furazolidone-resistant Gram-positive cocci from the skin surface of the human axilla and fourth toe cleft

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**Samples of skin surface bacteria from 28 healthy subjects plated directly on to selective and non-selective media revealed that the proportion of aerobic coryneforms and furazolidone-resistant Gram-positive cocci (FURECs) resistant to erythromycin was significantly greater in the fourth toe cleft than in the axilla ($P < 0.05$). There were more erythromycin-resistant bacteria than tetracycline-resistant bacteria at both sites ($P = 0.001$ for the toe cleft; $P < 0.01$ for the axilla). In total, 160 distinct isolates were obtained, of which 42 were FURECs and 118 were aerobic coryneforms. Of these, 153 (96%) were resistant to erythromycin and 66 (41%) to tetracycline. All except seven of the tetracycline-resistant strains were also resistant to erythromycin. The resistant isolates belonged to a variety of species. CDC group ANF corynebacteria were most numerous and composed 31% of all isolates. The majority (76%) of FURECs were identified as *Micrococcus luteus*. MIC determinations on selected strains revealed that tetracycline-resistant FURECs were sensitive to doxycycline and minocycline, as were most tetracycline-resistant coryneforms. Nine coryneform isolates were cross-resistant to all three tetracyclines. Only a minority of erythromycin-resistant FURECs (21%) demonstrated a macrolide–lincosamide–streptogramin type B (MLS)-resistant phenotype with inducible or constitutive cross-resistance to clindamycin and the type B streptogramin, pristinamycin $I_A$. Twenty-nine erythromycin-resistant FURECs had a novel phenotype distinct from MLS and macrolide–streptogramin type B resistance. In contrast, most coryneforms (79%) were MLS resistant. Among the remainder, two unusual erythromycin resistance phenotypes were apparent, both of which differed from the unusual phenotype in FURECs. This study has revealed that the non-staphylococcal aerobic flora of skin contains a considerable reservoir of tetracycline and erythromycin resistance determinants. The three unusual macrolide resistance phenotypes may be associated with novel resistance mechanisms.**

## Introduction

The resident commensal microflora of human skin comprises several bacterial genera, including staphylococci, micrococci, corynebacteria, brevibacteria and propionibacteria.[1–4] The genus formerly known as *Micrococcus* has been dissected into five separate genera; *Micrococcus* Cohn 1872 gen. emend., *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov. and *Dermacoccus* gen. nov.[5] These organisms form a group of furazolidone-resistant Gram-positive cocci (FUREC). The prevalence of antibiotic-resistant coagulase-negative staphylococci (CNS) at various skin sites in both healthy and hospitalized patients has received considerable attention because of the role of these organisms as nosocomial pathogens in compromised hosts. It is known that they harbour a considerable reservoir of resistance genes even in untreated individuals and that they share a gene pool with *Staphylococcus aureus*.[2,6–8] The non-staphylococcal components of the skin flora are phylogenetically much more closely related to one another than any of them are to staphylococci. In contrast to the staphylococci, their chromosomal DNA is G+C-rich. It

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seemed to us likely that these organisms may also share a resistance gene pool, distinct from that in staphylococci, which has been little characterized.

The aim of this study was to determine the prevalence of different erythromycin and tetracycline resistance phenotypes in populations of aerobic coryneform bacteria and FURECs from the human axilla and fourth toe cleft. These moist skin sites are the major habitats of these organisms.[9] One reason for selecting these particular antibiotics is that we already know that cutaneous populations of staphylococci are commonly resistant to them, as are propionibacteria from treated acne patients.[10–12] Thus it seemed likely that the other major resident skin bacteria would demonstrate similar resistance. A second reason for looking at erythromycin resistance was to detect novel phenotypes that were incompatible with the possession of either macrolide–lincosamide–streptogramin type B (MLS) or macrolide–streptogramin type B (MS) resistance determinants (*erm* genes or *msrA*).

## Materials and methods

### *Bacterial strains*

Aerobic coryneform bacteria and FURECs were isolated from either the fourth toe cleft of the right foot or from both axillae of 28 healthy volunteers (22 male, six female; age range 21–46 years), none of whom were receiving antibiotic therapy at the time of sampling. Only three volunteers were sampled from both sites, to minimize the chance of re-isolating the same strains. Samples from the fourth toe cleft were taken, using sterile cotton-tipped swabs moistened in full-strength wash fluid, from the base of the cleft and along the sides of the toes forming the cleft.[13] Axillary bacteria were collected using the detergent scrub technique of Williamson & Kligman.[13] Volunteers had been instructed to stop using deodorants for 1 week before sample collection.

### *Growth media*

Total viable counts were obtained by plating 0.1 mL aliquots of decimal dilutions of wash fluid on to coryneform agar as described by Leeming *et al.*,[14] with the inclusion of furazolidone 6 mg/L to inhibit the growth of staphylococci.[15] The same medium with the addition of either erythromycin 5 mg/L or tetracycline 10 mg/L was used to obtain differential counts of resistant bacteria.[11] Plates were examined after 48 h incubation at 37°C followed by 48 h at room temperature to enhance pigmentation and make it easier to discriminate colony types. All distinct colony types from the selective media were Gram-stained to differentiate Gram-positive cocci and Gram-positive pleomorphic rods. Isolates of different morphology were excluded. Following primary isolation, strains were purified and maintained on either Direct Sensitest Agar (DST) for Gram-positive cocci or DST plus 5% sterile defibrinated horse blood (E&O Laboratories, Bonnybridge, UK) and 0.1% Tween-80 (DST-BT) for coryneforms. Strains were stored in liquid nitrogen.

Gram-positive cocci were typed using API ID 32 Staph strips (bioMérieux, Basingstoke, UK) and coryneforms using API Coryne strips (bioMérieux). In addition all isolates were screened for resistance to mupirocin using 5 μg discs.

### *Antibiotic susceptibility and MICs*

The antibiogram of each isolate was determined by disc testing on DST or DST-BT as appropriate using 20 mL volumes of medium in 90 mm diameter Petri dishes. The antibiotics and concentrations used are shown in Table I. *Micrococcus luteus* NCTC 9341 and *Corynebacterium glutamicum* R163 were used as controls. Erythromycin-resistant strains were further tested for erythromycin-inducible resistance to pristinamycin $I_A$, spiramycin and clindamycin. Blunting of the inhibition zone proximal to the erythromycin disc was taken as evidence of inducibility. A minority of isolates of both FURECs and coryneforms which gave small zone diameters ($\leqslant$15 mm) or grew up to penicillin discs were qualitatively screened for $\beta$-lactamase production using the chromogenic substrate, nitrocefin.

MICs of MLS and tetracycline antibiotics were determined for selected strains by agar dilution on DST or DST-BT as appropriate. Inocula were prepared by diluting overnight cultures to give a standard inoculum of $10^4$ cfu/spot delivered by a multipoint inoculator. The MIC of each antibiotic for each organism was recorded after overnight incubation at 37°C as the lowest concentration yielding no growth or a barely visible haze.

Clindamycin and spiramycin were dissolved and diluted in distilled water. Erythromycin and azithromycin were dissolved in ethanol and diluted in distilled water. Pristinamycin was dissolved in dimethylsulphoxide, and furazolidone in acetone.

### *Media components, chemicals and testing kits*

All growth media and nitrocefin were obtained from Oxoid, Basingstoke, UK; all other chemicals and antibiotics were from Sigma–Aldrich, Poole, UK with the exceptions of pristinamycin $I_A$ and azithromycin which were gifts from Rhône–Poulenc Rorer (De Vitry-Alfortville, France) and Pfizer (Sandwich, Kent), respectively. Antibiotic-impregnated discs were from Mast Laboratories, Bootle, UK.

### *Data analysis*

Within-group differences in median bacterial counts were determined using Wilcoxon's matched pairs. Between-

**Table I.** Interpretation of disc tests for antibiotic susceptibility

| Antibiotic | Disc concentration (μg) | Zone diameter (mm) indicating resistance for | |
|---|---|---|---|
| | | Gram-positive cocci | coryneforms |
| Tetracycline[a] | 10 | ≤10 | ≤10 |
| Minocycline | 30 | ≤10 | ≤15 |
| Rifampicin | 5 | ≤10 | ≤10 |
| Norfloxacin | 10 | ≤10 | ≤15 |
| Novobiocin | 5 | NDZ | NDZ |
| Vancomycin | 5 | NDZ | NDZ |
| Fusidic acid | 10 | ≤10 | ≤10 |
| Chloramphenicol | 10 | NDZ | ≤10 |
| Trimethoprim | 2.5 | ≤10 | N/A |
| Gentamicin | 10 | ≤15 | ≤15 |
| Netilmicin | 10 | ≤10 | ≤10 |
| Mupirocin[b] | 5 | NDZ | NDZ |
| Penicillin G | 1 IU | | |
| Cloxacillin | 5 | | |
| Cefuroxime | 30 | ≤15 | ≤15 |

IU, international units; NDZ, no detectable zone.
[a]Some strains producing intermediate zone sizes (11–15 mm) were found to be tetracycline sensitive and others tetracycline resistant when MIC determinations were carried out. This variation was species dependent.
[b]All aerobic coryneforms, micrococci and related Gram-positive cocci should be intrinsically resistant to mupirocin.

**Table II.** Total viable counts and numbers of erythromycin- and tetracycline-resistant bacteria from the axilla and fourth toe cleft

| Site sampled | Number of subjects | $Log_{10}$ total viable count[a] | | $Log_{10}$ erythromycin-resistant bacteria | | $Log_{10}$ tetracycline-resistant bacteria | |
|---|---|---|---|---|---|---|---|
| | | median (95% CL)[b] | range | median (95% CL) | range | median (95% CL) | range |
| Fourth toe cleft | 18 | 6.25 (5.72, 6.54) | 4.21–7.72 | 4.87[c] (4.12, 6.23) | 2.61–6.99 | 3.24[c] (2.65, 3.69) | 1.90–4.92 |
| Axilla | 13 | 5.50 (4.65, 6.29) | 2.13–6.53 | 2.72[d] (1.69, 3.26) | 0.52–5.12 | 1.84[d] (0.52, 2.91) | 0.00–3.68 |

[a]Counts are per swab for the toe cleft and per $cm^2$ for the axilla.
[b]95% confidence limits derived using the sign interval.
[c,d]Significantly more bacteria were resistant to erythromycin than tetracycline ([c]$P = 0.001$ or [d]$P < 0.01$); Wilcoxon matched pairs.

group differences in the proportion of resistant bacteria were determined using the Mann–Whitney U test. All computations were performed on release 8.0 of Minitab.

## Results

Table II summarizes the bacterial counts for the axilla and fourth toe cleft. Although axillary samples were collected using a quantitative technique, such methodology was not possible for sampling toe clefts. Despite this difference in sample collection, the median number of viable bacteria detected at each site was similar and there were significantly more bacteria resistant to erythromycin than tetracycline at both sites. When the number of resistant bacteria was transformed into a percentage of the total viable count at each site, the same trend was apparent (Table III) in that there were proportionally more bacteria resistant to erythromycin than tetracycline at both sites and the proportion of bacteria resistant to erythromycin was significantly higher in the fourth toe cleft than in the axilla. In total, 160 distinct isolates were obtained, of which 42 were FURECs and 118 were aerobic coryneforms (Table IV). Of these, 157 (98%) were resistant to erythromycin and 66 (41%) to tetracycline. Only seven strains resistant to tetracycline were not also resistant to erythromycin. The

resistant isolates belonged to a variety of species (Table IV). CDC group ANF corynebacteria were most numerous and composed 31% of all isolates. The majority (76%) of FURECs were identified as *M. luteus*.

Before co-resistance of the isolates to other antibiotics could be determined, it was necessary to plot frequency distributions of zone diameters obtained in disc tests for each antibiotic used. These distributions, most of which were bimodal, were used to determine the zone diameter cut-off points to distinguish sensitive and resistant isolates as shown in Table I. The distribution of zone sizes for penicillin and cloxacillin did not reveal two populations and could not be used to discriminate between sensitivity and resistance. Using the chromogenic cephalosporin, nitrocefin, none of the isolates (corynebacterial or FUREC) with small (<15 mm) zone diameters produced penicillinase. The most commonly encountered co-resistance in FURECs was to the fluoroquinolone, norfloxacin

**Table III.** Proportion of the total viable bacterial count resistant to erythromycin or tetracycline in the axilla compared with the fourth toe cleft

| Site sampled | Number of subjects | % erythromycin-resistant | | % tetracycline-resistant | |
|---|---|---|---|---|---|
| | | median (95% CL)[a] | range | median (95% CL) | range |
| Fourth toe cleft | 18 | 6.93[b,d] (4.29, 18.3) | 0.02–100 | 0.06[b] (0.01, 0.92) | 0.001–10.88 |
| Axilla | 13 | 0.14[c,d] (0.015, 4.22) | 0.00–100 | 0.02[c] (0.007, 0.22) | 0.00–4.82 |

[a]95% confidence limits derived using the sign interval.
[b,c]A significantly greater proportion of bacteria were resistant to erythromycin than tetracycline ([b]$P < 0.01$ or [c]$P < 0.05$; Wilcoxon matched pairs).
[d]The proportion of bacteria resistant to erythromycin in the fourth toe cleft was significantly greater than in the axilla ($P < 0.05$).

**Table IV.** Details of isolates

| Bacterium | Number of isolates | Number resistant to | |
|---|---|---|---|
| | | erythromycin | tetracycline[a] |
| *Micrococcus luteus* | 32 | 32 | 2 |
| *Kocuria rosea* | 5 | 5 | 1 |
| *Kocuria varians* | 3 | 3 | 2 |
| *Kocuria kristinae* | 1 | 1 | 0 |
| *Kytococcus nishinomiyaensis* | 1 | 1 | 1 |
| *Brevibacterium* | 5 | 5 | 3 |
| *Corynebacterium aquaticum* | 11 | 7 | 10 |
| *Corynebacterium jeikeium* | 7 | 7 | 3 |
| *Corynebacterium minutissimum* | 3 | 2 | 3 |
| *Corynebacterium pseudodiphtheriae* | 1 | 1 | 0 |
| *Corynebacterium striatum* | 2 | 2 | 2 |
| *Corynebacterium xerosis* | 1 | 1 | 1 |
| *Corynebacterium* | | | |
| group A | 5 | 5 | 4 |
| group ANF | 50 | 48 | 21 |
| group D2 | 1 | 1 | 0 |
| group F | 1 | 1 | 0 |
| group G1 | 5 | 5 | 2 |
| group G2 | 16 | 16 | 3 |
| *Oeskovia* | 1 | 1 | 0 |
| *Rhodococcus equi* | 1 | 1 | 0 |
| Unidentified coryneforms | 8 | 8 | 8 |
| All isolates | 160 | 153 | 66 |

[a]Only three isolates (one *C. minutissimum* and two group ANF corynebacteria) were resistant to tetracycline and sensitive to erythromycin.

(Table V). The only other resistances detected in more than one Gram-positive coccal isolate were to chloramphenicol and trimethoprim. As far as the coryneforms were concerned, resistance to fusidic acid, chloramphenicol and norfloxacin was the most frequent. A minority of isolates (<1%) were resistant to novobiocin or netilmicin. Nine of 60 tetracycline-resistant coryneforms (15%) were also resistant to minocycline.

In order to characterize the tetracycline and erythromycin resistance more fully, MIC determinations were carried out for selected strains. The six strains of tetracycline-resistant FURECs were inhibited by tetracycline 8–64 mg/L but remained sensitive to doxycycline and minocycline (MICs $\leqslant$ 1 mg/L). The MICs of various tetracyclines for sensitive and resistant coryneforms are shown in Table VI. The strains fell into two classes: those that were resistant to tetracycline only, for which the modal MIC of tetracycline was 16 mg/L, and secondly, nine strains that were cross-resistant to all three tetracyclines for which the modal MIC of tetracycline was much higher, 256 mg/L.

**Table V.** Summary of co-resistance in erythromycin- and/or tetracycline-resistant isolates

| | Number (%) of resistant isolates of | |
|---|---|---|
| Antibiotic[a] | Gram-positive cocci | coryneforms |
| Minocycline | 0 | 9 (8) |
| Rifampicin | 0 | 1 (1) |
| Norfloxacin | 25 (60) | 23 (19) |
| Novobiocin | 0 | 4 (3) |
| Vancomycin | 0 | 0 |
| Fusidic acid | 1 (2) | 28 (24) |
| Chloramphenicol | 13 (31) | 27 (23) |
| Trimethoprim | 16 (38) | N/A[b] |
| Gentamicin | 1 (2) | 1 (1) |
| Netilmicin | 0 | 5 (4) |
| Cefuroxime | 1 (2) | 1 (1) |

[a] Data for penicillin and cloxacillin are not given because results could not be obtained from zone size distributions.
[b] Coryneform isolates could not be screened for resistance to trimethoprim because the growth medium contained blood.

The majority of erythromycin-resistant FURECs exhibited an unusual phenotype in that they were sensitive to clindamycin and pristinamycin $I_A$ but inducibly resistant, albeit weakly, to spiramycin in disc tests. The MICs of erythromycin for these isolates (designated group A) were low in comparison with those for isolates exhibiting an MLS-resistant phenotype (Table VII). MICs of azithromycin were four-fold higher than MICs of erythromycin. Without induction, the MICs of spiramycin were similar to those for fully sensitive strains. Of the isolates for which MICs were determined, 23 exhibited the unusual phenotype, two had a constitutive MLS-resistant phenotype and three had phenotypes which could not be classified into either group. Of the 14 isolates for which MICs were not determined, six had the unusual phenotype and seven had either an inducible or a constitutive MLS resistance phenotype in disc tests. The results for one strain suggested that it did not belong to either group. Two of the atypical strains showed reversion of erythromycin resistance in the presence of pristinamycin. There is insufficient evidence to comment reliably on the distribution of resistance phenotypes among the micrococci as most of the isolates were *M. luteus* and inevitably, therefore, the group A phenotype was predominantly associated with this species (data not shown).

In contrast to the FURECs, most of the aerobic coryneforms were MLS resistant (Table VIII). In total, 89 isolates (75%) demonstrated an MLS-resistant phenotype.

**Table VI.** MICs of various tetracyclines for sensitive and resistant coryneforms

| | Range (mode) of MICs, mg/L | |
|---|---|---|
| Antibiotic | sensitive strains ($n$ = 20) | resistant strains ($n$ = 31) |
| Tetracycline | 0.25–2.0 (1) | 8–256 (16, 256)[a] |
| Minocycline | 0.25–2.0 (1) | 64–256 (256) |
| Doxycycline | 0.125–4 (2) | 16–128 (128) |

[a] The first value is the modal MIC for strains resistant to tetracycline only. The second value is the modal MIC for strains cross-resistant to all three tetracyclines.

**Table VII.** MICs of various MLS antibiotics for sensitive and resistant Gram-positive cocci

| | MIC range (mode) mg/L | | |
|---|---|---|---|
| Antibiotic | sensitive strains ($n$ = 6) | MLS-resistant strains ($n$ = 2) | group A strains ($n$ = 23) |
| Erythromycin | 0.06–0.5 (0.06, 0.12) | 256, $\geqslant$512 | 8–32 (16) |
| Spiramycin | 0.5–2 (1) | 256, $\geqslant$512 | 0.5–8 (1) |
| Azithromycin | 0.12–1 (0.12) | 128, $\geqslant$512 | 64–256 (64) |
| Clindamycin | 0.12–0.5 (0.5) | $\geqslant$512 | 0.25–1 (0.5) |

In disc tests the majority of isolates showed constitutive expression of erythromycin, spiramycin and clindamycin resistance but erythromycin-inducible expression of pristinamycin $I_A$ resistance. In addition, a minority of isolates showed inducibility of clindamycin or spiramycin resistance or were constitutively resistant to pristinamycin. The resistance phenotype was labile in that the same isolates tested on different occasions produced different results with respect to the inducibility of resistance. The MIC results revealed that the degree of resistance to erythromycin was eight to 128 times lower than that to azithromycin and/or clindamycin for 12 of the 32 isolates tested (38%). Although MICs varied between isolates, this variation was not species dependent.

Those isolates that did not express MLS resistance could be assigned to one of two phenotypic classes. One group (designated B1) comprised 11 strains that expressed low-level resistance to erythromycin and azithromycin but which were sensitive to clindamycin and/or spiramycin (Table VIII). In disc tests, the strains were sensitive to pristinamycin $I_A$ (Table IX). The second group (designated B2 because of their similarity to B1 strains) comprised 11 strains that demonstrated low-level resistance to erythromycin, spiramycin, clindamycin and, usually but not always, azithromycin (Table VIII). In disc tests, group B2 strains were usually sensitive to pristinamycin $I_A$ (Table IX). Neither group demonstrated erythromycin-inducible resistance to any other antibiotic. The phenotypic charac-

**Table VIII.** MICs of various MLS antibiotics for sensitive and resistant coryneform bacteria

| | MIC range (mode) mg/L | | | |
|---|---|---|---|---|
| | | | non-MLS-resistant strains | |
| Antibiotic | sensitive strains ($n$ = 10)[a] | MLS-resistant strains ($n$ = 32) | group B1 ($n$ = 11) | group B2 ($n$ = 11) |
| Erythromycin | 0.06–0.25 | 4–⩾512 (⩾512) | 4–32 (16) | 4 – 16 (4) |
| Spiramycin | 0.25–2 | 4–⩾512 (⩾512) | 0.25–2 (0.5) | 4–8 (4) |
| Azithromycin | 0.03–0.5 | 64–⩾512 (⩾512) | 8–32 (32) | 0.5 – 16 (4) |
| Clindamycin | 0.06–0.5 | 16[b]–⩾512 (⩾512) | 0.25–4 (0.5) | 4–16 (8) |

[a]Includes the *C. glutamicum* control strain.
[b]One inducible strain was inhibited by clindamycin 0.12 mg/L (uninduced).

**Table IX.** Characteristics of group A and B erythromycin-resistant isolates

| Group | Antibiotic and disc strength (μg) | Sensitive (S) or resistant (R)? | Zone diameter (mm)[a] | Inducible by erythromycin? |
|---|---|---|---|---|
| A | erythromycin 5 | R | NDZ–10 | N/A |
| | spiramycin 5 | R | 30–38/27–33[b] | yes |
| | clindamycin 2 | S | 18–35 | no |
| | pristinamycin 50 | S | 20–35 | no |
| B1 | erythromycin 5 | R | 9–15 | N/A |
| | spiramycin 5 | S | 22–33 | no |
| | clindamycin 2 | [S][c] | [28–34][d] [16–24][d] | No |
| | pristinamycin 50 | S | 17–28 | no |
| B2 | erythromycin 5 | R | NDZ–15 [23][e] | N/A |
| | spiramycin 5 | R | 11–19 | no |
| | clindamycin 2 | R | NDZ–14 | no |
| | pristinamycin 50 | S or R | 8–21 | no |

NDZ, no detectable zone.
[a]Zone diameters for sensitive strains were ⩾25 mm, except for pristinamycin $I_A$ in which case they were ⩾16 mm.
[b]The first value represents the maximum diameter and the second value represents the diameter where the zone had been blunted by proximity to an erythromycin disc.
[c]A minority of isolates were resistant.
[d]The zone diameters given first refer to those isolates which were sensitive to clindamycin (MIC 0.25–0.5 mg/L). Those given second refer to the minority of isolates which were resistant to clindamycin (MIC 2–4 mg/L).
[e]One isolate for which the erythromycin MIC was 4 mg/L gave an inhibition zone of 23 mm.

**Table X.** Phenotype of four *Corynebacterium aquaticum* isolates with low-level resistance to clindamycin and spiramycin

| | Antibiotic[a] | | | | |
|---|---|---|---|---|---|
| | erythromycin | spiramycin | azithromycin | clindamycin | pristinamycin $I_A$ |
| MIC range (mg/L) | <0.06[b] | 2–4 | <0.06 | 8–16 | N/T |
| Zone diameter (mm) | 28–32[b] | 17–26 | N/T | NDZ–15 | 15–27 |

NDZ, no detectable zone; N/T, not tested.
[a]For disc concentrations see Table IX.
[b]One of the four strains was inhibited by erythromycin 0.25 mg/L and gave a zone diameter of 23 mm around a 5 μg erythromycin disc.

teristics of group B coryneforms are summarized in Table IX which also shows how their resistance phenotype differs from that of group A FURECs. Among the coryneforms, the B1 and B2 resistance phenotypes were not associated with particular species (data not shown). Four tetracycline-resistant isolates of *Corynebacterium aquaticum* selected as controls for MIC determinations with MLS antibiotics demonstrated low levels of resistance to clindamycin and spiramycin but were fully sensitive to erythromycin and azithromycin (Table X).

One unusual feature of many of the isolates with low-level resistance to one or more MLS antibiotics was that disc zone diameters could not be used reliably as predictors of resistance. This can be seen, for example, by comparing the zone diameters in Table IX with the MICs in Table VIII (see also Table X). Isolates that required ⩽16 mg/L of antibiotic to inhibit their growth in MIC determinations demonstrated considerable inhibition zones around discs impregnated with lower concentrations of the corresponding antibiotic.

## Discussion

This study has shown that, even in the absence of selective pressure, the axilla and fourth toe cleft of healthy individuals support large populations of antibiotic-resistant aerobic coryneforms and FURECs. Significantly more of these bacteria were resistant to erythromycin than to tetracycline at both body sites. This is consistent with our previous findings for the cutaneous propionibacteria, the other major constituent of the skin flora with G+C-rich DNA,[16] but contrasts with the low G+C cutaneous CNS in which prevalence of resistance to tetracycline is greater than that to erythromycin.[2] Comparison between the two main groups of cutaneous Gram-positive cocci revealed an even more marked difference between CNS and FURECs. The prevalence of tetracycline resistance in the FURECs was unusually low at 14.3% (6/42) compared with our previous report where 87% of untreated subjects carry tetracycline-resistant CNS.[10] It was also significantly lower than the prevalence of tetracycline resistance in aerobic coryneforms at 50.8% (60/118) (Table IV). More generally, antibiotic-resistant bacteria could be isolated in greater numbers from the fourth toe web than in the axilla. Thus a picture emerges where, in the absence of selective pressure, the skin surface carries a significant reservoir of antibiotic-resistant bacteria and in which the prevalence of individual resistance determinants is dependent on both bacterial type and body site.

Although breakpoint data are not given for cutaneous aerobic coryneforms and FURECs in the recent NCCLS guidelines,[17] comparison of data given for other organisms, e.g. *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp., with the zone sizes used in this study to assign co-resistance shows that the criteria used to define resistance were rigorous (see Table V). Analysis of co-resistance adds an extra dimension of complexity to the picture outlined above. Although all the antibiotic resistance identified could be traced either to agents used topically or to antibiotics that reach intact skin, the resistance profiles were highly dependent upon bacterial type. For example, the most common co-resistance in the cutaneous aerobic coryneforms was to fusidic acid and chloramphenicol, antibiotics that are used topically. The resistance pattern in the FURECs differed from that in both the CNS[10] and the cutaneous propionibacteria,[16] with a higher prevalence of resistance to chloramphenicol and trimethoprim. Notably, the most common co-resistance in FURECs was to the fluoroquinolone, norfloxacin. In the other groups of skin bacteria, the aerobic *Propionibacterium* spp. and the CNS, there is a paucity of information on the prevalence of fluoroquinolone resistance in untreated subjects. Further studies are also required to quantify the effects of fluoroquinolone treatment on the acquisition of fluoroquinolone resistance by propionibacteria, aerobic coryneforms and FURECs, although it has been shown that the cutaneous CNS acquire resistance to ciprofloxacin rapidly as a result of excretion of the drug in sweat.[18] This route of delivery of quinolones to the skin surface may be significant in that FURECs preferentially colonize moist skin surface sites.[9] Unfortunately, it was not possible to discriminate between sensitivity and resistance to penicillins in either cutaneous aerobic coryneforms or FURECs. Measurement of zone

sizes of all the sensitive and resistant strains included in this study failed to reveal a bimodal distribution to distinguish the two populations. A lack of breakpoint data for coryneform suseptibility to $\beta$-lactams has also been noted by other workers[19] and this, together with the observation that none of the isolates with smaller zones of inhibition produced penicillinase, may point to an alternative resistance mechanism in these isolates. One possibility could be a succession of mutations leading to steadily increasing resistance with no clear distinction between sensitive and resistant strains.

The six tetracycline-resistant FURECs (MICs 8–64 mg/L) were sensitive to the other tetracyclines, doxycycline and minocycline. In contrast, the aerobic cutaneous coryneforms fell into two groups: those that were resistant to tetracycline alone (modal MIC 16 mg/L), and those (comprising nine strains) that were resistant to all three tetracyclines, for which the modal MIC of tetracycline was much higher (256 mg/L). Resistance to tetracycline alone would suggest carriage of either determinants encoding tetracycline efflux systems of the major facilitator type[20] or, as recently reported in *Corynebacterium striatum*, an ABC transporter mediating drug efflux encoded by *tetAB*.[21] Alternatively, coryneform resistance to all three tetracyclines appears to mirror the phenotype identified in staphylococci mediated via ribosomal protection in which *TetO/TetM* encodes a homologue of elongation factor G.[22]

Analysis of the MLS resistance phenotypes suggested the presence of several different antibiotic resistance mechanisms, some of which may be novel. Most cutaneous aerobic coryneforms and a number of FURECs showed classic MLS resistance, easily explained either by expression of inducible/constitutive erythromycin ribosomal methylase (*erm*) genes[23] or by a mutation at base 2058 in the 23S ribosomal RNA.[24] However, some isolates exhibited unusual phenotypes which could not be placed in these categories and are not compatible with other known erythromycin resistance mechanisms. Examples of these included (i) the group A FURECs which were erythromycin resistant, sensitive to clindamycin and pristinamycin $I_A$ and weakly inducibly resistant to spiramycin (the majority of erythromycin-resistant FURECs fell into this group); (ii) the group B1 aerobic cutaneous coryneforms which expressed low-level resistance to erythromycin and azithromycin, but were sensitive to pristinamycin $I_A$, clindamycin and/or spiramycin; and (iii) the group B2 aerobic cutaneous coryneforms which expressed low-level resistance to erythromycin, spiramycin and clindamycin and usually to azithromycin and pristinamycin $I_A$. Resistance to the MLS antibiotics in the latter two groups was non-inducible and, because of the low level of some of the resistance identified, it was found that MIC determinations provided a more reliable indicator of resistance phenotype than the use of disc tests. It is likely that genetic analysis of these strains will identify previously unreported antibiotic resistance genes and that this approach would be facilitated by the development of genetic technology using *C. glutamicum*.[21]

There was strong evidence for a link between tetracycline and erythromycin resistance in the aerobic cutaneous coryneforms. In both axilla and toe cleft the vast majority of these bacteria were sensitive to erythromycin. Erythromycin-resistant strains represented $<10\%$ of the median count in the toe cleft and $<1\%$ in the axilla (Table II). In the absence of linkage, a majority of tetracycline-resistant strains would, therefore, be expected to be erythromycin sensitive. Only seven isolates were tetracycline resistant without also being erythromycin resistant (Table IV). This undoubtedly reflects both antibiotic usage as well as genetic linkage of the resistance determinants themselves. For example, in *C. striatum*, the plasmid pTP10, which carries the tetracycline resistance determinant *tetAB*, also carries genes encoding resistance to kanamycin, erythromycin and chloramphenicol.[21] There are clearly many examples of linked antibiotic resistance genes in cutaneous CNS.[25] Interestingly, mobile genetic elements have yet to be identified in the cutaneous propionibacteria and resistance to both erythromycin and tetracycline has been shown to be caused by mutations in genes encoding ribosomal RNA.[24,26]

In addition to the short-term use of antibiotics for the treatment of acute infections, the long-term use of antibiotics in dermatology, especially for the treatment of acne vulgaris, represents a significant source of selective pressure for the evolution of antibiotic-resistant organisms.[16] The results of this study show that the axilla and toe cleft of untreated subjects harbour a significant reservoir of G+C-rich antibiotic-resistant bacteria. In addition many of these express novel antibiotic resistance phenotypes. It is certain that during antibiotic treatment the proportion of antibiotic-resistant cutaneous aerobic coryneforms and FURECs will increase significantly. Clearly they share a common niche with the G+C-rich cutaneous propionibacteria and the A+T-rich CNS. Thus it is likely that, under conditions of prolonged selection, gene exchange between these groups of bacteria will provide a significant impetus for the further evolution and spread of antibiotic resistance among cutaneous bacteria.

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