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Use of Selective Broth Enrichment to Determine the Prevalence of Multiply Resistant JK Corynebacteria on Skin

LUCY S. TOMPKINS,[1,2]* FAWZIYAH JUFFALI,[2] AND WALTER E. STAMM[3]

Departments of Laboratory Medicine,[1] Microbiology and Immunology,[2] and Medicine,[3] University of Washington School of Medicine and Harborview Medical Center, Seattle, Washington 98195

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Use of a selective broth enrichment procedure disclosed a low prevalence (1%) of multiply resistant JK corynebacteria on the skin of healthy adults.

Recent attention has been directed to infections caused by a new *Corynebacterium* species called group JK. Common features of the first 50 reported cases included hospital acquisition (5, 7, 9) and multiple antibiotic resistance of nearly all of the JK strains isolated from blood or deep tissue infection. Despite the apparent nosocomial acquisition of JK infections, we have found little evidence of person-to-person transmission during prospective colonization studies of hospitalized patients (9). A hypothesis reconciling these observations is that resistant JK corynebacteria are normally present on the skin in low numbers and proliferate under the selective pressure of antibiotic therapy. Alternatively, susceptible JK strains might be constituents of the skin flora, with only rare resistant mutants proliferating during antibiotic therapy.

In a previous survey, we attempted to isolate group JK from inguinal and perirectal skin by employing a premoistened swab and selective agar containing gentamicin and cephalothin (9). Directly plating swabs onto this medium, we found that the prevalence of resistant JK strains in healthy individuals is very low (1 of 94) (9). However, this low recovery rate does not preclude the possibility that group JK strains are present in concentrations too low to be detected in a selective agar medium without prior broth enrichment. When compared with selective agar medium alone (1), selective broth enrichment substantially enhances the recovery rate of other bacteria, including group B streptococci.

To determine whether lower concentrations of group JK are present on the skin, we developed a selective enrichment broth which promotes the growth of multiply resistant strains while inhibiting other skin flora. In addition, we modified our method of obtaining skin cultures and sampled more skin sites, including those from which other lipophilic diphtheroids have been recovered. Using these three modifications, we surveyed two patient groups: 51 adults attending an adult health clinic, none of whom had received antibiotics, and 12 adults hospitalized at Harborview Medical Center, Seattle, many of whom had received broad-spectrum antibiotics before the survey. Axilla, inguinal region, toeweb, and forehead skin was cultured by swabbing a circular area, 4 cm in diameter, through an aluminum foil template for 60 s, using a sterile cotton swab (Swabe; Falcon Plastics) (3) premoistened in 0.1% Triton X-100 (Rohm and Haas; Sigma Chemical Co.) in 0.075 M phosphate buffer, pH 7.9 (10). Swabs were then agitated for 1 min in 8 ml of selective broth medium. After aerobic incubation at 35°C for 18 to 24 h, 0.1 ml of the broth was subcultured onto selective agar medium, and the plates were held at 35°C for another 48 to 96 h. Colonies which were catalase positive and had a coryneform morphology as determined by Gram staining were stocked and later identified as JK group by the criteria of Riley et al. (8).

The selective broth medium, which supported the growth of a multiply resistant JK strain (HD119) and inhibited normal skin flora, included Trypticase soy broth (BBL Microbiology Systems) containing 1% yeast, 0.5% Tween 80 (Sigma Chemical Co.), and 15 μg of gentamicin per ml (Schering Corp.). HD119 increased by 3 to 4 logs in selective broth medium after 18 h of incubation at 35°C when inoculated alone or in the presence of higher concentrations of normal skin flora. The selective agar medium which we employed contained 10 μg of gentamicin per ml and 1 μg of cephapirin (Bristol Laboratories) incorporated in Trypticase soy agar containing sheep blood. Failure to incorporate a cephalosporin in selective agar medium allowed some *Staphylococcus epidermidis* isolates to overgrow the JK corynebacteria.

In spite of the broth enrichment procedure (Table 1), we found the prevalence of resistant JK corynebacteria to be low (1 of 51 patients) and not significantly different from the prevalence found in our previous survey (1 of 96; $P = 0.5$, determined by Fisher exact test). The prev-

TABLE 1. Skin colonization of multiresistant group JK corynebacteria in healthy adults and hospitalized patients

Study group	No. of individuals cultured	No. of culture-positive patients (%)
Present study		
Healthy adults	51	1 (2)
Hospitalized patients	12	3 (25)
Stamm et al. (9)		
Healthy adults	94	1 (1)
Hospitalized patients	82	11 (13)
Bone marrow transplant patients	42	17 (40)
Gill et al. (4)		
Healthy adults	17	2 (12)
Hospitalized patients	20	7 (35)

alence rate of 25% (3 of 12) from hospitalized adults (Table 1), however, was significantly higher than the rate for healthy adults ($P = 0.0197$) and correlated with previous antibiotic therapy. The three hospitalized patients who were colonized had received broad-spectrum antibiotics, including cephalosporins, aminoglycosides, or tetracycline before culturing was performed. The toeweb was the most commonly colonized site of the four culture-positive individuals; the inguinal area was the next most frequently colonized area.

These data indicated that multiply resistant JK corynebacteria are only rarely present on the skin of healthy adults but are more often found in hospitalized patients on antibiotics. Gill et al. (4) found similar results (Table 1). However, the recent recovery of susceptible JK corynebacteria from a high percentage of infected prosthetic heart valves (6) suggests that, unlike the multiply resistant strains, susceptible strains of JK may be constituents of the normal adult flora. The most likely explanation for the increased prevalence of resistant organisms on the skin of antibiotic-treated patients and the paucity of resistant strains on the skin of healthy individuals is that rare antibiotic-resistant mutants of indigenous susceptible strains are selected by antibiotic therapy and proliferate when the competing, susceptible flora are reduced. No studies of the prevalence of antibiotic-susceptible strains of JK corynebacteria on skin have been reported. Undoubtedly, the lack of differential characteristics of this group is an impediment to the development of a selective medium for their recovery.

It seems clear that less emphasis should be placed on multiple antibiotic resistance as an identification characteristic of this group of corynebacteria. Although 98% of the first 50 isolates reported were determined to be resistant to cephalosporins and aminoglycosides (5, 7, 9), another survey of 95 isolates submitted to the Centers for Disease Control has revealed that only 40 and 51% were resistant to gentamicin and cephalothin, respectively (8). Until further definitive taxonomic studies are performed on the strains included in the JK group, we suggest that all *Corynebacterium* isolates sharing certain biochemical properties (nitrate, urease, and gelatin hydrolysis negative; slow fermentation of glucose and maltose but not of sucrose or starch [2]), should be tentatively assigned to the JK group regardless of antibiotic susceptibility profile.

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