Isolation and Characterization of Corynebacteria from Burned Children

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A total of 221 strains of corynebacteria were isolated and characterized by methods which included tests encompassing five schemes proposed for grouping cutaneous diphtheroids. Seventy-one strains (group I) were isolated from the hospital air in patient areas and from the normal skins of children admitted for reconstructive surgery of old healed burns and from the normal skins of nursing personnel. One hundred and fifty strains (group II) were isolated from various clinical specimens and from normal skins of a population of acutely burned children. The majority of the strains in group I were lipophilic and contained the largest number of fluorescent strains. Among the group II strains, there was a subgroup which was nonsusceptible to oxacillin, lincomycin, erythromycin, and kanamycin and also had in common the fermentation of glucose and galactose, reduction of both nitrate and nitrite, and growth on 40% bile agar. These strains were the most commonly recognized types isolated from acutely burned patients and possibly originated from the patient's intestinal tract. Data indicated that the air was not a means of transmission for these corynebacteria among acute patients. Corynebacteria were isolated from 11% of the burned wound cultures by using a selective medium but were found in 66% of the acute patients. Over 90% of the strains in groups I and II did not conform sufficiently with described characteristics of common human indigenous corynebacteria to be accurately speciated.

Human indigenous corynebacteria species, commonly called diphtheroids, inhabit various body sites and are occasionally isolated in clinical specimens. In the latter case, their detection is almost always regarded as contamination. Some of these species, however, including C. xerosis and C. hoffmannii (pseudodiphtheriticum), together with nonspecified strains of corynebacteria, have been reported to cause serious human infections (6).

The inability of most laboratories to identify diphtheroids is due, at least in part, to the lack of taxonomic recognition of human indigenous diphtheroids at the species level. This has led several investigators to characterize hundreds of human strains of diphtheroids and select those tests which best place newly isolated strains into groups based on some arbitrary but reproducible scheme. This has been the case in studies on diphtheroids isolated from oral (9) and cutaneous sources (4, 5, 8, 10, 12).

Burn units generally monitor each acute patient by culturing multiple specimens from each patient on a weekly basis for the purpose of infection control. The qualitative and quantitative characteristics of the burn wound microflora are also of interest in judging the efficacy of topical antimicrobial agents in controlling or eradicating organisms from burns and to ascertain the origin of the organisms found therein.

This study was conducted to characterize the diphtheroids in a population of acutely burned children and included methods of five investigators who have developed grouping schemes for cutaneous diphtheroids. Efforts were also made to determine the incidence of the diphtheroids in the patient population and the possible origins of the strains in burns by isolating and characterizing strains from the hospital air and the normal skins of nursing personnel and children hospitalized for reconstructive surgery of old burns.

MATERIALS AND METHODS

Isolation of corynebacteria. Airborne strains were isolated on Columbia blood agar (BBL) by using a TDL air sampler (15). Strains from patients with acute burns were isolated from burn wound, fecal, urine, blood, and catheter tip specimens (14). The primary isolation medium was Furoxone-Tween 80-
Acid
Medium
Oil Red O (FTO) agar (13) prepared with Letheen agar (BBL) as the basal medium. Corynebacteria were isolated on FTO medium after 24 to 48 h of aerobic incubation at 37 C. Representative colonies were Gram stained and transferred to Letheen broth. After incubation in this medium for 48 h, broth cultures were streaked on blood plates. This procedure was used to determine the purity of each strain by Gram staining and examining colonies under a low-power stereomicroscope. Strains were further separated to lipophilic and nonlipophilic types by the methods of Smith (12). Lipophiles were maintained on slants of Letheen agar and nonlipophiles were maintained on brain-heart infusion agar (BBL). Corynebacteria species from normal skins of reconstructive burned children and hospital personnel were taken with saline-moistened cotton swabs and streaked directly on FTO medium.

**Characterization tests.** A minimal number of tests and media were chosen to accommodate those features needed to identify or group corynebacteria species according to five reported schemes (Table 1) used in cutaneous bacteriology studies, with several tests being common to all the schemes.

**Table 1. Comparative methods for the identification of cutaneous diphtheroids**

| Medium and test                                  | Evans (4) | Holt (5) | Marples (8) | Somerville (10)* | Smith (12)* |
|-------------------------------------------------|-----------|----------|-------------|------------------|-------------|
| Tween 80 agar, enhanced growth and hydrolysis, morphology |           |          | +           | +                | +           |
| Tween 20 agar, growth and morphology             | +         |          |             | +                | +           |
| Medium 199, fluorescence                         | +         |          |             | +                | +           |
| Nitrate reduction                                | +         | +        | +           | +                | +           |
| Gelatinase                                       | +         |          |             | +                | +           |
| Acetoin production                               | +         |          |             | +                | +           |
| Methyl red reaction                              | +         |          |             | +                | +           |
| Catalase                                         | +         |          |             | +                | +           |
| Urease                                          | +         | +        |             | +                | +           |
| Acid from glucose                                | +         | +        | +           | +                | +           |
| sucrose                                          | +         | +        | +           | +                | +           |
| maltose                                          | +         | +        |             | +                | +           |
| lactose                                          | +         |          |             | +                | +           |
| galactose                                        | +         |          |             | +                | +           |
| fructose                                         | +         |          |             | +                | +           |
| Groups identified                                | 7         | 15       | 8           | 8*               | 7           |

* All groups fluorescent on medium 199.
* All groups lipophilic.
* Catalase usually included in all schemes as test for aerobic corynebacteria, but author recognized a catalase-negative group of diphtheroids.
* Eight additional subgroups recognized.

Growth enhancement by Tween 80 or hydrolysis of Tween 80 and ability of strains to grow on Tween 20 agar were determined by adding 0.5% Tween 80 and Tween 20 separately to Trypticase soy agar (BBL). These two media were also used to describe colonial morphology. Production of coral red fluorescence on medium 199 agar was determined by the method of McBride et al. (7). Gelatin hydrolysis was detected by flooding nutrient agar (BBL) plates containing 0.4% gelatin with acidic mercury bichloride. Methyl red and acetyl methyl carbinol tests were performed in MRVP broth. Acid production of strains inoculated into 1% carbohydrate broth was tested by using phenol red broth base (BBL). Urease production was detected by the method of Cowan and Steel (2).

Additional tests included lipase production on Spirit Blue agar (Difco), the oxidase test (8), phosphatase activity, and nitrate and nitrite reduction (2). Nitrate semisolid agar was used to detect denitrification with formation of N₂ gas noted. Media used to test for reduction of nitrite usually contain 0.01 g of NaNO₂ per liter because higher concentrations of NaNO₂ have been found to be toxic for many bacteria (2). This concentration was used initially, but it was
later found that media with 0.5 g of NaNO₃ per liter added to the medium did not inhibit growth of the organisms. Acid production from mannose, trehalose, mannitol, glycerol, starch, and dextrin was also included. Utilization of inorganic nitrogen was tested by using Hucker ammonium phosphate agar slants (BBL). Growth on 40% bile agar was determined by using bile esculin agar (BBL). All test media, with the exception of Tween 80 and Tween 20 agar, were supplemented with 0.1% Tween 80 for lipophilic strains. The Tween was not added to media for nonlipophilic strains. Inoculated media were incubated aerobically at 37 °C for a maximum of 7 days. Antibiotic susceptibility tests were performed by using the Kirby-Bauer agar disk diffusion method and Mueller Hinton agar (BBL). Selected antibiotics were also tested in Tryptcase soy broth (BBL) to measure minimal inhibitory concentrations.

Reference strains. Corynebacterium xerosis ATCC 373 was obtained from the American Type Culture Collection, Rockville, Md. Two additional strains of C. xerosis were obtained from the Midwest Culture Service, Terre Haute, Ind. Two strains of Corynebacterium minutissimum (ATCC 23347 and 23349) were obtained from D. Taplin, Miami, Fla.

RESULTS

A total of 221 strains of corynebacteria were studied. Group I consisted of 71 strains cultured from the normal, unscarred skins of 26 of 42 reconstructive patients and from the normal skins of 10 of 16 hospital personnel in contact with both acute and reconstructive patients. The remainder of the strains in this group were from the air sampled six times in both patient areas during a 5-month period. The total airborne counts never exceeded 10 viable particles/ft³ of air per min, but the diphteroid colonies identified on blood agar plates ranged from 9 to 40% of the total counts obtained. Group II consisted of 150 strains isolated from the burns, urine, rectal swabs, oral cavity, and normal unburned skin of acute patients (Table 2). During this study, none of the corynebacteria strains was isolated from blood cultures or from intravenous or Foley catheter tips. Urine specimens with corynebacteria were all less than 10³/ml. The isolation rate of corynebacteria from burns (11% of specimens) was based on isolation of the organisms on FTO agar. By comparison, strains were isolated from only 3% of the burn wounds on Columbia blood agar and none of the organisms was isolated on phenethyl alcohol blood agar. The greater frequency of isolation of corynebacteria from burns by using FTO agar was considered to be the result of furoxone inhibition of gram-positive bacteria predominant in burns. More than 75% of the burn wound corynebacteria isolates were obtained from acute patients who had been hospital-

| Source          | Total no. of specimens cultured | Specimens with corynebacteria No. | Percent positive | Patients with corynebacteria No. | Percent positive |
|-----------------|---------------------------------|-----------------------------------|------------------|----------------------------------|------------------|
| Burns           | 778                             | 88                                | 11.0             | 33                               | 66.0             |
| Fecal swab      | 186                             | 36                                | 19.0             | 22                               | 44.0             |
| Urine           | 176                             | 19                                | 7.4              | 13                               | 26.0             |
| Oral swab       | 50                              | 1                                 | 2.0              | 1                                | 2.0              |
| Normal skin     | 50                              | 6                                 | 12.0             | 5                               | 10.0             |

* A total of 50 acutely burned children was cultured during a 6-month period.

Table 2. Frequency and distribution of corynebacteria isolated from acutely burned children
| Test or reaction | Group I' (71 strains) | Group II' (150 strains) | Reference strains |
|-----------------|----------------------|-------------------------|------------------|
|                 | a N (11) | b L (14) | c L (11) | Air L (35) | Total percent positive | a N (72) | b L (16) | c N (21) | d L (15) | e N (11) | f L (8) | g N (2) | h L (4) | i C. xerosis (3) | j C. minutissimum (2) |
| Tween 80        | 7 6 7 6 7 38   | 44 4 5 5 9 4 2 1 49 1 2 |
| Tween 20        | 6 6 7 7 7 37   | 24 7 4 6 8 3 1 1 36 2 2 |
| Nitrate         | 11 6 6 6 7 42  | 64 3 11 4 9 5 2 3 67 1 0 |
| Nitrite         | 3 0 3 0 0 08   | 48 0 8 0 3 0 1 0 40 0 0 |
| 40% bile        | 1 4 4 4 7 23   | 28 3 16 12 2 4 0 1 44 0 0 |
| Urease          | 1 1 0 0 4 08   | 4 4 3 2 1 0 0 0 09 0 0 |
| Glucose         | 6 11 9 31 80   | 65 5 17 13 8 8 2 1 80 3 2 |
| Sucrose         | 6 6 3 9 34     | 52 4 7 4 5 4 2 0 52 2 2 |
| Maltose         | 0 4 5 12 29    | 45 1 17 8 7 5 1 0 56 2 2 |
| Galactose       | 2 0 4 0 08     | 53 1 5 2 4 3 2 0 47 1 2 |
| Fructose        | 5 7 5 27 62    | 64 3 17 10 6 6 2 1 73 2 2 |
| Mannose         | 5 8 0 21 48    | 58 2 16 9 7 6 2 1 67 2 2 |
| Glycerol        | 0 0 0 0 0      | 0 0 3 0 1 2 0 0 04 0 1 |
| Methyl red      | 1 0 0 8 06     | 49 3 7 8 5 2 2 1 51 0 0 |
| Fluorescent     | 3 7 2 14 37    | 1 2 1 0 0 0 0 0 03 0 2 |
| Nonfermenting   | 5 3 2 4 20     | 7 12 4 2 3 0 0 3 20 0 0 |
| Phosphatase     | 0 2 3 8 09     | 5 2 6 11 3 2 0 0 19 0 0 |

* Abbreviations: N, nonlipophile; L, lipophilic. Number in parentheses is number of strains tested. Number under each column indicates strains positive.

* From normal skin. Strains a and b were from reconstructive patients and c strains were from hospital personnel.

* From acutely burned patients.

* Percentage of all strains positive in groups I and II.
solid agar, indicating denitrification. The nitrite-reducing strains were all fermentative and nonlipophilic and did not fluoresce. There were 72 nonfluorescent strains from groups I and II that grew rapidly on 40% bile esculin agar without demonstrating esculin hydrolysis.

There was no apparent correlation between morphology of colonies of any of the corynebacteria on Tween 80 or Tween 20 media and results of other biochemical tests.

Five reference strains of C. xerosis and C. minutissimum were found to be susceptible to 12 of the antibiotics tested. Virtually all of the 221 isolates were susceptible to penicillin, methicillin, gentamicin, ampicillin, and cephalothin (Table 4). It was determined that among 106 nonlipophiles isolated from acute patients (group II), there were 39 strains—28 wound, five urine, and six rectal—which were nonsusceptible to oxacillin, lincomycin, erythromycin, and kanamycin. Minimal inhibitory concentrations against these strains were 10 μg/ml for oxacillin and greater than 100 μg/ml for lincomycin and kanamycin. Erythromycin was not tested for minimal inhibitory concentration values. These 39 strains also had in common the fermentation of glucose and galactose, reduction of both nitrate and nitrite, growth on bile agar, and nonfluorescence on medium 199. The strains were isolated from the burns of 15 individuals, the urine of four, and rectal swabs of five. Five patients had this type of corynebacterium isolated simultaneously from rectal swabs and burns at least once.

DISCUSSION

The primary purpose of this study was to characterize strains of corynebacteria isolated from the wounds of acutely burned children. Additional strains, isolated from other than wounds from reconstructive burn patients, nursing personnel, and the air in patient areas, were also collected for comparison.

Corynebacteria in group II were infrequently encountered in burn cultures compared with other gram-positive organisms (14), but were isolated from 66% of the acute patients at least once, particularly after the patients were hospitalized 3 or 4 weeks, corresponding in most cases to a period when xenografting or autografting began. At this time, the majority of these organisms may become established in burns. Oxacillin was given to the acute patients before grafting, as a prophylaxis against graft rejection or infection. One group of burn and rectal isolates shared a nonsusceptibility to oxacillin, together with galactose fermentation and several other biochemical characteristics. The corynebacteria in the air did not appear to be a significant means of spreading the fecal strains because the airborne strains did not have characteristics similar to the burn-fecal types.

Although some data was obtained that indicated the origins of some corynebacteria studied, the classification or identification of the strains was not clear. Somerville (11) stated that because of the varied morphological and biochemical characteristics of cutaneous diphtheroids, and because of the overlap of several schemes to identify or classify these organisms, it was only possible to divide the organisms into anaerobic, nonlipophilic, lipophilic, fluorescent, and keratinolytic types. The scheme of Evans (4) used nitrate, urease, glucose, sucrose, and maltose tests to classify diphtheroids. These tests are sufficient to identify C. diphtheriae, C. xerosis, and C. hoffmanii according to the methods of some authors (2). Organisms that are aerobic, catalase-positive, pleomorphic bacilli, regardless of colonial morphology, and that have certain reactions (i.e., NO₃⁻, urease, acid from glucose, maltose and sucrose) are considered to be C. xerosis (2, 4, 12). On this basis, only six of the 221 strains studied here had these minimal combined characteristics, but they also fermented fructose and mannose as did many other strains. McBride et al. (7) found that eight reference strains of C. xerosis varied in the fermentation of fructose, mannose, and galactose. These authors also reported that none of the C. xerosis strains examined conformed to the description of C. xerosis given in Bergey's Manual, 1957. Smith (12) showed that many lipophilic diphtheroids isolated from normal adult skin were not identical with C. xerosis. Marples (8) identified strains that hy-

![Table 4. Antibiotic susceptibility of corynebacteria]({{site_media_url}})

| Antibiotic (μg or units per disk) | Group I* (71 strains) | Group II* (150 strains) |
|----------------------------------|-----------------------|------------------------|
| Sulfadiazine (250)               | 100                   | 100                    |
| Penicillin (10)                  |                       |                        |
| Oxacillin (1)                    | 20                    | 56                     |
| Neomycin (30)                    | 100                   | 13                     |
| Methicillin (5)                  | 100                   | 100                    |
| Lincomycin (2)                   | 6                     | 57                     |
| Kanamycin (30)                   | 2                     | 47                     |
| Gentamicin (10)                  | 100                   | 100                    |
| Furadantin (300)                 | 16                    | 24                     |
| Erythromycin (15)                | 100                   | 55                     |
| Cephalothin (30)                 | 100                   | 100                    |
| Ampicillin (10)                  | 100                   | 100                    |

* By the Kirby-Bauer agar disk diffusion method.

* Expressed as percentage of strains susceptible.
drolyzed urea as \textit{C. xerosis}. This character is not recognized as a positive test for \textit{C. xerosis} by others (2, 4, 5, 7, 12).

Corynebacteria species that reduce nitrate and hydrolyze urea but do not ferment carbohydrates are commonly identified as \textit{C. hoffmannii} (2, 4). Only three strains in this study from group I could be so classified.

Fluorescent strains, considered to be \textit{C. minutissimum}, were demonstrated by Somerville (10) to vary considerably in biochemical characteristics. None of the 30 isolated fluorescent strains were identical to those of the two \textit{C. minutissimum} strains, but presently there are no other established correlate or confirmatory tests to identify all fluorescent diphtheroids as \textit{C. minutissimum}.

Lactose fermentation by corynebacteria also remains a problem in corynebacterial identification. Barksdale (1) maintained that true \textit{Corynebacterium} species did not ferment lactose with the possible exception of \textit{C. pseudotuberculosis}. Lactose-fermenting diphtheroids or corynebacteria species have been reported, however, from cutaneous (6) and oral (9) sources.

Growth on bile agar and nitrite reduction used in conjunction with lipophilic, fluorescent, and fermentative properties demonstrated the probable origins of some of the strains isolated in the burn unit, but the bile-nitrite reactions do not clarify specification of the strains involved.

Davis and Newton (3) found that \textit{C. renale}, \textit{C. ulcerans}, and \textit{C. michiganense} reduced nitrite but not nitrate and differed in other biochemicals tests from any of the other strains studied here. Over 90% of the strains isolated did not conform sufficiently with published descriptions of \textit{C. xerosis}, \textit{C. minutissimum}, or any other commonly recognized human indigenous \textit{Corynebacterium} species. The five schemes used for grouping cutaneous diphtheroids collectively utilized a total of 15 different tests. The complete test battery produced a series of corynebacterial biotypes in arbitrary units yielding a wide variety of reactions, few of which are currently recognized as named species of the genus \textit{Corynebacterium}.

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