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Phenotypic Characteristics of 31 Strains of *Corynebacterium striatum* Isolated from Clinical Samples

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During a 34-month period (January 1991 to October 1993), 31 *Corynebacterium striatum* strains recovered from clinical samples from 24 patients were characterized. Twenty (64%) strains were isolated from wound exudates, 5 (16%) were isolated from bronchial aspirates, 2 (7%) were isolated from urine, 2 (7%) were isolated from endotracheal tubes, 1 (3%) was isolated from a catheter, and 1 (3%) was isolated from empyema. The organisms were identified by conventional culture and phenotypic characterization, the API CORYNE system, and cellular fatty acid composition analyses. The colonies of *C. striatum* could be confused with those of coagulase-negative staphylococci upon primary isolation from clinical material. A consistent phenotypic pattern was observed: all strains reduced nitrate, hydrolyzed tyrosine, and produced acid from glucose, fructose, and sucrose but not from maltose. API CORYNE profile numbers were 3100105 (28 strains) and 3000105 (3 strains). Susceptibility testing of *C. striatum* was performed by disk diffusion. All strains were susceptible to both imipenem and vancomycin and resistant to fosfomycin; most strains were susceptible to ampicillin and cephalosporins and resistant to clindamycin, erythromycin, and tetracycline. Performing a Gram stain of fosfomycin-resistant "*Staphylococcus*-like" colonies was critical in order to identify *C. striatum*.

Coryneform bacteria are members of the normal flora of skin and cutaneous membranes. These organisms are being increasingly isolated from immunocompromised hosts and patients with vascular or urinary catheters (10, 18). The importance of *Corynebacterium diphtheriae* declined in western countries after the introduction of an effective vaccine, but in recent years isolation of these coryneform bacteria from patients with infections other than classical diphtheria has been described (10). Both *Corynebacterium jeikeium* and *Corynebacterium urealyticum* are currently recognized as important human pathogens (1, 3, 15, 27), but the significance or prevalence of other *Corynebacterium* species as causative agents of disease is not as well understood.

Although appreciation of the pathogenic potential of nondiphtherial coryneform bacteria has increased, the identification of these bacteria, including *Corynebacterium striatum*, continues to present difficulties for the clinical microbiology laboratory. Definitive classification of bacteria as *Corynebacterium* species sensu stricto necessitates the use of sophisticated tests, such as cell wall composition analyses and nucleic acid studies, which are often performed only at reference centers (7, 9, 12, 17, 18). The problem is compounded by the suggestion that conventional phenotypic schemes (7, 10, 16, 18) identify only about 60 to 70% of all gram-positive bacilli. Therefore, other methods, such as cellular fatty acid (CFA) composition analysis (5) and the API CORYNE system (13, 14, 29, 30), have been recently described to assist in the identification of coryneform bacteria.

Microbiological and clinical information specifically concerning *C. striatum* infections is scarce. Previously, there have been several case reports (4, 6, 8, 11, 19, 21, 23), and two series (25, 31) have been described, whereby three and six cases, respectively, of *C. striatum* infections were reported. Person-to-person transmission of *C. striatum* in intensive care units has also been described, but limited information on the clinical charts of these patients was presented (19). In this report, we describe the microbiological identification and susceptibility testing of 31 *C. striatum* strains isolated from clinical samples of 24 patients. Clinical information concerning these patients will be reported separately (21a).

MATERIALS AND METHODS

Bacteria. Samples referred to the Microbiology Laboratory at the Hospital Universitario "Virgen Macarena," Seville, Spain (referred to below as HUS), in the period from January 1991 to October 1993 were processed according to standard methods for microbiological culture. Blood samples were processed with the BACTEC NR860 instrument (Becton Dickinson, Paramus, N.J.). All other samples were routinely cultured on Columbia agar base with 5% sheep blood and, for some of them, in thioglycolate broth, as nonselective media. Aerobic and facultative anaerobic gram-positive, pleomorphic, nonbranched, non-spore-forming, non-acid-fast rods were included for identification to species level. Microorganisms isolated from sterile fluids, catheters ($\geq$15 CFU by the rolling-plate method), urine culture ($\leq$2 organisms, $\geq 10^4$ CFU/ml), and pure or predominant culture from wound exudates and respiratory samples were included. *C. striatum* ATCC 6940 was included as a reference strain.

Conventional identification. The Centers for Disease Control (CDC) methods and identification scheme (16) were used by the Laboratory Centre for Disease Control (LCDC), Ottawa, Canada, for all reference and patient strains. The phenotypic traits indicated in Table 1 were studied. Acid production from carbohydrates was determined by using peptone water with Andrade's indicator and 1% carbohydrate. The CAMP test (with *Staphylococcus aureus* ATCC 25922 as the marker strain), tests for production of pyrazinamidase and pyrrolidonyl arylamidase, growth in peptone water with 0 or 0.5% Tween 80, and hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) were performed at the HUS by standard methods. Eight randomly chosen strains were also evaluated by a slide test (Staphylex, Oxford, United Kingdom) for production of clumping factor and/or protein A, as usually used for *Staphylococcus* species.

API CORYNE. Each strain was tested at the HUS by using the API CORYNE system (API Bio-Mérieux, Inc., La Balme Les Grottes, France) in parallel with conventional methods described above. A 7-digit code was obtained for each strain, and this code was interpreted by using the profile index provided by the manufacturer (API CORYNE Analytical Profile Index [1990, 1st edition]). If

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TABLE 1. Conventional phenotypic traits of 31 *C. striatum* isolates from clinical samples

Test of characteristic	Result (% isolates with positive result)
Growth on Tinsdale agar	Black, no halo
Growth on 0% NaCl	+ (100)
Growth on 6% NaCl	+ (100)
Catalase	+ (100)
Oxidase	− (0)
Oxidation/fermentation of glucose	+/+ (100)
Triple sugar iron slant/butt	Acid/acid (100)
Motility	− (0)
DNase	− (0)
Hydrolysis of:	
Tyrosine	+ (100)
PYR[a]	− (0)
Pyrazinamide	+ (100)
Esculin	− (0)
Urea	− (0)
Gelatin	− (0)
Citrate (Simmons)	− (0)
Nitrate reduction	+ (100)
Indole production	− (0)
H_2S triple sugar iron butt/paper	−/+ (0/100)
CAMP test	− (0)
Lysine decarboxylase	− (0)
Ornithine decarboxylase	− (0)
Arginine dehydrolase	− (0)
Acid from:	
Glucose	+ (100)
Ribose	− (0)
Xylose	− (0)
Mannitol	− (0)
Maltose	− (0)
Fructose	+ (100)
Galactose	+ (93)
Lactose	− (0)
Sucrose	+ (100)
Raffinose	− (0)
Glycogen	− (0)
Salicin	− (0)
Starch	− (0)
Trehalose	− (0)

[a] PYR, pyrrolidonyl arylamidase.

supplemental tests were required, they were performed as indicated by conventional methods. Growth in 6% NaCl was tested by using heart infusion broth.

CFA composition. Each strain was analyzed for CFA composition by methods reported previously (5). Fatty acid methyl esters were analyzed by gas-liquid chromatography with the MIDI system (Microbial Id Inc., Newark, Del.) and Library Generation System software (MIDI) as previously described (13), except that AEROBE version 3.7 was used as the root for the in-house method LCDCAER2 attached to the in-house library LCDC1.

Identification of *C. striatum* with Pasco and MicroScan systems. As *C. striatum* colonies may be confused with coagulase-negative staphylococci, eight *C. striatum* strains were studied (at the HUS) with two semiautomated identification systems: Pasco (Difco, Detroit, Mich.) and MicroScan (Baxter, West Sacramento, Calif.). The inoculum was prepared by using the PROMPT System (Baxter), and then the methodology recommended by the manufacturer was followed to inoculate type 3P (Pasco) or Pos Combo 4I (MicroScan) panels. Panels were incubated at 35°C for 18 to 20 h and read with a laser pointer (Pasco) or an automatic reader (MicroScan). In either case, the organism to be identified was considered catalase positive. The biotype and final identification were recorded for further evaluation.

Antimicrobial susceptibility testing. A disk diffusion assay was used for antimicrobial susceptibility testing. A suspension in saline equivalent to a turbidity of 0.5 of the McFarland standard was prepared by using bacteria grown on Columbia agar base with 5% sheep blood at 35°C for 18 to 20 h. This inoculum was applied with a cotton swab to plates containing Mueller-Hinton agar. Disks (Difco) of the antimicrobial agents indicated in Table 2 were applied to the inoculated plates. Plates were incubated at 35°C for 18 to 20 h. Zones of inhibition around the disks were measured. As there are no accepted breakpoints that specifically assign coryneform bacteria to interpretative categories, breakpoints recommended by the National Committee for Clinical Laboratory Standards (24) for organisms other than *Haemophilus* strains and *Neisseria gonorrhoeae* have been used. For penicillin and ampicillin, breakpoints for *Listeria monocytogenes* (the only gram-positive rod included by the National Committee for Clinical Laboratory Standards) were used. Because studies of the clinical effectiveness of vancomycin against coryneform bacteria are lacking, the breakpoints for "other gram-positive" organisms (not for enterococci) were used. Organisms for which inhibition zones were ≥15 mm were considered susceptible to fosfomycin (2).

TABLE 2. Percentages of *C. striatum* strains isolated from clinical samples susceptible, intermediate, and resistant to 15 antimicrobial agents ($n = 31$)[a]

Antimicrobial agent (amt on disk)	% Isolates		
	S	I	R
Penicillin (10 U)	93	0	7
Ampicillin (10 μg)	93	0	7
Cephalothin (30 μg)	93	7	0
Cefoxitin (30 μg)	90	3	7
Cefotaxime (30 μg)	93	7	0
Imipenem (10 μg)	100	0	0
Clindamycin (2 μg)	0	3	97
Erythromycin (15 μg)	3	19	78
Ciprofloxacin (5 μg)	7	13	80
Tetracycline (30 μg)	3	0	97
Gentamicin (10 μg)	45	7	48
Amikacin (30 μg)	87	0	13
Rifampin (5 μg)	32	0	68
Fosfomycin (50 μg)	0	0	100
Vancomycin (30 μg)	100	0	0

[a] Abbreviations: S, susceptible; I, intermediate; R, resistant.

RESULTS

Thirty-one *C. striatum* isolates from 24 patients were isolated: 20 (64%) from wound exudates, 5 (16%) from bronchial aspirates, 2 (7%) from urine, 2 (7%) from endotracheal tubes, 1 (3%) from a catheter, and 1 (3%) from empyema.

All isolates grew at 37, 42, and 25°C in air and 3 to 5% CO_2. Growth in thioglycolate was dense throughout the tube at 24 h. Colonies in Columbia agar base with 5% sheep blood were nonhemolytic; on this medium and on tryptic soy agar they were creamy white to yellowish, with an entire edge. Growth was not stimulated in the presence of Tween 80. A Gram stain showed short gram-positive rods, with an occasional striped pattern. Conventional biochemical reactions are summarized in Table 1. As noted in Table 1, a consistent pattern was obtained from our isolates. Three isolates were initially categorized as pyrazinamidase negative in conventional tests, but all three were positive when the test was repeated. Eight organisms tested negative for coagulase production. All 31 strains were correctly identified as *C. striatum* by the API CORYNE system. The codes obtained were 3100105 (28 strains) and 3000105 (3 strains).

All strains were observed to have CFA compositions both qualitatively and quantitativey consistent with those described previously for a large collection of Canadian patients and reference strains (5). A peak with an equivalent chain length of or near 14.966, which usually failed the MIDI system's internal standard for maximum peak width, was observed for most samples but not *Corynebacterium xerosis*, as described previously (5). Similarity index (SI) values for all strains except two (LCDC 93-0490 [HUS 151] with an SI of 0.16 and LCDC 93-0495 [HUS 203] with an SI of 0.21) ranged from 0.50 to 0.90 relative to the LCDC1 *C. striatum* entry (data not shown).

The code number obtained with the Pasco system for the

eight strains tested was 010162. The corresponding identifications were as follows: *Staphylococcus auricularis*, 59%; *Staphylococcus capitis*, 33%; *Micrococcus* spp., 8%. Similarly, when the MicroScan system was used the code number obtained was 306000, corresponding to the following identifications: *Staphylococcus hominis*, (63%); *S. auricularis*, 14%; *S. capitis*, 13%; *Staphylococcus epidermidis*, 7%; and *Staphylococcus haemolyticus*, 2%.

Results of antimicrobial susceptibility testing are presented in Table 2. All strains were susceptible to both imipenem and vancomycin, and most were susceptible to ampicillin and cephalosporins. All strains were resistant to fosfomycin, and most of them were resistant to clindamycin, erythromycin, and tetracycline. Only 7 and 32% were susceptible to ciprofloxacin and rifampin, respectively. Forty-five percent of our strains were susceptible to gentamicin, and 87% were susceptible to amikacin.

DISCUSSION

Until recently, *C. striatum* rarely has been reported as a cause of human disease. The organism has been described as causing respiratory disease (4, 6, 8, 11, 19, 23, 25), but it has also been isolated in cases of endocarditis, septicemia, osteomyelitis, infections of central lines, ulcers and wounds, and eye infections (11, 21, 31). In our hospital, *C. striatum* was the coryneform bacterium most frequently isolated from clinical samples during the period from 1990 to 1993 (21a). A significant number of isolates in that study were obtained from respiratory samples (26%), but the majority (64%) of isolates were recovered from wound exudates and the remaining strains were from venous catheters and urinary tracts. In a separate study (22), we found that *C. striatum* was the causative agent in a case of fatal pulmonary disease in a young, previously healthy man who was hospitalized in the intensive care unit at HUS. Leonard et al. had also reported (19) person-to-person transmission of a pigmented strain of *C. striatum* in intensive care units.

It is clear that the recognition of *C. striatum* as a possible human pathogen requires rapid and efficient methods for its identification from clinical samples. In our experience, colonies of *C. striatum* on blood agar media commonly used in clinical laboratories can resemble those of *Staphylococcus* spp., as also observed by Leonard et al. (19). If a slide coagulase test (but not a Gram stain) is performed, the organism may be misidentified as a coagulase-negative *Staphylococcus* sp. The use of commercial panels for identification of gram-positive organisms could indeed support this misidentification. As shown here for the eight strains tested with the Pasco and MicroScan systems, low probability values for several staphylococcus or micrococcus species should alert the clinical microbiologist to the possibility of the presence of *C. striatum*, especially if the strains are also observed to be resistant to fosfomycin. This reenforces the need for a Gram strain after primary isolation in order to correctly and rapidly recognize potentially pathogenic coryneform bacteria.

Final identification of a gram-positive, *C. striatum*-like bacillus may be done on the basis of conventional or biochemical tests and (if possible) the study of CFAs. However, Coyle et al. (9) found that schemata to identify *C. striatum* phenotypically differed, depending on whether one used the scheme described by the CDC, based on strains with reactions similar to that of the type strain, ATCC 6940 (16), or that described in *Bergey's Manual of Systematic Bacteriology* (7), which is based on the 1954 description of *C. striatum* by Munch-Petersen. Coyle and coworkers also found in that study that three reference strains for phenotypically similar *C. xerosis* were found to be genetically identifiable as *C. striatum*. API CORYNE identifications are based on the CDC scheme (whereby *C. striatum* isolates reduce nitrate and ferment sucrose but not maltose). In our experience, several conventional tests may help to differentiate *C. striatum* from *C. xerosis*. *C. striatum* is a short gram-positive rod, grows as creamy, pale-yellow colonies, hydrolyzes tyrosine, ferments sugars rapidly (24 to 36 h), and is commonly susceptible to ampicillin and cephalothin, while *C. xerosis* is a diphtheroid-like organism, grows as dry colonies with a sugary texture, does not hydrolyze tyrosine, ferments sugars slowly (72 to 96 h), and is commonly resistant to ampicillin and cephalothin.

In the present study (Table 1), all strains of *C. striatum* were found to be consistent with the scheme as described by Hollis and Weaver (16). All strains were identifiable by the API CORYNE system, with only two codes being generated: 3100105 (28 strains) and 3000105 (3 strains). These identifications were considered "good to species levels" or "very good to genus level," with confidence levels of 97.1 and 89.1%, respectively. Unlike Leonard et al. (19), who reported a positive CAMP test for pigmented and several nonpigmented strains of *C. striatum*, we did not obtain a positive CAMP reaction for any strain. All *C. striatum* isolates in this study were found to have CFA compositions consistent with that described previously (5) and demonstrated respectable similarity to the LCDC entry for that species as first (or, in two instances, second) choice.

Information on activities of drugs against *C. striatum* is also limited. The isolates of *C. striatum* we tested were susceptible to both vancomycin and imipenem and resistant to fosfomycin. Most of the isolates were susceptible to penicillin G, ampicillin, and cephalosporins and resistant to clindamycin, erythromycin, and tetracycline. Roberts et al. (26) have reported that 15 of 17 strains (carrying the *erm* Cd gene) were resistant to clindamycin and either resistant or intermediate to erythromycin and that 13 of these strains (carrying the *tetM* gene) were resistant to tetracycline. Similar rates of resistance to these antimicrobial agents have been reported by Soriano et al. (28), who studied 11 strains of *C. striatum*; these authors also found that all 11 strains were susceptible to doxycycline and fusidic acid and resistant to nitrofurantoin; most of them were oxacillin resistant. MICs of gentamicin for these 11 strains ranged from 0.03 to 4 μg/ml (MIC at which 90% of the isolates are inhibited, 2 μg/ml); for our isolates, however, only 45% of strains were susceptible (as determined by disk for diffusion) to this drug although 87% were susceptible to amikacin, which suggests different activities of the two aminoglycosides. Although ciprofloxacin is not active against most *C. striatum* strains (this report and references 22 and 28), previous results (22) indicate that newer fluoroquinolones (i.e., clinafloxacin) are highly active against the organism. This pattern of susceptibility is different from that of *C. jeikeium* and *C. urealyticum*, which are commonly multiresistant organisms (28). Resistance to clindamycin, erythromycin, and tetracycline has also been reported for other coryneform bacteria (28) but was not noted in a recent report on *C. diphtheriae* isolated from northwestern Russia (20).

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