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Characteristics and Sites of Infection of *Eubacterium nodatum*, *Eubacterium timidum*, *Eubacterium brachy*, and Other Asaccharolytic Eubacteria

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Three new species, *Eubacterium nodatum*, *Eubacterium timidum*, and *Eubacterium brachy*, were described, primarily from subgingival samples taken from patients with moderate and severe adult periodontitis. Except for the isolation of *E. brachy* from a pleuropulmonary infection, these species have not been reported from other infected body sites. We report on the isolation of these species and an undescribed group (D-6) of asaccharolytic eubacteria also found in periodontal disease from numerous different sites of infection, mostly the head and neck. A similarity in cellular morphological properties of *E. nodatum* and *Actinomyces* sp. was noted previously. Additional similarities, particularly to *Actinomyces israelii*, that we found are the formation of molar tooth colonies and the isolation from cases of lumpy jaw and from the genital tract of women in association with the use of an intrauterine contraceptive device. *E. timidum* and *E. brachy* did not occur more often from any particular site outside of the head, neck, and respiratory tract. The group D-6 strains came from a variety of sites in the trunk and pelvis. These species are all obligately anaerobic, asaccharolytic, and generally nonreactive, and they grow poorly and slowly on media commonly used to isolate anaerobic bacteria. L-Lysine (0.5%) markedly stimulated the growth of *E. nodatum* and, to a lesser extent, another acetate- and butyrate-producing group, *Eubacterium* sp. group D-6, but we did not find comparable stimulants for the other species. We found the production of phenyl acetate to be a helpful marker in the identification of *E. timidum* and *Eubacterium* sp. group D-6. Although the isolation and identification of most of these species remain somewhat difficult, the evidence from dental infections and the present report suggests that these species are potential pathogens that are likely to be overlooked in infected clinical material without special attention to more prolonged incubation and use of enriched isolation media.

Eubacterium nodatum, *Eubacterium timidum*, and *Eubacterium brachy* were recently described as new species principally isolated from subgingival areas in association with moderate or severe adult periodontitis (4). A pathogenic role for these three species was indicated by their high incidence (in 35 to 42% of the subgingival periodontitis samples cultured) and concentration (3 to 57% of the cultivable flora) in the subgingival sites and their infrequent isolation from adjacent supragingival areas. All three species were obligately anaerobic, gram-positive rods that were nonsaccharolytic and mostly nonreactive in other biochemical tests. These species were identified on the basis of characteristics of growth, morphological properties, and metabolic products. It was presumed that the slow and often minimal growth of these species on laboratory media and the fact that they shared certain cultural, biochemical, or morphological characteristics with well-known species of anaerobic bacteria had prevented their earlier recognition in periodontitis. Only *E. brachy* has been reported from another body site, empyema fluid (10). We report the isolation of these three species and other asaccharolytic eubacteria from numerous infections other than periodontitis and on additional features of their growth and metabolism which may aid in their isolation and identification in a clinical laboratory. A morphological similarity of *E. nodatum* to *Actinomyces* species has been noted (4); we report additional similarities, in particular to *Actinomyces israelii*, which extend to certain cultural aspects and to the clinical settings in which these organisms are found.

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MATERIALS AND METHODS

Media. Anaerobic blood agar (BA) was prepared from brucella agar (BBL Microbiology Systems, Cockeysville, Md.) to which was added, at final concentrations, 10 μg of hemin (Eastman Kodak Co., Rochester, N.Y.) per ml, 1 μg of vitamin K_1 (Sigma Chemical Co., St. Louis, Mo.) per ml, and 5% sheep blood. Supplemented Proteose Peptone broth (SPP) consisted of 2.5% Proteose Peptone no. 3 (Difco Laboratories, Detroit, Mich.), 0.5% yeast extract (BBL), 0.15% Na_2HPO_4 (Mallinckrodt, Inc., St. Louis, Mo.), 0.05% $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific Co., Fair Lawn, N.J.), 1 μg of vitamin K_1 per ml, 10 μg of hemin per ml, 0.05% cysteine HCl · H_2O (Calbiochem-Behring, La Jolla, Calif.), and 10% horse serum (Granite Diagnostics, Inc., Burlington, N.C.) in deionized water; the cysteine hydrochloride and serum (sterile) were added after the rest of the rehydrated ingredients had been autoclaved. The final pH of SPP was 6.9 to 7.0. We subsequently found that 0.5% L-lysine added to the SPP formulation gave the best growth for strains of *E. nodatum* and *Eubacterium* sp. group D-6, and we now include it in SPP. Agar (1.5%) was added to SPP to make SPPA. Prereduced anaerobically sterilized media which included chopped meat agar (CMA) slants, chopped meat broth (CM), peptone yeast extract broth (PY), and all carbohydrate and certain other test media using PY as a base (5) were obtained from Carr-Scarborough Microbiologicals,

Inc., Atlanta, Ga. At final concentrations, 0.5% L-arginine hydrochloride (Sigma), 0.5% L-lysine monohydrochloride (Sigma), 0.1 or 0.5% L-leucine (Mann Research Laboratories, New York, N.Y.), and 0.1 or 0.5% L-phenylalanine (Mann) were tested for stimulation of growth in PY, usually, or other media. Whenever noted, horse serum, rabbit serum (both from Granite Diagnostics), or fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.) was added to PY and other media at a final concentration of 10%. Aside from the prereduced anaerobically sterilized media, plates and broth were either freshly prepared or stored in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) before use.

Bacterial strains. Asaccharolytic, anaerobic, gram-positive rods that resembled *E. nodatum*, *E. timidum*, and *E. brachy* were subcultured from frozen stock cultures (−70°C) for tests to establish or confirm their identities. All of these strains had been isolated from patients' specimens submitted for anaerobic culture to the Clinical Microbiology Laboratory at Duke University Medical Center. Known strains kindly supplied for comparative purposes by Lillian Moore of Virginia Polytechnic Institute (VPI), Blacksburg, were *E. nodatum* D10B-8, D13B-5, and D40D-21, *E. timidum* D12B-28, D10B-17, and D84B-2, and *E. brachy* D6B-23, D4A-24, 12415, and D1A-30. All of the known strains were from gingival-crevice samples except 12415, which was a referred culture isolated from pleural fluid.

Frozen stock strains generally were subcultured to broth (CM with added horse serum) and one or two solid media (CMA or BA). These strains were subcultured two or three times to check for purity and to obtain actively growing cultures before various tests for characterization were begun.

Identification and stimulation tests. A total of 12 of 32 strains of *E. nodatum*, 1 of 5 strains of *Eubacterium* sp. group D-6, and 1 of 8 strains of *E. timidum* were tested for their reactions on the 28 different types of media used previously (4) to identify *E. nodatum*, *E. timidum*, and *E. brachy* strains. These and other tests, i.e., utilization of lactate or pyruvate, growth stimulation and ammonia production from arginine, effects on bacterial growth of Tween 80, heme, or bile, conversion of threonine to propionate, and metabolic products from PY, PY with glucose, and CM, were performed as previously described (5). Phenyl acetate and hydrocinnamate (both control standards from Sigma) also were sought, among other metabolic products. The remaining strains of each of these species and all other strains were tested in an abbreviated set of biochemicals, usually for all of the following: fermentation of glucose, fructose, and sucrose; production of indole; reduction of nitrate; production of ammonia from PY and PY with added arginine; utilization of pyruvate; stimulation by lysine, arginine, or pyruvate; and production of organic acids and alcohols in PY, PY with glucose, CM, SPP, and various other media. For nine of the strains of *E. nodatum*, two strains of *Eubacterium* sp. group D-6, and five strains of *E. timidum*, polyacrylamide gel electrophoresis was performed as an additional confirmation of the species (courtesy of Lillian Moore).

Note was made of any medium (see section on Media) which appeared to improve the growth of these strains. Tests of potential stimulants (see section on Media) were made by introducing equivalent inocula of organisms into media (usually PY) with and without the additive and subsequently comparing the turbidities (bacterial growth) at timed intervals and the amounts of various organic acids produced as analyzed by gas-liquid chromatography profiles after incubation of broths for 5 to 7 days. Organic acids were extracted from broth media using methyl *tert*-butyl ether (Fisher) as a solvent (12).

RESULTS

E. nodatum. We first became aware of *E. nodatum* during attempts to isolate *Actinomyces* species from pus aspirated from a patient with lumpy jaw and later from intrauterine contraceptive devices (IUD) submitted for culture. In these instances, organisms morphologically indistinguishable from *A. israelii* were observed on Gram-stained smears made from the specimen submitted and also from colonies that resembled those of *Actinomyces* species. However, gas-liquid chromatography of the metabolic products of pure cultures of these isolates revealed acetate and butyrate rather than acetate, lactate, and succinate as expected for the members of the genus *Actinomyces*; also, these isolates were nonsaccharolytic. These and similar strains from other sources isolated before and after the description of *E. nodatum* (4) were frozen for further study.

(i) Isolation. In our clinical laboratory, *E. nodatum* strains generally were recovered from primary BA but on occasion were found on brain heart infusion agar which had been included for the purpose of isolating *Actinomyces* species. Colonies often were described as molar tooth or heaped or, less often, raspberry in appearance. Colonies were white or tan and often were embedded in or stuck to the agar. Usually, 5 to 10 days of growth were required to form these characteristic-appearing colonies. Less distinctive small, entire, convex colonies also were formed by many *E. nodatum* strains, and these often predominated compared with the more distinctive forms just mentioned. Colonies on BA incubated for 3 to 4 days generally were less than 0.5 to 1 mm in diameter. Subcultures from the primary colonies onto BA or into supplemented thioglycolate broth usually grew slowly and poorly. Growth usually could be obtained on CMA slants or in CM. Growth in broth media often was grainy in appearance or in clumps or balls.

(ii) Microscopic appearance. In a Gram-stained smear, gram-positive rods with a branching, often filamentous and beaded appearance and occurring in clumps suggested the presence of *Actinomyces* species. In most of these clinical specimens, the gram-positive rods with this form and later identified as *E. nodatum* were approximately equal in number to the other species present and were in high concentrations. The Gram-stain morphological characterizations were quite similar for 29 of the 31 strains, but 2 strains appeared shorter, with minimal branching when grown in broth culture, although branching rods were clearly present when these strains were grown in solid media. The typical branching shape was an important characteristic in identifying *E. nodatum* because other species of anaerobic gram-positive rods are asaccharolytic and share certain biochemical characteristics with *E. nodatum*.

(iii) Characteristics and growth stimulation. Characteristics previously described (4) and others demonstrated by us for the identification of *E. nodatum* are shown in Table 1. It earlier (4) was reported that pyruvate was not utilized by *E. nodatum*, but all 29 of our strains tested and the 3 reference strains partially or completely utilized pyruvate, which also stimulated growth in PY. The amounts of acetate and butyrate in PY-pyruvate cultures were approximately two-fold greater than those in PY cultures, and proportionally more acetate was produced. This utilization of pyruvate by

TABLE 1. Characteristics of *E. nodatum*, *E. timidum*, *E. brachy*, *Eubacterium* sp. group D-6, and *E. lentum* (anaerobic, nonsporeforming, gram-positive, asaccharolytic rods)[a]

Eubacterium species	Morphological properties		Metabolic products[b]	Utilization of pyruvate	NH_3 from PY/PYARG[c]	Nitrate	Growth stimulant(s)[d]
	Cellular	Colonial					
E. nodatum	Branched, filamentous, or club-shaped	Molar tooth, heaped, or raspberry	aB (fls)	+	+/++	−	0.5% Lysine 0.5% Arginine 0.5% Pyruvate
D-6 group	Regular, variable lengths, occasional chaining, beading, or diphtheroidal	Circular entire convex	aBpaa (fls)	−	+/+	−	0.5% Lysine
E. brachy	Short or coccoidal, chaining	Circular entire low convex, occasionally rough	ibivic (aflshc)	−(+)	+/+	−	
E. timidum	Short, regular to diphtheroidal, occasional clumps	Circular entire low convex	paa (afls)	−	−(+)/−(+)	−	10% Egg yolk
E. lentum	Short or coccoidal, occasional chains	Circular entire convex	(afls)	−	+/++	+	0.5% Arginine

[a] Our observations and from references 4 and 5. All strains were nonreactive for indole, starch, esculin, gelatin, meat, milk, and catalase except *E. lentum*, which occasionally was catalase positive. All were susceptible (tested by broth-disk method; 5) to clindamycin (1.6 μg/ml) and chloramphenicol (12 μg/ml), and all except rare *E. timidum* strains were susceptible to penicillin (2 U/ml). Some strains of *E. timidum* and, rarely, *E. nodatum* were resistant to tetracycline (6 μg/ml). +, Positive; −, negative; − (+), usually negative but occasional strain positive.

[b] a, Acetic; f, formic; B, butyric; ib, isobutyric; iv, isovaleric; ic, isocaproic; l, lactic; s, succinic; paa, phenyl acetic; hc, hydrocinnamic. Capital letters indicate that most of the strains produce >1 meq of acid per 100 ml of broth, and lowercase letters indicate <1 meq/100 ml of broth. Products sometimes produced are shown in parentheses.

[c] PYARG, PY with arginine (0.5% [wt/vol]); ++, ammonia production increased in PYARG compared with PY.

[d] Ten percent serum apparently improved the viability of most strains but was not frankly stimulatory to growth.

E. nodatum strains subsequently was confirmed by Lillian Moore (personal communication), and the basis for this discrepancy with the earlier report is unclear. Oxgall (2%) was inhibitory to growth, and growth was not affected by 0.02% Tween 80, as reported previously (4). Ten percent (vol/vol) rabbit, bovine, or horse serum did not stimulate growth as measured by any increase in metabolic products but appeared to enhance success in subculturing, particularly of frozen stock cultures.

We sought other growth stimulants. Growth was unchanged by increased heme (50 μg/ml) or sodium bicarbonate (1 mg/ml), but arginine (0.5% [wt/vol]) stimulated growth in PY (metabolic products approximately twofold increased) and increased ammonia production for 27 of our 29 strains tested and for the 3 reference strains. Based on the metabolic end products which are produced by *E. nodatum*, we tested 0.5% (wt/vol) lysine, which proved to be an even more active stimulant of growth than arginine or pyruvate. For all 15 strains tested, 0.5% lysine markedly increased (three- to fourfold) the amounts of acetate and butyrate in PY (or in CM) and also enhanced the production of ammonia.

(iv) Improved growth medium. During the work on characterization of these strains of *E. nodatum*, the desirability of an improved growth medium, particularly an agar medium, became apparent. One of us (G.B.H.) devised a medium (SPPA) which utilized Proteose Peptone, based on the assumption that these organisms grow on peptides or amino acids. Supplements, such as yeast extract, vitamin K_1, hemin, and cysteine, that are commonly used to improve the growth of many anaerobic organisms were added without performing an analysis of their individual effects on growth. A buffer system was incorporated to poise the initial pH and to prevent a marked increase in alkalinity from ammonia which might inhibit growth. Colonies of *E. nodatum* on SPPA were usually cream in color, and they appeared sooner, were larger, and were smoother in appearance, with fewer of the heaped colonial forms than observed on BA. Likewise, growth in SPP was good, approximately equal to that observed in CM. Lysine (0.5%) also increased metabolic products by approximately fourfold in SPP and markedly enhanced colonial growth on SPPA. Thus, 0.5% lysine has been incorporated in the formulation of SPP or SPPA.

(v) Clinical sources. Seventeen of our thirty-two clinical strains of *E. nodatum* were from infections of the head and neck; only a few isolates were cultured from the trunk or an extremity (Table 2). The other major source (11 isolates) was the female genital tract in association with the presence of a foreign body, particularly an IUD (10 of 11 isolates). Of these 10 patients, 5 presented with pelvic inflammatory disease and 4 others had various signs and symptoms that contributed to a decision to remove the IUD. Actinomyces-like organisms had been reported from Papanicolaou smears performed on two of these symptomatic patients and also from the smear of one asymptomatic patient whose IUD was subsequently removed, apparently because of the smear report. Generally, the IUD was sent for culture, although one *E. nodatum* isolate each was obtained from abscess drainage and an endometrial sample. Another type of foreign body, nonabsorbable suture material used for a cervical cerclage, was associated with the recovery of *E. nodatum* from amniotic fluid in a woman with premature rupture of membranes.

Multiple facultative and anaerobic species usually accompanied *E. nodatum* in specimens, although in five instances *E. nodatum* was present with exclusively anaerobic or microaerophilic species. Accompanying species were typical of those usually recovered from mixed infections, depending on the particular body site(s) involved. In four specimens, i.e., amniotic fluid, pelvic abscess, tooth abscess, and lumpy jaw, *Actinomyces* species also were present. Black granules

TABLE 2. Infected sites of isolation of *E. nodatum*, *E. timidum*, *E. brachy*, and *Eubacterium* sp. group D-6

Source	No. of isolates			
	E. nodatum	*E. timidum*	*E. brachy*	D-6 group
Brain abscess or empyema	1		1	
Infected sinus (operative specimen)	4	1		
Osteomyelitis of mandible	1		1	
Abscessed tooth	2			
Buccal abscess or cellulitis	2	1		
Lumpy jaw	3			
Ludwigs angina	1	1		
Tonsillar abscess	2			
Neck abscess	1		1	
Parotid gland abscess		1		
Lung aspirate or pleural fluid	1	1	1	
Breast abscess				1
Liver abscess	1		1	
Peritoneal fluid		1		
Aortic graft				1
Blood		1		
Thumb wound	1			
Necrotizing fasciitis, thigh or groin	1	1		1
Pelvic abscess	1			1
Endometrium or IUD	9			1
Amniotic fluid	1			

containing long filamentous forms were present in a liver aspirate from which *E. nodatum*, *E. brachy*, and black-pigmenting *Bacteroides* spp. (*Bacteroides asaccharolyticus* and *Bacteroides intermedius*) were isolated; no actinomyces were found.

***Eubacterium* sp. group D-6.** We isolated a *Eubacterium* strain biochemically similar to *E. nodatum* from a pelvic abscess, but this strain usually appeared as short- to medium-length gram-positive rods, sometimes in chains, without any branching. The length of these rods varied considerably in different media, and occasionally a pronounced beading along faintly stained rods resembled chains of cocci. The rods were occasionally irregular or diphtheroidal on plated media. This strain was identified as *Eubacterium* sp. group D-6 by polyacrylamide gel electrophoresis (courtesy of Lillian Moore). We identified four additional strains of the D-6 group (Table 2). Characteristics of the D-6 group that we found useful for identification and for differentiation from *E. nodatum* are shown in Table 1. In particular, our D-6 strains produced phenyl acetate in addition to acetate and butyrate, whereas none of 13 *E. nodatum* strains tested produced phenyl acetate (or hydrocinnamate). This finding was subsequently confirmed with additional D-6 strains (personal communication, Lillian Moore). Our D-6 strains usually did not utilize pyruvate and were not stimulated by arginine (as measured by increased metabolic products), although minimal stimulation by arginine occasionally was observed. Growth and metabolic products were moderately enhanced by added lysine.

***E. timidum*. (i) Isolation and microscopic appearance.** *E. timidum* colonies usually appeared round, entire, and cream or white and occasionally had a rough appearance. Holdeman et al. (4) encountered difficulties in obtaining growth on blood agar plates of their *E. timidum* strains and reported that these strains grew better on egg yolk agar plates, although neither lecithinase nor lipase was produced. Our isolates usually grew on BA (brucella base), although growth was slow and the colonies were generally still less than 1 mm in size after 5 days. All of eight strains tested grew better on CMA, and good growth (five of five strains tested) also was obtained on SPPA with added egg yolk suspension (10% final concentration), but these media were not directly compared with each other. In broth culture, *E. timidum* produced, at best, only 2+ turbidity, and occasionally no turbidity was observed. Turbidities and metabolic products were highest when strains were grown in CM and somewhat better in SPP than in PY. On Gram stain, *E. timidum* appeared as short, gram-positive rods that often were regular but, particularly in broth, sometimes diphtheroidal or arranged in clumps.

(ii) Characteristics. *E. timidum* has been described as generally nonreactive in a large variety of biochemical tests in addition to being asaccharolytic and producing no or only trace amounts of acetate, formate, or succinate as metabolic end products. Its characteristic nonreactivity and lack of very unique morphological characteristics, taken with the poor growth of many strains, create problems in its definitive identification (Table 1). We found that *E. timidum* (eight of eight strains tested) produces phenyl acetate (best in CM), which has been a useful marker for identification. This finding was subsequently confirmed with additional strains of *E. timidum* (personal communication, Lillian Moore).

Holdeman et al. (4) reported that 0.02% Tween 80, 5% rumen fluid, or 10% serum with cocarboxylase did not stimulate growth of *E. timidum* and that 2% oxgall inhibited growth. For four strains tested, we observed no increased turbidity with additional heme (50 μg/ml), lysine (0.5%), or phenylalanine (0.1%). Rabbit or horse serum appeared to marginally improve growth in PY broth. Also, for eight strains tested, turbidity and metabolic products were not increased by arginine (0.5%) or pyruvate (5 mg/ml), except that very small amounts of acetate were more commonly observed in pyruvate cultures. Pyruvate, in fact, reduced the amount of phenyl acetate produced by approximately half.

(iii) Clinical sources. Of the eight clinical isolates identified as *E. timidum*, five were recovered from infections of the head, neck, or lung (Table 2); these were in mixture with multiple strains of facultative and other anaerobic species. A strain isolated from a peritoneal abscess was present only with microaerophilic streptococci. The *E. timidum* strain isolated from blood was accompanied by facultative alpha-hemolytic streptococci, not group D. *Streptococcus morbillorum* also was isolated from another blood culture set taken at approximately the same time from this patient. We suspect that these isolates represented a transient bacteremia but cannot be certain. This patient had severe congestive heart failure and had been intubated the previous day. The blood culture was drawn because of fever; he had been extubated that same day.

During this work, we noted four *Eubacterium* strains which resembled *E. timidum* in cellular morphological characteristics but were somewhat more coccoidal and chaining, particularly in broth culture, than the *E. timidum* strains. These strains differed biochemically from the *E. timidum* strains by their production of ammonia, occasionally in PY but particularly in PY-arginine cultures, and by the lack of phenyl acetate as a metabolic product. Like *Eubacterium lentum* (Table 1), these strains produced no detectable products or, usually, a trace of acetate, infrequently with succinate or lactate. By polyacrylamide gel electrophoresis,

they were found to be different from each other and different from any known species or provisionally described *Eubacterium* spp. (personal communication, Lillian Moore). The sites of isolation were peritoneal fluid (one strain), brain (empyema [one strain]), and blood (two strains). One of the strains from blood was present with diphtheroids and *Peptostreptococcus asaccharolyticus*. The other isolate from blood was in pure culture, collected on the day of admission from a patient diagnosed to have an infection posthysterectomy. Two days later, the patient underwent an operation for drainage of a pelvic abscess from which multiple species, mostly anaerobes, were cultured. No similar organism was isolated from this culture, but it easily could have been missed.

***E. brachy*. (i) Isolation.** On primary BA plates, *E. brachy* colonies generally were noted after 4 to 6 days of incubation; colonies were less than 1 mm in diameter, round, convex, entire, white or cream, and sometimes rough in appearance. Several of our strains were difficult to subculture, particularly on solid media. One strain grew only in broth subcultures; growth could never be obtained on plated media. Turbidity in broth ranged from none to 2+ at best, usually 1+. Strains grew better and produced increased amounts of metabolic products in CM and, to a lesser extent, in SPP compared with PY. On Gram-stained smears, *E. brachy* appeared as short- to medium-length gram-positive rods, often coccoidal, and usually in chains, particularly in broth cultures.

(ii) Characteristics. Several compounds commonly used to stimulate the growth of anaerobes, as mentioned above for *E. timidum*, failed to improve the growth of *E. brachy* (4). We performed limited trials of additional potential growth stimulants, many of which were similar to those noted for *E. timidum*, but our results with *E. brachy* were inconclusive. The typical metabolic products for *E. brachy* are isocaproate, isovalerate, isobutyrate, and, usually, acetate, formate, and succinate (4). We also found that minor amounts of lactate usually were present. Additionally, we found hydrocinnamate to be present in cultures of all four reference strains of *E. brachy* and in cultures of both of our strains tested for hydrocinnamate (Table 1). We noted another gas-liquid chromatography peak, identity unknown, that has a retention time between that of succinate and phenyl acetate; it does not appear to be one of the volatile or nonvolatile acids commonly used to provide standard chromatography peaks for the identification of anaerobes. Ammonia production in PY, although reported as positive for *E. brachy* (4), was somewhat variable, probably a function of poor growth. Two of our *E. brachy* strains tested for utilization of pyruvate were positive, and metabolic products were increased.

(iii) Clinical sources. With the exception of an isolate from a liver abscess, our five strains of *E. brachy* were isolated from infections of the head, neck, and lung (Table 2). All of these strains were present in mixed infections, although the accompanying species from a neck abscess and pleural fluid were exclusively anaerobes.

DISCUSSION

The slow and minimal growth of many strains of *E. nodatum*, *E. timidum*, *E. brachy*, and certain other, provisionally designated, asaccharolytic species of *Eubacterium* on the media commonly used in clinical microbiology laboratories suggests that these isolates often are missed. Also, the time and effort required to isolate and identify all anaerobic bacteria from mixed infections involving numerous species means that, in many instances, no attempt at isolation even is made. Even if isolated, anaerobic gram-positive rods often have presented problems with identification, leading clinical laboratories to report nonclostridial isolates only by Gram stain or to give very presumptive identifications. There appear to be many eubacteria that are poorly characterized and apparently represent unnamed species. Additionally, the role in the pathogenesis of infection of most *Eubacterium* species is poorly understood. This report, taken with previous studies of periodontal disease (4, 7), suggests that *E. nodatum*, *E. timidum*, *E. brachy*, and *Eubacterium* sp. group D-6 are opportunistic pathogens which may be overlooked for the above-stated reasons.

The most likely candidate for isolation among these species is *E. nodatum*, since it may produce molar tooth colonies and many strains grow somewhat better than strains of these other *Eubacterium* species. The striking cellular and colonial morphological similarity of *E. nodatum* to *Actinomyces* species in particular might prompt efforts at isolation but also could lead to an erroneous identification of *Actinomyces* species if limited tests are used for identification. The common failure of *E. timidum* to produce noticeable turbidity in broth media and to grow readily on plates complicates the identification of some strains, since it becomes more difficult to ensure that nonreactivity in biochemical tests is not due just to a lack of viability of the strain. If gas-liquid chromatography is available, the production of phenyl acetate in broth culture is an aid to identification as well as an assurance of viability of the organism. Also, there are other fastidious, asaccharolytic, nonreactive strains of eubacteria (VPI groups D-8 and D-11) that have not been fully described with which these organisms could be confused (personal communication, Lillian Moore). Of the species more commonly encountered in the clinical laboratory, *E. timidum* most closely resembles *E. lentum* but easily can be differentiated from *E. lentum*, which produces ammonia (5), reduces nitrate (5), and is stimulated by arginine (11) but does not produce phenyl acetate (none of four strains tested), all in contradistinction to *E. timidum*. Additionally, the four different species (by polyacrylamide gel electrophoresis) discussed above which produced ammonia but were negative for nitrate and did not produce phenyl acetate suggest that there are other undescribed asaccharolytic species which might be assumed to be *E. lentum* (or perhaps *E. timidum*) if identification is based only on cellular morphological characteristics or very limited tests. As pointed out previously (4), *E. brachy* strains might be confused with *P. anaerobius* since chaining coccoidal cells are often observed with *E. brachy*, their metabolic products are very similar, and *P. anaerobius* is often asaccharolytic or only weakly saccharolytic.

So far, these species of *Eubacterium* have been reported as part of the microflora only in the mouth (4, 7). The endogenous mouth flora, particularly in the presence of oral infections such as periodontitis, can serve as a reservoir of opportunistic species that can infect sites in the head, neck, and lung, so that our report of numerous sites of infection involving *E. nodatum*, *E. timidum*, *E. brachy*, and *Eubacterium* sp. D-6 is not unexpected. Although these species have not been reported to be members of the gastrointestinal tract flora, they could have been missed or they may appear in the flora only as a result of being swallowed and then proliferating in certain pathologic states. The gastrointestinal tract most probably was the immediate source of the *E. nodatum* and *E. brachy* strains that occurred together with other

species in a liver abscess, the *E. timidum* from peritoneal fluid, and other examples.

The microecological characterization of *E. nodatum* appears to be especially similar to that of *Actinomyces* species. We have not isolated this species in studies of the vaginal microflora (2, 3), but we presume it must at least transiently colonize the vagina or cervix of some women since we isolated it from the genital tract. Likewise, *Actinomyces* species are considered part of the microflora of the mouth but are infrequently isolated from the vaginal microflora. However, with special culture and fluorescent-antibody techniques, *Actinomyces* species can be found in women not using IUDs (8, 9), although the incidence (and concentration, most probably) is higher in IUD users (1, 6, 9). The use of an IUD has been associated with an increased incidence of pelvic inflammatory disease and pelvic actinomycosis. Although *E. nodatum* has been found in very similar clinical settings, we do not know its incidence and concentration in the vaginal and cervical microflora of IUD users and nonusers and whether it is potentially pathogenic in the pelvis. If *E. nodatum* has a pathogenic potential in the pelvis similar to that of *Actinomyces* species, then it may be advantageous to remove the IUD even though the *E. nodatum* is mistaken for *Actinomyces* species on Papanicolaou smears. If *E. nodatum* lacks such pathogenicity, then some better appreciation of its incidence in IUD users and a means to selectively recognize *E. nodatum* might prevent instances of unnecessary removal of an IUD. We believe that these *E. nodatum* strains probably were on or around the IUD in situ, but they also may have been picked up from the cervix or vagina when the device was being removed.

The most compelling evidence that *E. nodatum*, *E. timidum*, *E. brachy*, and *Eubacterium* sp. group D-6 are anaerobic pathogens comes from studies of patients with periodontitis (4, 7). The present report supports the concept of the potential pathogenicity of these organisms and extends the range of infected body sites that may harbor them. Although these eubacteria were present with other species in infections, most of the recognized opportunistic pathogens among the anaerobes, including the actinomyces, also are uncommonly found in pure culture. Only a single strain, an unidentifiable *Eubacterium* sp., was isolated in pure culture, from blood. A pathogenic role for two of the *E. nodatum* strains isolated from two patients with lumpy jaw (cervicofacial actinomycosis) was suggested by their high (in one instance, clearly the predominant species) numbers in the apparent absence of *Actinomyces* species in the infected material. In each instance, numerous colonial types were picked from primary plates, resulting in multiple isolations of the *E. nodatum* strain. This point and the fact that *Actinomyces* species generally grow more readily than *E. nodatum* make it less likely that actinomyces were present but were simply missed. This finding suggests that *E. nodatum* also is etiologic in typical-appearing cases of lumpy jaw and may have been mistaken, at times, for actinomyces.

Further understanding of the role of *E. nodatum*, *E. timidum*, *E. brachy*, *Eubacterium* sp. group D-6, and other fastidious *Eubacterium* spp. in human disease is dependent on an improved opportunity for their isolation. Prolonged incubation of plates and broth and the use of enriched media are key factors. Lysine, in particular, should be added to media, SPP or other, to enhance growth if *E. nodatum* or D-6 strains are suspected. Identification of specific growth-stimulating compounds for the other species would be a significant aid to working with these organisms.

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