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Genotypic Diversity of Clinical *Actinomyces* Species: Phenotype, Source, and Disease Correlation among Genospecies

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We determined the frequency distribution of *Actinomyces* spp. recovered in a routine clinical laboratory and investigated the clinical significance of accurate identification to the species level. We identified 92 clinical strains of *Actinomyces*, including 13 strains in the related *Arcanobacterium-Actinobaculum* taxon, by 16S rRNA gene sequence analysis and recorded their biotypes, sources, and disease associations. The clinical isolates clustered into 21 genogroups. Twelve genogroups (74 strains) correlated with a known species, and nine genogroups (17 strains) did not. The individual species had source and disease correlates. *Actinomyces turicensis* was the most frequently isolated species and was associated with genitourinary tract specimens, often with other organisms and rarely with inflammatory cells. *Actinomyces radingae* was most often associated with serious, chronic soft tissue abscesses of the breast, chest, and back. *Actinomyces europaeus* was associated with skin abscesses of the neck and genital areas. *Actinomyces lingnae*, *Actinomyces gravenitzii*, *Actinomyces odontolyticus*, and *Actinomyces meyeri* were isolated from respiratory specimens, while *A. odontolyticus*-like strains were isolated from diverse sources. Several of the species were commonly coisolated with a particular bacterium: *Actinomyces israelii* was the only *Actinomyces* spp. coisolated with *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans*; *Actinomyces meyeri* was coisolated with *Peptostreptococcus micros* and was the only species other than *A. israelii* associated with sulfur granules in histological specimens. Most genogroups had consistent biotypes (as determined with the RapID ANA II system); however, strains were misidentified, and many codes were not in the database. One biotype was common to several genogroups, with all of these isolates being identified as *A. meyeri*. Despite the recent description of new *Actinomyces* spp., 19% of the isolates recovered in our routine laboratory belonged to novel genospecies. One novel group with three strains, *Actinomyces houstonensis* sp. nov., was phenotypically similar to *A. meyeri* and *A. turicensis* but was genotypically closest to *Actinomyces neuii*. *A. houstonensis* sp. nov. was associated with abscesses. Our data documented consistent site and disease associations for 21 genogroups of *Actinomyces* spp. that provide greater insights into appropriate treatments. However, we also demonstrated a complexity within the *Actinomyces* genus that compromises the biochemical identification of *Actinomyces* that can be performed in most clinical laboratories. It is our hope that this large group of well-defined strains will be used to find a simple and accurate biochemical test for differentiation of the species in routine laboratories.

Microbiological identification of *Actinomyces* to the species level is difficult in the clinical laboratory. Even with biochemical tests such as the RapID ANA II test, whole-cell fatty acid analysis, or gas-liquid chromatography, assignment to a species is difficult (1, 2, 3, 5, 8, 13). Thus, based only on Gram staining, the catalase reaction, and better growth under anaerobic conditions than aerobic conditions, strains may be assigned to the genus *Actinomyces*. An excellent paper addressing this problem and seeking an easily accessible scheme for reliable differentiation at the species level has recently been published (13).

It is not clear that assignment to a species is clinically important, although there have been some excellent previous reports of the frequencies of occurrence and site associations of *Actinomyces* species (4, 5, 7, 9, 12, 13, 14). *Actinomyces neuii* has been associated with abscesses; *Actinomyces naeslundii* has been reported to cause hip prosthesis infection (5); and in recent studies of three newly described species, *Actinomyces turicensis* was reported to be associated with genital, skin-related, and urinary tract infections, whereas *Actinomyces radingae* was found only in skin-related infections and *Actinomyces europaeus* was detected in patients with urinary tract infections (12, 13, 14). Hall et al. (6) examined a large collection of organisms and found that *Actinomyces israelii* and *A. turicensis* were most prevalent and were most commonly associated with intrauterine contraceptive devices. In textbooks, *A. israelii* is usually identified as the cause of actinomycosis, with other *Actinomyces* cited as causative agents in various unspecified diseases (11) and as normal flora.

However, when the species are distinguished, the data from different institutions show variations in species distributions and clinical associations. Sometimes this is because of the type of laboratory (public health, reference, or oral microbiology laboratory) or because of the relative proportion of isolates examined that are strict anaerobes (7, 13). Thus, the frequency of occurrence of *Actinomyces* spp. in a routine clinical laboratory and their clinical significance are largely unknown. In the study described in this paper we characterized by genotypic and phenotypic methods a large group of presumptively significant isolates that were isolated in the routine laboratory but not as strict anaerobes. We also reviewed patient data. We thus wished to determine the frequency of occurrence and association with disease of the aerotolerant *Actinomyces* spp. isolated

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in a clinical laboratory which were accurately identified by 16S ribosomal DNA (rDNA) sequence analysis.

MATERIALS AND METHODS

Organisms. We sequenced 100 presumptively clinically significant strains of putative *Actinomyces* spp. that were recovered in a routine clinical laboratory on Trypticase soy agar with sheep blood or Columbia colistin-nalidixic acid agar (BBL, Becton Dickinson, Cockeysville, Md.) by incubation at 35°C with CO_2 (7%) added but without the use of special anaerobic conditions. Of these strains, 15 strains were not in the *Actinomyces* cluster and were not further studied; most were in the *Bifidobacterium* and *Gardnerella* groups, with at least one strain each of the genera *Streptococcus*, *Erysipelothrix*, and *Abiotrophia*. Thirteen strains were in the related *Arcanobacterium-Actinobaculum* taxon and were included in the study. In addition, we identified by 16S rDNA sequence analysis six strains as *Actinomyces* spp. that were originally identified as *Lactobacillus*, *Streptococcus*, or coryneform organisms. Thus, we analyzed 91 strains: 82 strains were obtained from the Microbiology Laboratory of the Houston Veterans Affairs Medical Center (VAMC) and 9 strains were from other clinical laboratories and were referred to the Houston VAMC for identification. All strains were stored frozen at −70°C. During the periods when we were most alerted to this group of organisms, the noted occurrence was about 15 to 20 cases per year. When available, patient records were reviewed for the clinical significance of the isolates. We made the assumption of possible significance if the isolate had one of the follow features: (i) was cultured from a normally sterile site, (ii) was the predominant organism or a copredominant organism from a wound infection or abscess, (iii) was found from a urine specimen culture at $>10^4$ CFU/ml with no more than one other isolate at $>10^4$ CFU/ml, or (iv) was the predominant organism isolated from a purulent sputum specimen. A careful review of medical notes was used to confirm clinical significance. The presence of polymorphonuclear leukocytes (PMNs), the use of surgical drainage, and a record of antibiotic treatment were primary indications of clinical significance. Although gram-positive branching rods were frequently noted on the original Gram stain, at times what were later shown on review to be *Actinomyces* were interpreted as gram-positive cocci in short chains.

Biochemical identification. All strains were grown at 35 to 37°C on sheep blood agar plates (Remel, Lenexa, Kans., and BBL, Becton Dickinson) with CO_2 added. Presumptive phenotypic identification was performed by Gram staining, evaluation of the colony morphology, and catalase reaction. None of the isolates was a strict anaerobe; all grew in an atmosphere with elevated CO_2 concentrations (5 to 8%). Biochemical testing was performed with RapID ANA II identification kits (RapID ANA; Remel, Inc., Norcross, Ga.). The interpretation of tests was done according to the manufacturer's instructions.

16S rDNA sequence analysis. 16S rRNA gene sequence identification was performed at the Houston VAMC laboratory and MIDI Labs (Newark, Del.) with the MicroSeq 500 gene kit (Applied Biosystems, Foster City, Calif.) according to the specifications of the manufacturer. Approximately 500 bp in both the forward and the reverse senses was sequenced for each isolate. Test strain sequences were compared with sequences in both the MicroSeq and the GenBank 16S rRNA gene sequence databases. The MicroSeq database contains sequences from 1,297 different species (1,187 type strains), including 9 type strains from the genus *Actinomyces*. Sequence data obtained from the strains in GenBank were included in the analysis. Sequences were compared in dendrogram form by using the neighbor-joining method (J. E. Clarridge, Q. Zhang, and S. Heward, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr., C-42, p. 157, 2001).

Nucleotide sequence accession numbers. The partial 16S rDNA sequence of the type strain of *A. houstonensis* Houston VAMC 3971 is deposited in GenBank under accession no. AF457638. To help compare isolates, we are depositing in GenBank one 500-bp sequence from each of the genospecies 2, 6, 13, and 17 (strains VAMC Ref113, VAMC 3971, VAMC Ref103, and S3672, respectively) as AF457642, AF457638, AF457641, and AF457640, respectively.

RESULTS

Genotypic identification and phylogenetic relationships. The dendrogram (Fig. 1) based on gene sequencing shows the overall relatedness of the *Actinomyces* spp. We show the type strains of 21 *Actinomyces* spp. and 4 related type strains (11 from the MicroSeq database, 13 from the GenBank database, and a challenge strain from the College of American Pathologists [CAP]) and representative clinical strains for each of the major groups. To present the sequences in a single comparative dendrogram, three or more clinical isolates that differed by no more than 5 bp are represented by a single entry. We note the number of isolates in the group; e.g., in genospecies 5 (*A. europaeus*), there are seven clinical strains. Because the *Actinomyces odontolyticus-Actinomyces meyeri* group was so diverse, the isolates are presented individually in Fig. 2.

The 91 strains were clustered into 18 major genogroups and 3 minor genogroups (Fig. 1 and Fig. 2). Table 1 shows the distribution of species by the number of isolates in each genogroup, designated as in the dendrogram in Fig. 1. Table 2 shows the same type of data for the *A. meyeri-A. odontotyticus* groups, as shown in Fig. 2, except that the data are presented separately for each strain of this diverse group. Twelve genogroups correlated with a known species: *A. turicensis* (23 strains), *A. radingae* (13 strains), *A. europaeus* (7 strains), *A. lingnae* (4 strains), *Actinomyces graevenitzii* (4 strains), *A. neuii* (3 strains), *A. odontolyticus* (4 strains) *A. meyeri* (3 strains), *A. israelii* (3 strains), *Arcanobacterium haemolyticum* (9 strains), and 1 strain each of *Actinomyces funkii* and *Arcanobacterium bernardiae*. These are so designated in both Tables 1 and 2. Nine genogroups (17 strains; 19%) did not correlate closely with a known type strain. For these, the closest strain with at least 95% similarity is listed and the genogroup is called "novel." The most common sources and associated diseases are listed.

Phenotypic characterization. The biochemical reactions obtained with the RapID ANA II system, coded as a biotype number, for the species sequenced are also presented in Tables 1 and 2. Although the biochemical identifications with this system frequently indicate an inaccurate species name (as indicated in the tables), many of the different genogroups show distinctive and reproducible biochemical profiles. For example, 4 *A. europaeus* strains had similar code numbers (421670, 421671, and 421070), 2 isolates of genogroup 10 generated similar codes (671671 and 671470), 18 of the 21 *A. turicensis* strains tested generated the same code (020671), and most of the *A. radingae* isolates generated a code similar to 677671 (Table 1). The same may be true for other genogroups, but due to the limited number of isolates in these other groups, we do not believe that the data are sufficiently robust for reliable identification.

The full description of the biochemical significance of the biotype numbers is presented in the literature accompanying the RapID ANA II system. Briefly, to summarize some important differences found in this study: most isolates shared the last three digits of 671 in their profile numbers, which indicates positive reactions for the cleavage of leucyl-glycine, glycine, proline, phenylalanine, arginine, and serine and negative reactions for alkaline phosphate, pyrrolidine, and indole. *A. turicensis* was usually additionally positive only for hydrolysis of aryl-substituted α-glucoside. *A. europaeus* was positive for carbohydrate hydrolysis of aryl-substituted arabinoside, α-glucoside, and galactoside. Genogroup 10 was additionally positive for β-disaccharide, *o*-nitrophenyl-β-D-galactopyranoside, and β-glucoside. It is clear that, according to the RapID ANA II system database, many genospecies would be identified as *A. meyeri* (e.g., genospecies 2, 6, and 15). All strains except *A. neuii* and genospecies 9 were negative for catalase; *A. neuii* has previously been reported to be positive for catalase, and genospecies 9, a strain from gallbladder fluid with an unusual biotype profile, which we report here for the first time, was pos-

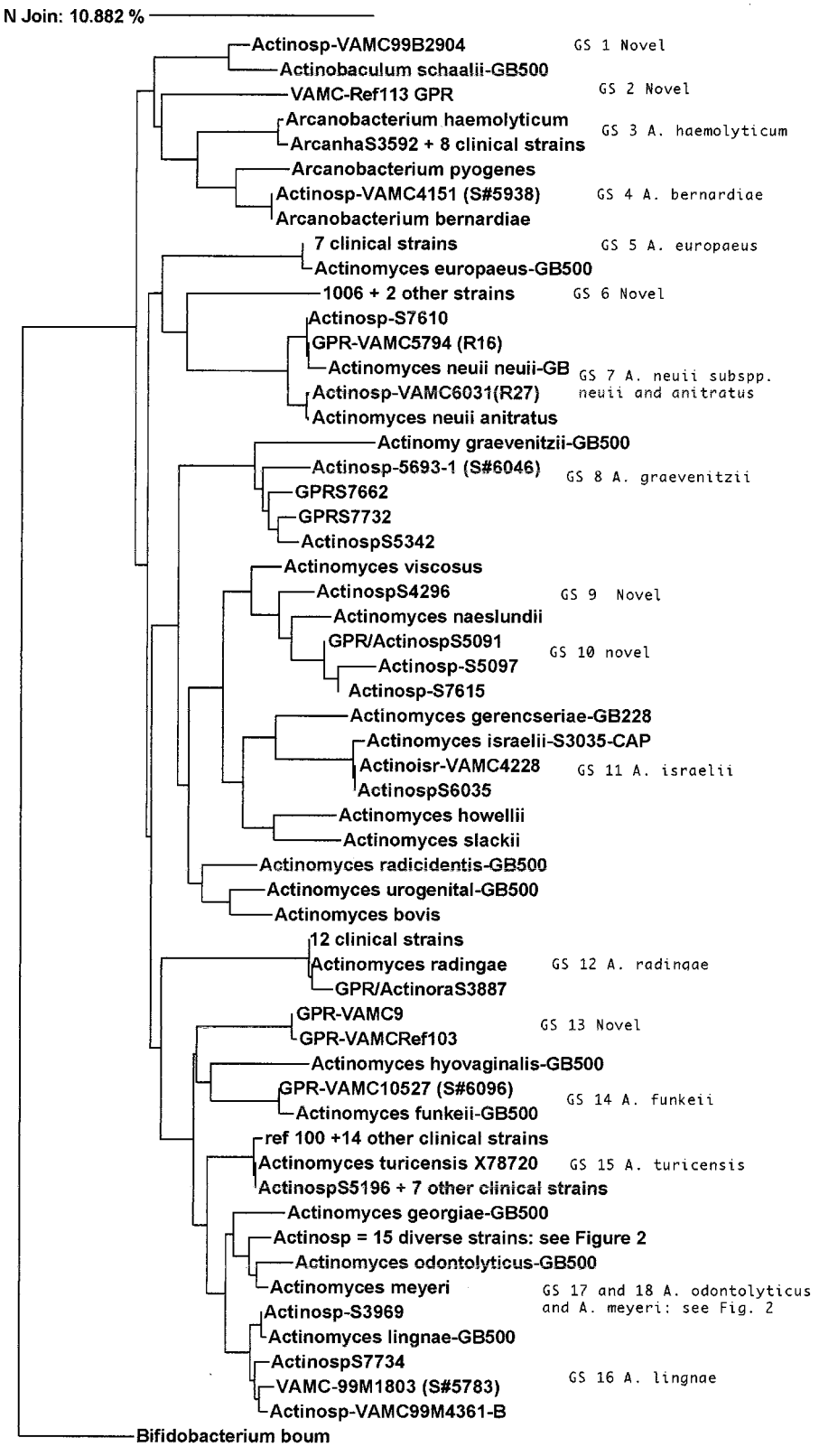

FIG. 1. Dendrogram showing the genospecies (GS) clusters based on our sequence data for clinical isolates and some *Actinomyces* type strains from MicroSeq and GenBank databases with *Bifidobacterium boum* as an outgroup. All type strains are represented by the name written out in full. We note the type strain sequences from GenBank by GB and the number of base pairs that were used or available for comparison. The single strain from CAP has the suffix CAP, and the strains from the MicroSeq database do not have a suffix.

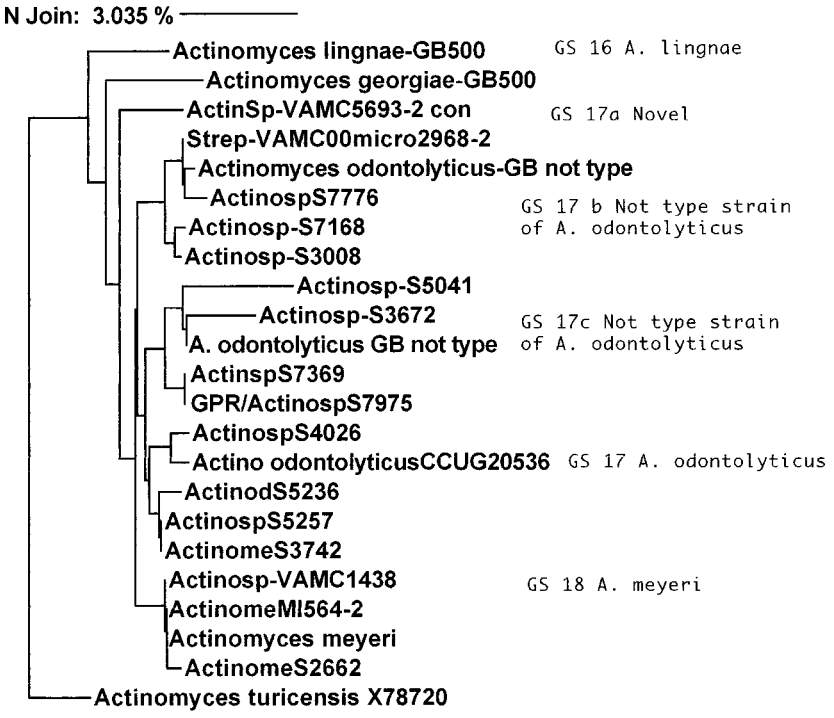

FIG. 2. Dendrogram showing the genospecies clusters based on our sequence data for clinical isolates within the *A. odontolyticus-A. meyeri* branch. In addition to all 14 clinical strains and the *A. meyeri* type strain, we show one *A. odontolyticus* type strain and two other sequences that were called *A. odontolyticus* in GenBank but which have 16S rDNA sequences different from that of the *A. odontolyticus* type strain. *A. turicensis* is the outgroup.

itive for catalase. Genospecies 9 and 10 cluster with the *Actinomyces viscosus* and *A. naeslundii* groups, which are variable for catalase (10). Most isolates in the *A. odontolyticus* clusters were identified as either *A. odontolyticus* or *A. meyeri* by the RapID ANA II system.

Sources and disease associations. Table 3 summarizes the sources and disease associations of the more common *Actinomyces* species and genospecies. *A. turicensis* was the most frequently isolated species and was associated with genitourinary tract specimens, often with other organisms and only rarely with inflammatory cells. In a few instances *A. turicensis* was isolated as a pure culture: from two urine specimens, one specimen from a wound, and one specimen from a breast abscess. It was the only *Actinomyces* sp. isolated from urine and urethral exudates. *A. radingae* was usually associated with abscesses; almost all of these were serious, chronic soft tissue abscesses of the breast, chest, and back. *A. radingae* was often recovered in large numbers and was usually associated with PMNs. *A. europaeus* was associated with soft tissue abscesses of the neck and genital areas. The newly described species *A. lingnae* was found in low numbers in respiratory specimens from compromised hosts.

Both *A. israelii* and *A. meyeri* were associated with pulmonary actinomycosis and sulfur granules in histological specimens. *A. israelii* was the only *Actinomyces* sp. coisolated with *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans*. *A. meyeri* was coisolated with *Peptostreptococcus micros*. Because these two coisolates are well-known pathogens in their own right, it is interesting to speculate on their contribution to disease.

In Table 4 we present our deductions of the normal niches of the various *Actinomyces* spp. by correlating both the site of isolation (indicated in Tables 2, 3, and 4) and the known niche of the common coisolates. *A. radingae* and *A. europaeus* were isolated with low numbers of coagulase-negative *Staphylococcus* spp. and *Corynebacterium* spp., suggesting skin contamination and that the niche for these organisms is the skin. *A. lingnae*, *A. graevenitzii*, *A. odontolyticus*, *A. meyeri*, and genospecies 10 are associated with respiratory specimens. The two strains in genospecies 10 were cultured from a mandible and a face, and their sequences had 98% similarity to an unnamed oral clone of *Actinomyces* sp. from GenBank (accession no. AF287749). *Streptococcus intermedius* and *Eikenella corrodens* were also found in these cultures, further suggesting an oral source. The *A. odontolyticus*-like strains (genospecies 17a to 17c) were isolated from heterogeneous sources, some of which suggest hematogenous spread and a genitourinary or gastrointestinal source.

In both instances in which two different *Actinomyces* spp. were recovered in the same specimen, the presumed niches agreed: the respiratory site-associated species, *A. graevenitzii* and *A. lingnae*, were recovered together, both in low numbers, from bronchial wash specimens; and the skin- and abscess-associated species, *A. europaeus* and *A. radingae*, were recovered together from a back abscess.

Two genetically well-separated genospecies, genospecies 6 and 13, had multiple strains. Below we describe genospecies 6 as *A. houstonensis* sp. nov.

DISCUSSION

Using 16S rRNA gene sequencing to identify clinically derived *Actinomyces* spp., we determined the frequency distribu-

TABLE 1. Characteristics of the genogroups of clinically isolated *Actinomyces* strains

Genospecies cluster[a]	Type strain or closest strain (% related)	No. of isolates	Most common source(s)	Catalase reaction	Most common RapID ANA II biotype(s)	Most probable identification with RapID ANA II system (probability [%])
1	Novel, resembles *Actinobaculum schaalii*	1	Blood	−	420673[b]	None
2	Novel	1	Wound	−	020671	*A. meyeri* (92.4)
3	*A. haemolyticum*	9	Wound of foot and leg; see Table 3	−	Identified with Coryne API system	
4	*A. bernardiae*	1	Bone, sacral	−	024051	*Eubacterium aerofaciens* (99.9)
5	*A. europaeus*	7	Abscess; see Table 3	−	421670 421671, 421070[b]	*Propionibacterium granulosum* (89.9, 97.8), *Mobiluncus* spp. (99.6%)
6	Novel	3	Abscess, trunk; see Table 3	−	020671	*A. meyeri* (92.4)
7	*A. neuii*	3	Abscess	+	021051	*Eubacterium aerofaciens* (89.9)
8	*A. graevenitzii*	4	Bronchial wash, sputum	−	414673[b]	None
9	Novel	1	Gallbladder fluid	+	061030[b]	None
10	Novel; GenBank accession no. AF287749 is closest	2	Mandible, face fluid	−	671671, 671470	*A. israelii* (84.8)
11	*A. israelii*	2	Pleural fluid	−	271671	*A. israelii* (83.2)
12	*A. radingae*	13	Abscess; see Table 3	−	677671, 07/63671	None
13	Novel; GenBank accession no. AJ243891 is closest	2	Bronchial wash, hand	−	020671, 022671	*A. meyeri* (92.4), *A. odontolyticus* (99.9)
14	*A. funkeii*	1	Urine	−	022671	*A. odontolyticus* (99.9)
15	*A. turicensis*	23	See Table 3	−	020671	*A. meyeri* (92.4)
16	*A. lingnae*	4	Bronchial wash, blood, pleural fluid	−	024671, 434671[b]	*A. meyeri* (99.6)
17–18	*A. odontolyticus* and *A. meyeri* groups	15	See Tables 2 and 3	−	See Table 2	See Table 2

[a] The genospecies numbers are the same as those in the dendrogram in Fig. 1.
[b] No identification was suggested.

tions of species isolated in a single laboratory and their site and disease associations. As in some other studies, *A. turicensis* was the most commonly isolated species (7, 12). However, *A. radingae* and *A. europaeus* were the second and third most frequently isolated species, respectively, in contrast to *A. israelii* and *A. naeslundii*, respectively, in the study by Hall et al. (7). Sabbe et al. (12) found twice as many *A. europaeus* isolates as *A. radingae* isolates.

A. turicensis was most commonly associated with the urinary tract infections and skin-related infections of the lower body. In contrast to Funke et al. (5), some of the *A. turicensis* strains were isolated as pure cultures, and several caused abscesses. *A.*

TABLE 2. Characteristics of genogroups 17 and 18, *A. odontolyticus*, and *A. meyeri* strains

Genospecies[a]	Type strain or closest strain	Isolate no.	Source and comments[b]	RapID ANA II biotype	Most probable identification with RapID ANA II system (probability [%])
17a	Novel	5693-2	Sputum, COPD, community-acquired pneumonia with mixed respiratory flora, inadequate sputum	030671	*A. meyeri* (93.6)
17b	Novel, matches a non-type strain of *A. odontolyticus*	7776	Sinus, chronic sinusitis, with *Escherichia coli*	021671	*A. meyeri* (93.6)
		7168	Aortic graft, with *Lactobacillus*	270671	*A. viscosus* (76.3)
		3008	Tissue, diabetic foot ulcer, with group B *Streptococcus*, *Enterococcus*	030671	*A. meyeri* (93.6)
17c	Novel, matches a non-type strain of *A. odontolyticus*	3672	Aorta, cystic medial necrosis, Marfan's syndrome	020671	*A. meyeri* (92.4)
		5041	Leg swab, diabetic ulcer, with *Enterobacter cloacae* and *Staphylococcus aureus*	062671	*A. odontolyticus* (99.9)
		7369	Bronchial wash, lung cancer	062671	*A. odontolyticus* (99.9)
		7975	Blood, hepatitis C, myocardial intarction, GI bleed	060671	*Lactobacillus acidophilus* (77.7)
17	*A. odontolyticus*	4026	Breast abscess	012671	*A. odontolyticus* (99.9)
		5236/5257	Blood and sputum, HIV, COPD, adenocarcinoma	072671	*A. odonlyticus* (99.9)
		3742	Finger abscess	272671	*A. odonlyticus* (99.9)
18	*A. meyeri*	2662	Neck abscess, many PMNs	430671	*A. meyeri* (98.2)
		564-2	Pleural fluid, many PMNs, actinomycosis	020671	*A. meyeri* (92.4)
		1438-5316	Lung aspirate and brain abscess, many PMNs	020671	*A. meyeri* (92.4)

[a] The genospecies numbers are the same as those in the dendrogram in Fig. 2.
[b] Abbreviations: COPD, chronic obstructive pulmonary disease; GI, gastrointestinal; HIV, human immunodeficiency virus.

TABLE 3. Site of isolation or infection associated with *Actinomyes* spp. for which there were at least three strains

Site of isolation or infection	No. of isolates for the following species (total no. isolates):									
	A. turicensis (23)	*A. radingae* (13)	*A. europaeus* (7)	*A. graevenitzii* (4)	*A. lingnae* (4)	*A. neuii* (3)	*A. odontolyticus* (4)	*A. meyeri* (3)	"*A. houstonensis*" (3)	*A. haemolyticum* (9)
Urine, urethral exudate	9									
Abscess										
Face, neck, breast, chest, back	4	12	4			2	2		2	
Groin, perianal, scrotal, labial, abdominal	5	1	2			1			1	1[a]
Swab or abscess (leg, foot, decubitus, ulcer)	4									8
Bronchial wash, bronchial biopsy, sputum				4	1		1			
Pleural fluid, lung mass					2			2		
Blood					1		1			
Abscess, unknown location	1		1					1		

[a] Scrotal abscess.

radingae and *A. europaeus* were associated with soft tissue infections. Our data show a striking correlation of *A. radingae* with recurrent abscesses of the chest, back, and breast. Infections due to *A. europaeus* were also found in the genital area, such as scrotal abscesses and labia abscesses. *A. meyeri* and *A. israelii* were both associated with actinomycosis, sulfur granules, and a distinct coisolate.

The RapID ANA II system biochemical tests for *A. turicensis*, *A. radingae*, and *A. europaeus* were reproducible, yielding codes that were useful for identification; however, the code numbers either corresponded to another organism or were not in the database. The same biotype for *A. turicensis* was noted by Sabbe et al. (12). Our data confirm the revised description by Vandamme et al. (14) that *A. radingae* strains are positive for *N*-acetylglucosamine and β-glucosidase, while *A. turicensis* strains are negative. Our biochemical data do not support the identification scheme proposed by Sarkonen et al. (13), who used reagents from a different manufacturer.

In contrast to biochemical identification, which, as we have shown here, might be ambiguous, 16S rRNA sequence analysis assigns an unknown strain to a reproducible genocluster. Because we sequenced all isolates in a cluster, we were able to discern that some genogroups are more heterogeneous than others. For example, organisms in the *A. graevenitzii* and *A. viscosus-A. naeslundii* clusters were genetically heterogeneous, as was also determined from the data of Hall et al. (7). The heterogeneity in the *A. odontolyticus*-like groups allowed us to distinguish one group that seemed to have a gastrointestinal

TABLE 4. Discovery of the probable normal niche of *Actinomyces* spp. approached by using the known niche of specific coisolates and the sources of the *Actinomyces* strains in this investigation[a]

Species	Common coisolate(s)	Probable source of coisolates	Probable normal niche of species[b]
A. haemolyticum	Beta-hemolytic streptococci, usually group B or G	Not clear; possibly GU or skin flora	Skin?; it is not clear why we find *A. haemolyticum* in lower-limb infections, whereas others find them in respiratory specimens
A. europaeus	Coagulase-negative *Staphylococcus* spp., *Corynebacterium*; once isolated with *A. radingae*	Skin flora	Skin, upper body
Novel GS 6; *A. houstonii* sp. nov.	*Staphylococcus* spp., *Bacteroides fragilis*. *Fusobacterium* sp., *Peptococcus magnus*	Skin and GI flora	All serious infections; breast, chest, and surgical abdominal wound abscesses; Branching GPR seen on GS with PMN
A. neuii	Only one evaluable case; *Peptococcus prevotti, Corynebacterium urealyticum*		Not clear
A. graevenitzii	Usually in low numbers with mixed respiratory flora; once isolated with *A. lingnae*	Mixed respiratory flora	Oropharynx
A. israelii	*A. actinomycetemcomitans*	Respiratory flora	Oropharynx
A. radingae	Coagulase-negative *Staphylococcus* spp.; once isolated with *A. europeaus*	Skin flora	Skin, upper body
A. turicensis	*S. anginosis* (a GI clade), *E. coli, Enterococcus, Aerococcus urinae*	GI-GU flora	Gastrointestinal, genital or skin in that area
A. lingnae	Usually in low numbers with mixed respiratory flora; once isolated with *A. graevenitzii*	Respiratory flora	Oropharynx
Novel GS 17b; not type strain of *A. odontolyticus*	*E. coli, Lactobacillus, Enterococcus,* group B *Streptococcus*	GI-GU flora	Both the coisolates and the source of the isolates found here suggest a GI-GU site
A. odontolyticus	Alpha-hemolytic streptococcus, mixed respiratory flora	Respiratory flora	Oropharynx
A. meyeri	*Peptostreptococcus micros*	Respiratory flora	Oropharynx

[a] Only groups for which enough evaluable data were available are represented. Abbreviations: GU, genitourinary; GI, gastrointestinal; GS, genospecies; GPR, gram-positive rods; GS, Gram staining.

[b] The probable normal niche was determined on the basis of the information in the labeled "Probable source of isolates" in this table and the information in Table 3.

source. In contrast, genogroups that showed minimal variations in their 16S rDNA sequences and that were closely related to the type strain are *A. turicensis*, *A. radingae*, *A. europaeus*, and the newly described species *A. lingnae*.

Nine genogroups did not have a corresponding known type strain at the time of submission of the manuscript. However, as many new sequences are being deposited and new species are being described every day, it is probable that other investigators will find similar strains.

At present, clinical microbiologists and infectious disease specialists should be cautious in their acceptance of an identification as an *Actinomyces* species by testing that is usually performed in most clinical laboratories. Case reports based on identifications achieved prior to the use of 16S rDNA-based techniques (2, 6, 7, 9, 10) may not be accurate. Our data document consistent site and disease associations for 21 genogroups of *Actinomyces* spp. that provide greater insights into the clinical relevance of the genogroups. However, we also demonstrate a complexity within the genus *Actinomyces* that compromises the biochemical identification of *Actinomyces* that can be performed in most clinical laboratories. It is our hope that this large group of well-defined strains will be used to find simple and accurate biochemical tests for differentiation of the species in routine laboratories.

Description of *Actinomyces houstonensis* sp. nov. *Actinomyces* means ray fungus for the shape of the microcolonies; *houstonensis*, in honor of Houston, Texas, indicates the place where the bacterium was identified and described.

A. houstonensis is facultatively anaerobic and grows on sheep blood agar as α-hemolytic, gray colonies 0.2 mm in diameter after 48 h of incubation at 36°C with elevated CO_2 concentrations (8%). Growth is equal with elevated CO_2 and anaerobic conditions. By Gram stain, the cells are nonsporulating, gram-positive pleomorphic rods, which are more robust than those of *A. meyeri*, and have a tendency to form half circles. The organism is nonmotile and catalase negative. It is esculin, urease, and gelatin test negative. It reduces nitrate and produces positive reactions for the cleavage of aryl-substituted α-glucoside, leucyl-glycine, glycine, proline, phenylalanine, arginine, and serine and negative reactions for alkaline phosphate, pyrrolidine, and indole. It ferments glucose and sucrose but not xylose. The type strain was isolated from an abscess from the back of a patient. All three strains were associated with serious subcutaneous abscesses requiring drainage; two strains were associated with other organisms that tend to be associated with the gastrointestinal tract or skin. The type strain of *A. houstonensis* is Houston VAMC strain 3971 and is deposited in GenBank under accession no. AF457638.

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