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Identification of Clinical Isolates of *Actinomyces* Species by Amplified 16S Ribosomal DNA Restriction Analysis

VAL HALL,* P. R. TALBOT,† S. L. STUBBS,‡ AND B. I. DUERDEN

Anaerobe Reference Unit, Department of Medical Microbiology, and Public Health Laboratory, University Hospital of Wales, Cardiff CF14 4XW, United Kingdom

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Amplified 16S ribosomal DNA (rDNA) restriction analysis (ARDRA), using enzymes *Hae*III and *Hpa*II, was applied to 176 fresh and 299 stored clinical isolates of putative *Actinomyces* spp. referred to the Anaerobe Reference Unit of the Public Health Laboratory Service for confirmation of identity. Results were compared with ARDRA results obtained previously for reference strains and with conventional phenotypic reactions. Identities of some strains were confirmed by analysis of partial 16S rDNA sequences. Of the 475 isolates, 331 (70%) were clearly assigned to recognized *Actinomyces* species, including 94 isolates assigned to six recently described species. A further 52 isolates in 12 ARDRA profiles were designated as apparently resembling recognized species, and 44 isolates, in 18 novel profiles, were confirmed as members of genera other than *Actinomyces*. The identities of 48 isolates in nine profiles remain uncertain, and they may represent novel species of *Actinomyces*. For the majority of species, phenotypic results, published reactions for the species, and ARDRA profiles concurred. However, of 113 stored isolates originally identified as *A. meyeri* or resembling *A. meyeri* by phenotypic tests, only 21 were confirmed as *A. meyeri* by ARDRA; 63 were reassigned as *A. turicensis*, 7 as other recognized species, and 22 as unidentified actinomycetes. Analyses of incidence and clinical associations of *Actinomyces* spp. add to the currently sparse knowledge of some recently described species.

The genus *Actinomyces* comprises a heterogeneous group of anaerobic and facultatively anaerobic, nonspore-forming, nonmotile, non-acid-fast, gram-positive rods that have a G+C content of 55 to 71 mol% (1). Volatile and nonvolatile acid end products of glucose metabolism are acetic, lactic, and succinic acids (11). The natural habitats of many *Actinomyces* spp. are the mucous membranes of humans and other animals, particularly the oral mucosa. Members of the genus can cause classical actinomycosis, are associated with infections arising from tissue invasion by oral anaerobes, and may be instrumental in the development of periodontal diseases (21).

Historically, classification of *Actinomyces* spp. was based upon differentiation in a few phenotypic tests (11). However, application of modern chemotaxonomic and genotypic methods has demonstrated heterogeneity within the classic species and led to the recognition of several new species. Furthermore, these powerful tools have demonstrated the existence of several genera within the actinomyces group and the need for a major review of taxonomy (17, 22). Some actinomycetes have been reclassified as *Arcanobacterium* spp. or as *Actinobaculum* spp. (14, 17). However, the taxonomic positions of other species remain unsatisfactory, and further revisions are likely in the light of recently described species and additional taxa, as yet unnamed. Recently described actinomycetes isolated from human sources include *Actinomyces europaeus* (7), *Actinomyces graevenitzii* (16), *Actinomyces neuii* (8), *Actinomyces radingae* (27), *Actinomyces turicensis* (27), *Actinomyces urogenitalis* (15), and *Actinomyces radicidentis* (5). The clinical spectra of infections due to *A. turicensis*, *A. radingae*, and *A. europaeus* have been investigated by Sabbe et al. (20). However, for some species, few strains have been examined, and little is known of their natural habitats, clinical prevalence, and pathogenic potential.

Identification of *Actinomyces* spp. is notoriously difficult and unreliable (9), yet clinically important. Current taxonomy is based upon delineation in 16S ribosomal DNA (rDNA) sequence analysis, whole-cell protein profiling, and an extensive range of phenotypic characteristics. Clearly, this approach is impractical for routine identification of clinical isolates. Hence, commonly, identification in clinical laboratories is based solely upon a limited range of conventional biochemical tests. Even when a wider range of tests is performed, these lack discrimination at species level and are subject to method-dependent variations, and overlaps in phenotype between isolates of different genospecies may occur, e.g., potential misidentification of *A. turicensis* as *A. meyeri* in phenotypic tests has been demonstrated (10). Therefore, identification to species level is often tentative. Furthermore, few clinical laboratories have the ability to perform gas-liquid chromatography for end products of glucose metabolism. This valuable aid to genus-level identification of non-spore-forming gram-positive bacilli enables differentiation of *Actinomyces* spp. from morphologically similar but nonpathogenic isolates of *Propionibacterium*, *Bifidobacterium*, and *Lactobacillus* (11). Hence, clinical isolates are often misidentified, and many reported *Actinomyces* spp. are members of other genera. Amplified 16S rDNA restriction analysis (ARDRA) with enzymes *Hae*III and *Hpa*II has been shown to be a simple and highly discriminatory method for

* Corresponding author. Mailing address: Anaerobe Reference Unit, Department of Medical Microbiology and Public Health Laboratory, University Hospital of Wales, Cardiff CF14 4XW, United Kingdom. Phone: 44 (0)29 20742171. Fax: 44 (0)29 20744123. E-mail: hallv@cardiff.ac.uk.

† Present address: Marine BioProducts International, Delta, British Columbia, Canada V3M 6K8.

‡ Present address: Nycomed Amersham plc, Forest Farm Estate, Cardiff CF14 7YT, United Kingdom.

identification of *Actinomyces* isolates, including these recently described species (10).

The aims of this study were to evaluate ARDRA for identification of clinical isolates of putative *Actinomyces* spp., to create a robust library of ARDRA profiles for *Actinomyces* spp. of clinical origin, and to explore the prevalence of species and their associations with specific natural habitats, sites of infection, or particular pathogenic potential. ARDRA was applied to 176 fresh and 299 stored clinical isolates of putative *Actinomyces* spp. referred to the Anaerobe Reference Unit (ARU) of the Public Health Laboratory Service for confirmation of identity. Isolates were referred between 1983 and 1999 from hospital laboratories throughout England and Wales. Results were compared with those obtained for reference strains (10) and with conventional phenotypic reactions. Identities of some strains were confirmed by analysis of partial 16S rDNA sequences.

MATERIALS AND METHODS

Bacterial strains. A total of 475 strains, comprising 176 fresh and 299 stored clinical isolates, were examined. The fresh isolates were all of the putative *Actinomyces* spp. received by the ARU for confirmation of identity in 1998 and 1999. For these strains, ARDRA was performed blind, in parallel with conventional phenotypic tests. Stored isolates were selected from those referred during 1983 to 1997 and were identified at the time of submission by conventional phenotypic tests as *Actinomyces* spp. or as gram-positive rods of uncertain identity. Strains were selected to represent the range of morphological and biochemical diversity within the genus, and in light of previous findings, all strains (n = 113) previously identified as *A. meyeri* or resembling *A. meyeri* were examined. Strains were stored at −80°C on Microbank beads (Pro-lab Diagnostics, Wirral, United Kingdom) and were recovered on Fastidious Anaerobe Agar (LabM, Bury, United Kingdom) incubated anaerobically at 37°C for 48 h.

ARDRA. Tests were performed and analyzed as described previously (10). Each isolate was assigned a six-digit code, the first and second groups of three digits representing banding patterns obtained in *Hae*III and *Hpa*II digests, respectively. Novel banding patterns were assigned three-digit codes, as encountered, and added to existing HAE and HPA libraries (10). Resulting ARDRA profiles were equated with species where they contained a reference strain and clinical isolates with concordent phenotypic reactions, or where the identity of one or more representative strains was confirmed by 16S rDNA sequencing. For isolates that yielded ARDRA profile 001/003 (a pattern obtained for two species), a supplementary digest with *Ear*I was performed as described for *Hae*III and *Hpa*II digests (10). Resulting patterns were analyzed as above and assigned a three-digit code, listed after the HAE/HPA code, e.g., 001/003/001.

Conventional tests. Fresh clinical isolates were identified as members of the genus *Actinomyces* on the basis of volatile and nonvolatile fatty acid end products of glucose metabolism, detected by gas-liquid chromatography (11).

Cell and colonial morphologies, pigment production, fluorescence under longwave UV illumination, ability to grow in air and in air plus 5% CO_2, and production of catalase and indole were recorded. Hydrolysis of esculin and starch and production of acid from amygdalin, arabinose, cellobiose, glucose, mannitol, raffinose, ribose, salicin, sucrose, trehalose, and xylose were tested by the method of Phillips (18). Production of nitrate reductase, urease, pyrazinamidase, β-galactosidase, α-glucosidase, and β-*N*-acetylglucosaminidase were detected after incubation for 18 to 24 h with Rosco diagnostic tablets (BioConnections, Leeds, United Kingdom). Results were interpreted with reference to the identification tables of Funke et al. (7, 8), Lawson et al. (14), and Pascual Ramos et al. (16). Stored isolates had been similarly identified at the time of submission to the ARU, and results were interpreted according to the Anaerobe Laboratory Manual (11) and other publications available at the time of testing (8, 13).

Partial 16S rDNA sequencing. The 16S rDNA was extracted and amplified as for ARDRA (10). Amplified products were purified by Qiaquick spin-column (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions. A variable region of approximately 450 bp of the 16S gene was sequenced with a reverse primer targeting positions 536 to 516 (*Escherichia coli* numbering) and the ABI-PRISM Big Dye Terminator sequencing kit (Perkin Elmer, Warrington, United Kingdom) according to the manufacturer's instructions but in half volume. Sequences were compared to those in the EMBL database with BlastN 2.1.1 and analyzed further in DNASIS (Hitachi Software, Yokohama, Japan). The efficacy of this method as a screening tool, representative of full 16S gene sequence similarities, was demonstrated in preliminary experiments by application to some well-characterized clinical isolates of *Actinomyces* spp. (authors' data; not presented). Similarities of >98% were obtained with reference strains of the same species.

RESULTS

ARDRA profiles of fresh and stored isolates are listed in Table 1. The 475 clinical isolates produced 62 profiles containing from 1 to 59 strains (Table 1); 331 isolates (70%) were clearly assigned to recognized *Actinomyces* species, confirmed either by concurrence with ARDRA profiles obtained for reference strains and supportive results in conventional phenotypic tests or by >98% partial 16S rDNA sequence similarities of representative strains with those of reference strains (Table 2). Representative strains of 52 isolates in 12 ARDRA profiles showed ~N97% partial 16S rDNA sequence similarities with those listed for reference strains, so isolates in these groups were designated as apparently resembling recognized species (Tables 1 and 2). Forty-four isolates, in 18 novel profiles, were confirmed as members of genera other than *Actinomyces* (Tables 1 and 2). The identities of 48 isolates in nine profiles remain uncertain, and these may represent novel species of the actinomyces group. Thirty-nine of these isolates were contained in three ARDRA profiles, and within each group, isolates were morphologically and phenotypically similar. Group 1 strains (n = 12, ARDRA 001/016) resembled *Actinomyces odontolyticus* but grew poorly or not at all in air, produced pink rather than red colonies, and failed to ferment lactose. Group 2 strains (n = 19, ARDRA 025/026) resembled *A. meyeri* but grew moderately well in air plus CO_2 and poorly or not at all in air. Colonies were grey rather than white, and most strains reduced nitrate to nitrite. Group 3 strains (n = 8, ARDRA 035/023) were obligately anaerobic, yielding tiny grey colonies, were weakly saccharolytic, and reduced nitrate to nitrite. Partial 16S rDNA sequence analysis of representative strains of each group confirmed them as members of the genus *Actinomyces* but distinct from currently recognized species.

Profile 001/003 comprised isolates identified in phenotypic tests as *A. meyeri* (n = 28) and *A. odontolyticus* (n = 7) plus reference strains ATCC 35568 (*A. meyeri* type strain) and CCUG 32402 (APL 11), described by Wust et al. as resembling *A. odontolyticus* (27, 28) (ARDRA data unpublished). In *Ear*I digests, the two taxa were clearly differentiated, in concurrence with phenotypic tests, and as predicted by analysis of 16S rDNA sequences for reference strains; isolates resembling *A. odontolyticus* were not cut (profile 001), whereas *A. meyeri* isolates produced a band of approximately 130 bp (profile 002). Partial 16S rDNA sequencing of representative strains from this group and reference strains of *A. odontolyticus* and *A. meyeri* demonstrated a high level of similarity between the two species and the intermediate position of strain CCUG 32402 and clinical isolates R6084, R10717, and R10146 (Table 2).

Precise correlation of identities in ARDRA with those obtained in phenotypic tests was not possible, as the latter often lacked discrimination at species level, and some isolates produced variable carbohydrate fermentation reactions on repeat testing. For most fresh isolates, phenotypic reactions were consistent with published reactions and with identities obtained in

TABLE 1. ARDRA identification of fresh and stored isolates of putative actinomycetes[a]

Identification	No. of isolates	ARDRA profile	No. of isolates	
			Fresh	Stored
Resembling *A. denticolens*	5	011/027	1	4
A. europaeus	5	012/014	2	3
Resembling *A. europaeus*	3	042/014	0	3
A. georgiae	4	001/004	2	2
A. gerencseriae	41	006/008	20	21
A. graevenitzii	3	013/013	1	2
Resembling *A. graevenitzii*	6	031/013	2	4
A. israelii	72	008/009	18	15
		020/009	30	8
		040/009	1	0
Resembling *A. israelii*	6	018/009	2	4
A. meyeri	28	001/003/002	5	23
A. naeslundii genospecies 1	16	003/005	2	7
		009/005	1	6
A. naeslundii genospecies 2	37	014/005	2	5
		014/010	1	2
		017/005	0	4
		021/005	7	16
Resembling *A. naeslundii*/*A. viscosus*	8	011/005	1	2
		017/010	0	1
		021/010	1	1
		083/005	1	0
		054/040	1	0
A. neuii	8	022/019	3	5
A. odontolyticus	31	001/001	8	21
		073/001	0	1
		074/001	1	0
Resembling *A. odontolyticus*	24	001/003/001	5	2
		025/001	4	11
		025/003	0	2
A. radingae	3	028/023	2	1
A. turicensis	80	015/016	4	55
		015/032	3	10
		016/016	0	8
A. urogenitalis	3	024/011	1	2
Unidentified actinomycete group	48	001/016	1	11
		001/026	0	1
		019/017	0	1
		023/020	0	1
		025/026	6	13
		034/014	0	1
		035/023	3	5
		036/019	1	2
		041/020	0	2
A. schaalii	3	004/015	1	2
A. bernardiae	3	026/021	2	1
A. haemolyticum	10	027/022	3	7
A. vaginae	1	039/033	0	1
Bifidobacterium adolescentis group	1	060/045	1	0
Bifidobacterium bifidum	1	061/046	1	0
Bifidobacterium infantis group	1	058/041	1	0
Bifidobacterium longum	2	054/043	2	0
Bifidobacterium sp.	1	043/055	1	0
Lactobacillus acidophilus	1	049/037	1	0
Lactobacillus rhamnosus	1	064/047	1	0
Lactobacillus sp.	1	067/053	1	0
Propionibacterium acnes	2	033/028	2	0
Propionibacterium avidum	1	048/036	1	0
Propionibacterium granulosum	6	038/031	6	0
P. propionicum	8	052/038	7	0
		053/039	1	0
Streptococcus mutans	1	032/029	1	0
All isolates	475		176	299

[a] Fresh isolates comprise all putative *Actinomyces* spp. referred in 1998 and 1999, selected solely by submission from referring laboratories. Stored isolates were past referrals (1983 to 1997) selected for morphological and biochemical diversity.

ARDRA. For stored isolates identified in phenotypic tests as *Actinomyces israelii*, *Actinomyces gerencseriae*, or *Actinomyces naeslundii* or *A. viscosus*, 61 of 72 were confirmed in ARDRA. Four isolates were identified in ARDRA as *A. israelii* (profile 008/009) and in conventional tests as *A. gerencseriae* by virtue of their inability to ferment arabinose. Two of these fermented arabinose on repeat testing. Partial 16S rDNA sequences of the other two isolates (ARU strains R10167 and R9548) confirmed both to be *A. israelii* (Table 2). For three isolates, identified in ARDRA as *A. neuii* but originally identified as *A. viscosus*, phenotypic reactions were reassessed, and all were confirmed as *A. neuii* by their ability to ferment mannitol and xylose.

ARDRA confirmed the identities of 35 of 59 stored isolates identified in phenotypic tests as *A. odontolyticus*. The remainder were found to be *A. israelii* ($n = 5$), *A. naeslundii* or resembling *naeslundii* ($n = 4$), *A. geraevenitzii* ($n = 3$), and one each of *A. gerencseriae, A. meyeri*, and *Actinomyces georgiae*, and unidentified actinomycetes ($n = 9$, of which eight belonged to ARDRA group 1, profile 001/016). Dark pigmentation was

TABLE 2. Similarities to reference strains of partial 16S rDNA sequences of clinical isolates[a]

ARDRA profile	Strain (ARU no.)	Reference strain having highest similarity	Sequence similarity	
			EMOL accession no.	% similarity
011/027	R12391	*Actinomyces* sp. oral strain B19SC[b]	AF287748	97.5
		Actinomyces denticolens NCTC 11490^T	X80412	97.2
042/014	R4119	*A. europaeus* CCUG 32789A^T	Y08828	97.0
031/013	R12575	*A. graevenitzii* CCUG 27294^T	Y09589	97.3
008/009	R10167, R9548	*A. israelii* ATCC 12102^T	X82450	99.6
018/009	R3358	*A. israelii* ATCC 12102^T	X82450	97.4
020/009	R5753	*A. israelii* ATCC 12102^T	X82450	99.6
040/009	R11968	*A. israelii* ATCC 12102^T	X82450	99.3
003/005	R7710	*A. naeslundii* ser I NCTC 10301^T	X81062	98.4
009/005	R8152	*A. naeslundii* ser I NCTC 10301^T	X81062	98.4
011/005	R9108	*A. naeslundii* ser. I NCTC 10301^T	X81062	95.5
014/005	R4479	*A. naeslundii* ser. I NCTC 10301^T	X81062	96.8
014/005	R1265	*A. naeslundii* ser. II ATCC 49339^T	Authors' data	96.4
		Actinomyces sp. oral clone EP011[b]	AY008315	96.5
017/005	R2242	*A. viscosus* ser. II ATCC 27044^T	Authors' data	96.5
		Actinomyces sp. oral clone EP005[b]	AY008314	96.5
021/005	R2589	*A. viscosus* ser. II ATCC 27044^T	Authors' data	96.9
021/005	R11430	*A. viscosus* ser. II ATCC 27044^T	Authors' data	95.7
		Actinomyces sp. oral clone AP064[b]	AF287749	97.2
083/005	R13569	*A. naeslundii* ser. I NCTC 10301^T	X81062	97.0
		Actinomyces sp. oral clone EP011[b]	AY008315	97.0
054/040	R13724	*A. naeslundii* ser. I NCTC 10301^T	X81062	97.0
		Actinomyces sp. oral clone EP011[b]	AY008315	97.0
014/010	R1284	*Actinomyces* sp. oral clone AG004[b]	AF287747	100
		A. naeslundii ser. I ATCC 49339^T	Authors' data	99.0
017/010	R11372	*A. viscosus* ser. I NCTC 10951^T	X82453	95.3
021/010	R7437	*A. naeslundii*-like ATCC 49338	X81063	99.4
001/001	R5969	*A. odontolyticus* CCUG 20536^T	AJ234040	99.1
025/001	R6084	*A. odontolyticus* CCUG 20536^T	AJ234040	97.6
073/001	R5568	*A. odontolyticus* CCUG 20536^T	AJ234040	98.0
074/001	R13009	*A. odontolyticus* CCUG 28084	AJ234041	99.1
001/003/00	R10717	*A. odontolyticus*-like CCUG 32402	X78721	98.7
025/003	R10146	*A. odontolyticus*-like CCUG 32402	X78721	99.0
015/016	R8614	*A. turicensis* DSM 9168^T	X78720	99.8
016/016	R10672	*A. turicensis* DSM 9168^T	X78720	100
015/032	R5978	*A. turicensis* DSM 9168^T	X78720	99.8
024/011	R6344	*A. urogenitalis* CCUG 38702^T	AJ243791	99.4
001/016	R5571, R10394	*A. meyeri* ATCC 35568^T	X82451	91.1

Continued on following page

TABLE 2—*Continued*

ARDRA profile	Strain (ARU no.)	Reference strain having highest similarity	Sequence similarity	
			EMOL accession no.	% similarity
001/026	R5040	*A. odontolyticus* CCUG 20536^T	AJ234040	96.0
019/017	R5292	*A. naeslundii* ser. I NCTC 10301^T	X81062	91.6
		A. viscosus ser. I ATCC 27044^T	Authors' data	91.6
		Actinomyces sp. oral clone AP064[b]	AF287749	91.9
023/020	R7773	*A. israelii* ATCC 12102^T	X82450	93.5
025/026	R5307, R10236	*A. hyovaginalis* NCFB 2983^T	X69616	94.0
035/023	R5231	*A. neuii* DSM 8576^T	X71861	90.8
034/014	R5638	*A. neuii* DSM 8576^T	X71861	88.7
035/023	R12359	*A. neuii* DSM 8576^T	X71861	90.8
036/019	R11881	*A. neuii* DSM 8576^T	X71861	87.3
041/020	R7252	*A. israelii* ATCC 12102^T	X82450	94.9
039/033	R10307	*A. vaginae* CCUG 38953^T	Y17195	100
032/029	R2334	*S. mutans* NCTC 10449	AJ243965	99.2

[a] Abbreviations: ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures, London; England; CCUG, Culture Collection, University of Goteborg, Goteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; NCFB, National Collection of Food Bacteria, Reading, England; ser., serotype.

[b] Sequences submitted by Paster et al. (17a).

recorded in some mature cultures of *A. graevenitzii* and *A. georgiae*, and pink pigmentation was found in some *A. israelii* and *A. naeslundii* isolates. These findings, and the several carbohydrate fermentation tests listed as variable for *A. odontolyticus*, may account for some of these misidentifications in phenotypic tests.

Of 113 stored isolates originally identified as *A. meyeri* or resembling *A. meyeri* by phenotypic tests, only 21 were confirmed as *A. meyeri* by ARDRA. Seventy were reassigned as *A. turicensis* ($n = 63$), *A. europaeus* ($n = 1$), apparently resembling *A. europaeus* ($n = 2$), *A. georgiae* ($n = 1$), *A. israelii* ($n = 1$), *Actinobaculum schaalii* ($n = 1$), and *Arcanobacterium bernardiae* ($n = 1$). Twenty-two isolates in eight novel ARDRA profiles remain unidentified, but partial 16S rDNA sequencing of representative strains suggests that these are *Actinomyces* spp.

Of 55 isolates originally identified only as *Actinomyces* sp. or as gram-positive rods of uncertain identity, 47 were assigned in ARDRA to various recognized *Actinomyces* spp. ($n = 38$), of which 18 were in recently described species, *Arcanobacterium haemolyticum* ($n = 7$), *A. schaalii* ($n = 1$), or *Atopobium vaginae* ($n = 1$); eight remain unidentified. Failure to identify isolates confirmed in ARDRA as *A. israelii* ($n = 5$) and *A. gerencseriae* ($n = 2$) had been due to their weak or negative carbohydrate fermentation reactions.

All of the eight isolates identified in ARDRA as *A. europaeus* or apparently resembling *A. europaeus* reduced nitrate to nitrite, as did the *A. europaeus* type strain (CCUG 32789A) in our hands (10); three *A. europaeus* isolates and the type strain were asaccharolytic.

Clinical sources of *Actinomyces* spp. are summarized in Table 3. The classic species *A. gereneseriae*, *A. israelii*, *A. odontolyticus*, and the *A. naeslundii/viscosus* complex were isolated principally from cervicofacial sites and intrauterine contraceptive devices (IUCDs).

A. meyeri strains ($n = 28$) were from brain abscesses ($n = 7$), cervicofacial lesions ($n = 6$), pleural fluids ($n = 4$), and a chest abscess ($n = 1$). *A. europaeus* and organisms apparently resembling this species were from breast abscesses ($n = 2$) and one from each of brain, neck, and mastoid abscesses, Pouch of Douglas fluid, IUCD, and necrotizing fasciitis of the thigh. *A. graevenitzii* and organisms resembling this species were found in intraoral sources ($n = 4$), and one each in lung, bronchoalveolar lavage, sputum, neck abscess, and osteomyelitis of the jaw. *A. neuii* occurred in breast ($n = 3$), dental ($n = 2$), axillary ($n = 1$), and buttock ($n = 1$) abscesses and an IUCD. *A. radingae* was found in one each of breast and axillary abscesses and an IUCD. *A. urogenitalis* was isolated from a vaginal swab, a groin abscess, and a case of osteomyelitis, site unspecified. Principal sources of *A. turicensis* were penile lesions, mainly balanitis ($n = 24$), IUCDs ($n = 21$), abscesses of groin or rectal areas ($n = 6$), and pilonidal abscesses ($n = 5$). Six of eight isolates identified as *Propionibacterium propionicum* were from cases of lacrimal canaliculitis. The strain identified as *A. vaginae* was isolated from kidney tissue.

DISCUSSION

Analysis of 475 clinical isolates of putative *Actinomyces* spp. confirmed the efficacy of ARDRA for identification of members of this genus; 70% of strains were identified as *Actinomyces* to species level with confidence, 11% apparently resembled recognized species, 9% were members of other genera, and 10% were deemed to be unidentified actinomycetes. In gen-

TABLE 3. Sources of *Actinomyces* spp.

Source[b]	*Actinomyces* spp.[a]																	
	Den.	Eur.	Geo.	Ger.	Gra.	Isr.[c]	Mey.	Nae.	Neu.	Odo.	Rad.	Tur.	Uro.	Gp. 1	Gp. 2	Gp. 3	Oth.	Total
Neck-face	1	2	2	11	6	17	6	17	2	12		4		2	1	2	3	88
Eye			1	5		4		2		3								15
Thorax					3	3	5	2		4		1		1	1		1	21
Abdomen				3		7	3	2		1		4						20
Pelvis		1		2		4	1					1		1	1			11
IUCD	3	1		13		41	2	18	1	18	1	21		5	4	1	1	130
Vagina/penis	1			2				1		1		26	1	1	2	1	1	37
Superficial		2				1	2	1	5	4	2	15	1		7	3	2	45
Blood			1					9		7		3						20
Brain/CSF		1		1			7	1		1		2		1	2	1		17
Other/unknown		1		4		2	2	8		4		3	1	1	1		1	28
Total	5	8	4	41	9	79	28	61	8	55	3	80	3	12	19	8	9	432

[a] Abbreviations for *Actinomyces* spp.: Den., resembling *A. denticolens*; Eur., *A. europaeus* and resembling *A. europaeus*; Geo., *A. georgiae*; Ger., *A. gerencseriae*; Gra., *A. graevenitzii* and resembling *A. graevenitzii*; Isr., *A. israelii* and resembling *A. israelii*; Mey., *A. meyeri*; Nae., *A. naeslundii* genospecies 1 and 2 and resembling *A. naeslundii*/*A. viscosus*; Neu., *A. neuii*; Odo. *A. odontolyticus* and resembling *A. odontolyticus*; Rad., *A. radingae*; Tur., *A. turicensis*; Uro., *A. urogenitalis*; Gp. 1, unidentified actinomycetes ARDRA profile 001/016; Gp. 2, unidentified actinomycetes ARDRA profile 025/026; Gp. 3, unidentified actinomycetes ARDRA profile 035/023; Oth., unidentified actinomycetes ARDRA profiles 001/026, 019/017, 023/020, 034/014, 036/019, and 041/020.

[b] Superficial, soft tissue lesions including breast, axillary, limb, groin, buttock, rectal, and pilonidal abscesses; CSF, cerebrospinal fluid.

[c] One *A. israelii* isolate from lung and liver included in thoracic and abdominal sources.

eral, phenotypic data supported identifications made in ARDRA but, in many cases, lacked discrimination between species. Thus, identities of some isolates were amended upon reassessment of phenotypic data in the light of ARDRA findings. This was most notable in the reassignment to other species of 81% of isolates originally identified as *A. meyeri* or resembling *A. meyeri*. These data underline those of other studies in which isolates subsequently found to be *A. turicensis* were variously described on the basis of phenotypic data as resembling *A. meyeri* (2, 10), *Actinomyces* (now *Arcanobacterium*) *pyogenes* (28), or *Gardnerella vaginalis* (25, 26).

Reassuringly, all of 44 isolates found to be members of genera other than *Actinomyces* yielded distinct ARDRA profiles, and thus none was misidentified as an *Actinomyces* sp. Numbers tested were small, but the profiles obtained indicate the potential of this method for identification of a broader range of genera. These findings and the authors' data for reference strains (not presented) demonstrate the ability of ARDRA to differentiate *Actinomyces* spp. from other nonspore-forming gram-positive bacilli, obviating the need for gas-liquid chromatography and conventional biochemical tests. Furthermore, when molecular expertise and equipment are available, the simplicity and cost-effectiveness of ARDRA compare favorably with conventional tests and have enabled the examination of a large number of strains in this study. At the ARU, the current strategy for identifying unknown, clinically significant anaerobic or microaerophilic actinomycetes comprises observation of cell and colony morphologies and atmospheric requirements plus ARDRA. When the ARDRA profile is consistent with a recognized *Actinomyces* sp. and morphology concurs, no further tests are necessary. When the ARDRA profile is distinct or suggests that the isolate is an unidentified actinomycete or a member of another genus, appropriate conventional biochemical tests, including gas-liquid chromatography for end products of glucose metabolism, are performed.

Difficulties in identification of actinomycetes in the clinical laboratory are demonstrated by outcomes for putative *Actinomyces* spp. referred to the ARU in 1998 and 1999. Only 65% of the 176 isolates were confirmed as members of recognized *Actinomyces* spp. A further 10% were identified as apparently resembling recognized *Actinomyces* spp., and 6% were deemed to be unidentified actinomycetes. The remainder (19%) were found to be members of other genera. Of interest, 9% of the 176 isolates were members of recently described species *A. turicensis* ($n = 7$), *A. neuii* ($n = 3$), *A. europaeus* ($n = 2$), *A. radingae* ($n = 2$), and *A. graevenitzii* ($n = 1$).

A. neuii subsp. *neuii* and *A. neuii* subsp. *anitratus* were not distinguished in ARDRA but can be differentiated by nitrate reductase reactions. Isolates of *A. gerencseriae* ($n = 41$) and *A. meyeri* ($n = 28$) formed homogeneous taxa in ARDRA, but in some species, subspecies variations were seen. Distinct profiles obtained within the species *A. israelii* and *A. odontolyticus* may relate to biochemical, serological, or molecular differentiations noted by other workers (1, 12, 23, 24). The three ARDRA profiles obtained for members of *A. turicensis* may denote similar variation within this species. Indeed, the dendrogram derived from whole-cell protein patterns, published by Vandamme et al. (25), indicates several distinct groups within *A. turicensis*. Within the *A. naeslundii*/*A. viscosus* complex, numerous distinct ARDRA profiles and relatively low similarities in 16S rDNA sequences confirmed the wide diversity of this group. Interestingly, some of these taxa showed high homology with 16S rDNA sequences of oral clones catalogued recently in EMBL by Paster et al. (see Table 1, footnote *b*) but not otherwise published.

Chemotaxonomic methods and 16S rDNA sequencing have demonstrated the existence of several genera within the genus *Actinomyces* as currently recognized (17, 22) and classification of this actinomycete group is still in flux. In this study, 23% of isolates deemed to be members of the actinomycete group could not be assigned to recognized species with confidence. Further clarification of taxonomy of the group is necessary, including a review of classification at the genus level.

Analyses of incidence and clinical associations of *Actinomyces* spp. must be undertaken with caution, as the study strains were a selected population; first by referral from clinical laboratories to the ARU and, second by selection of stored isolates to represent phenotypic diversity. Furthermore, for some species, the numbers of strains were small. However, certain trends are apparent.

In terms of incidence, the classic species were well represented, and strains belonging to six recently described species were identified. Numbers of *A. europaeus*, *A. graevenitzii*, *A. neuii*, *A. radingae*, and *A. urogenitalis* were small, but *A. turicensis* represented 17% of study strains. Similarly, Sabbe et al. (20) found that *A. turicensis* was isolated much more frequently than *A. radingae* or *A. europaeus*. Given that *A. neuii* is an aerotolerant catalase producer, it is probable that many clinical isolates of this species are dismissed as *Corynebacterium* spp. We found no isolates of *A. radicidentis*. To date, this species has been isolated only from infected root canals of teeth. It may be specific to that site and not associated with the wide range of clinical sites from which isolates were received in this study. Of interest among strains designated as apparently resembling recognized *Actinomyces* spp., five resembled *Actinomyces denticolens*, a species isolated from dental plaque of cattle (6) but not previously reported from human sources. Three ARDRA groups of unidentified actinomycetes contained 19, 12, and 8 isolates, suggesting that these organisms are at least as common in clinical material as some currently recognized species.

Clinical sources of the classic species *A. gerencseriae*, *A. israelii*, *A. odontolyticus*, and the *A. naeslundii*/*A. viscosus* complex have been well documented and were, as expected, principally cervicofacial sites and IUCDs. However, 25% of *A. meyeri* strains, now reliably identified, were isolated from brain abscesses, 21% from cervicofacial lesions, and 17% from thoracic sites. These appear to be real associations, as the study included all *A. meyeri* isolates in the ARU collection, and the sources of these are remarkably similar to those of the 16 strains reported by Cato et al. (4). Conversely, *A. turicensis* was isolated predominantly from genital or skin-related sources, similar to those reported previously for this organism (20, 25, 27). This species was also isolated from bacteremias in this and other studies (20, 25).

With the exception of *A. turicensis*, few isolates of recently described species have been reported to date. Therefore, though numbers in this study were small, our data add significantly to current knowledge of clinical sources of these species. Unfortunately, little information regarding possible significance and concomitant organisms was available to us. The skin-related and urogenital tract sources of *A. europaeus* and organisms apparently resembling this species were similar to those reported previously for this species (7, 20). However, one isolate was from a left temporal brain abscess; concomitant organisms were *Fusobacterium nucleatum*, *Peptostreptococcus magnus*, *Prevotella loescheii*, and coliforms. We believe this to be the first report of *A. europaeus* from a brain abscess. *A. graevenitzii* and organisms resembling this species were found exclusively in head, neck, and thoracic sites. These data support those of Pascual Ramos et al. (16) and suggest an oral niche for this organism. *P. propionicum* was associated with cases of lacrimal canaliculitis, as previously noted (3). Among unidentified actinomycetes, three ARDRA groups of 12, 19, and 8 isolates were prominent. Isolates in groups 1 and 3 occurred in sources similar to those of classic species, whereas group 2 isolates were found in sites similar to those of *A. turicensis*. Further investigations of these and other unidentified actinomycetes are in progress.

ARDRA is a valuable tool to elucidate the incidence in clinical material of currently recognized *Actinomyces* spp. and to screen for novel species. Currently, the method is suitable principally for specialist laboratories. However, rapid advances in molecular technologies, including DNA chips (microarrays), to which ARDRA may be readily adaptable, may bring the technique within the abilities of routine clinical laboratories.

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ADDENDUM IN PROOF

Partial 16S rDNA sequences of representatives (R5307 and R10236) of the 19 strains in ARDRA profile 025/026 (ARDRA group 2) show 99% similarity to that published for the recently described species *Actinomyces funkei* (GenBank accession no. AJ404889) (P. A. Lawson, N. Nikolaitchouk, E. Falsen, K. Westling, and M. D. Collins, Int. J. Syst. Evol. Microbiol. **51:**853–855, 2001).

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