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Clinical Spectrum of Infections Due to the Newly Described *Actinomyces* Species *A. turicensis*, *A. radingae*, and *A. europaeus*

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Over a 7-year period, we isolated 294 *Actinomyces*-like organisms (ALOs) which were not clearly identifiable. Using well-defined probes coding for sequences specific for recently described *Actinomyces* species (*A. turicensis*, *A. radingae*, and *A. europaeus*), we were able to identify 128 strains. The majority belonged to the *A. turicensis* species. *A. radingae* was found only in patients with skin-related pathologies. *A. europaeus* was also detected in patients with urinary tract infections. The main sources of *A. turicensis* were genital infections, followed by skin-related and urinary tract infections. Additional clinical pictures were appendicitis, cholecystitis, ear, nose, and throat infections, and bacteremia. In a small number of patients these ALOs were found as the only pathogen. Strains of the three species were tested by two widely used biochemical identification methods. *A. turicensis* was easily identifiable by both these methods. We conclude that these ALOs are not infrequent pathogens and are found in a wide range of human infections. At least *A. turicensis* is easily identifiable by clinical diagnostic laboratories.

Gram-positive rods have long been disregarded as contaminants or as unimportant bystanders in infectious diseases in humans. Only clearly pathogenic species like *Listeria* spp., *Actinomyces israelii*, and *Arcanobacterium haemolyticum* were among the few aerobically growing gram-positive rods considered worth identification (4, 5). Corynebacteria are mostly considered irrelevant. Nevertheless, during the last decade, clinically important pathogens like *Arcanobacterium haemolyticum* and *Actinomyces pyogenes* have been separated from this genus. The main reason why this group of aerobic gram-positive rods has long been neglected is that they are not easily identifiable to the species level (4, 6).

Apart from the well-defined and well-known pathogens mentioned above, there remained a large group of *Corynebacterium-Lactobacillus-Actinomyces*-like organisms which seemed potentially clinically relevant (2, 10). As a result of molecular biology techniques such as 16S rRNA gene sequence analysis, new species are now described almost monthly. We have been studying some of the new *Actinomyces* species, which were catalogued earlier into different genera by different investigators, illustrating the problems with identification: they have been described by Piot et al. (14) and Van Esbroeck et al. (16) as *Gardnerella*-like organisms, by Hollis and Weaver (11) as *Corynebacterium* CDC group E2, and by Brander and Jousimies-Somer (2) and Miller et al. (13) as aerotolerant *A. meyeri*.

Since 1986 we have been interested in these species and have collected them from clinical specimens sent to our laboratory. A large number of isolates that were defined as gram-positive or gram-variable rods, that were sensitive to tellurite, and that lacked catalase activity were classified as *Actinomyces*-like strains. Using reverse line blot analysis with well-defined oligonucleotide probes, we have identified in our collection some of the recently described species, *A. turicensis*, *A. radingae*, and *A. europaeus*, and a number of heretofore unidentified *Actinomyces*-like species. Because the clinical relevance of these species is still unclear, we describe here the nature of the samples from which they were cultured and discuss their relevance in the various infections.

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MATERIALS AND METHODS

Collection of strains. Clinical specimens were sent by general practitioners and medical specialists at two hospitals to the laboratory, mostly on cotton-tip swabs in Stuart's transport medium. All specimens were processed within 24 h. They were collected between June 1986 and September 1993 in the Regional Laboratory of Public Health "Zeeland" (Goes, The Netherlands). Clinical specimens were inoculated on 5% sheep blood agar (Columbia agar base) and on Vitox (Oxoid)-enriched chocolate agar and incubated in a 5% CO_2 atmosphere. Anaerobically incubated sheep blood agar that was enriched with vitamin K or that was made selective with norfloxacin (0.5 g/liter) was used for all samples except urine samples. *Actinomyces*-like organisms (ALOs) could mostly be isolated from all of the inoculated plates. In a number of cases they were found only or more abundantly in cultures incubated in a CO_2-enriched atmosphere and/or incubated anaerobically. Selection criteria for ALOs were, apart from being gram-positive or -variable rods, the absence of catalase activity (determined with 3% H_2O_2) and susceptibility to tellurite (determined with Clauberg agar including 3% [wt/vol] potassium tellurite [Merck] and 0.001% [wt/vol] L-cystine [Merck]). All strains were kept at −70°C in glycerol until testing. API Coryne tests (API, La Balme les Grottes, France) and RapID ANA II microtest systems (Innovative Diagnostic Systems, Norcross, Ga.) were used in accordance with the manufacturers' instructions. The results of the API Coryne sugar fermentation tests were read after 72 h of incubation. Determination of the species of other bacteria was done by conventional methods including tests with the API series and the Rapid ANA II system and by gas-liquid chromatography of volatile acids with peptone-yeast-glucose as the basal medium.

Clinical data were recorded from the request form and were completed by telephonic inquiries.

DNA sequence analysis, probe design, and hybridization by reverse line blotting. The 5′ part of the 16S rRNA gene was amplified with broad-host-range primers 16S1F and 16S1RR (Table 1) (1). The resulting 600-bp DNA fragment

TABLE 1. Sequences of primers and oligonucleotides used in the reverse line blotting assay

Oligonucleotide	Sequence	Specificity	Position in 16S rRNA (source)[a]	Reference or source
16SIF	AGAGTTTGATCMTGGYTCAG	Eubacteria	8–27 (*E. coli*)	1
16SIR	CTTTACGCCCARTRAWTCCG	Eubacteria	556–575 (*E. coli*)	1
ARC1	CAACAAAGTTGGAGCATCATCG	*A. turicensis*	59–38 (X78720)	This study
ARC2	AGAAACCACAAAGGCCCCT	*A. radingae*	211–193 (X78719)	This study
ARC3	CCGCAAGCAGGAGCCTT	*A. haemolyticum*	59–43 (X73952)	This study
ARC4	CCACCAAAAACACCAAAAGTGTAT	*Actinomyces* species	213–190 (strain 8813)	This study
ARC5	CAGGCTTATCCCAAAGACAAG	*A. bernardii* and *A. pyogenes*	127–108 (X79224)	This study
ARC6	CCCCATGCGAAGACCAG	*A. odontolyticus*	97–81 (X8721)	This study
ARC7	CATGCGACCAGCCTGGA	*A. europaeus*	190–174 (Y08828)	This study

[a] Designations beginning with X or Y are GenBank accession numbers.

was purified and sequenced directly by using fluorescent dye terminators in the cycling sequence (Perkin-Elmer Cetus). The sequences were compared with other 16S rRNA gene sequences by using the MegAlign module of the DNAStar program (DNAStar Inc., Madison, Wis.).

For identification of the various strains, a reverse line blotting assay was developed. Briefly, amino-linked group-specific oligonucleotides were covalently bound in parallel lines to a negatively charged membrane (Biodyne C; Pall Europe Limited, Portsmouth, United Kingdom) with a miniblotter (Immunetics, Cambridge, Mass.). After binding, the membrane was removed from the miniblotter, turned 90°, and again placed in the miniblotter. The slots which were perpendicular to the oligonucleotide lines were filled with heat-denatured, biotin-labeled PCR products obtained by amplification with a biotinylated variant of primer 16S1F and primer 16S1RR. A 60-min hybridization in 2× SSPE (360 mM NaCl, 20 mM $Na_2HPO_4 \cdot H_2O$, 2 mM EDTA, 0.5% sodium dodecyl sulfate [SDS]) at 50°C was performed in the miniblotter, and the membrane was subsequently incubated for 60 min at 42°C with horseradish peroxidase-labeled streptavidin (Boehringer Mannheim, Germany) in 2× SSPE (1:4,000).

Hybridization was visualized by incubation with enhanced chemiluminescence detection liquid (Amersham, 's-Hertogenbosch, The Netherlands) and a 10-min exposure of a Hyperfilm (Amersham) to the membrane.

RESULTS

Determination of the 16S rRNA gene sequence and hybridization assay. A total of 294 ALOs fulfilling the criteria mentioned above were isolated during the collection period of 88 months. Only 180 of these were available for identification by reverse line blotting, since many isolates did not survive the freezing procedure (Table 2). A total of 128 isolates could be identified as one of the newly described *Actinomyces* species: 116 were *A. turicensis*, 4 were *A. radingae*, and 8 reacted with the oligonucleotide for *A. europaeus*. The morphological and biochemical features of these strains are described elsewhere (7, 15). The remainder could be divided into five groups: 7 *A. haemolyticum* isolates, 4 strains reacting with oligonucleotide ARC4 (Table 1), 3 strains reacting with oligonucleotide ARC5, 19 nonreacting strains, and 19 strains that could not be amplified with the primers that we used.

There was a slight preponderance of female patients: for *A. turicensis*, 76 of 116 infections occurred in female patients; for *A. radingae*, 4 of 4 infections occurred in female patients; and for *A. europaeus*, 4 of 8 infections occurred in female patients.

The different strains were found in patients with the pathologies and infections described below (Table 2 and 3).

(i) Genital pathology. The genital organs were apparently the sites most frequently infected with ALOs in general and *A. turicensis* in particular. *A. radingae* and *A. europaeus* were not detected at these locations in either males or females.

The pathology is especially obvious in males. *A. turicensis* was found in eight patients with mild to severe balanitis, in-

TABLE 2. Occurrence of newly described *Actinomyces* species in different clinical infections

Infection	No. of isolates					
	Total ALOs	Tested by RLB[a]	*A. turicensis*	*A. radingae*	*A. europaeus*	Other
Genital infections						
Males	33	19	16	0	0	3
Females	104	65	49	0	0	16
Urinary tract infections	25	14	6	0	2	6
Skin-related infections						
Pilonidal sinus infection	22	13	7	1	1	4
Perianal abscess	25	16	11	1	1	3
Omphalitis	10	4	3	1	0	0
Crural and decubital ulcer	21	14	5	0	2	7
Postoperative wound infection	11	7	3	0	0	4
Primary abscess	30	18	9	1	2	6
Appendicitis or cholecystitis	6	6	3	0	0	3
Ear, nose, and throat infections	4	2	2	0	0	0
Bacteremia	3	2	2	0	0	0
Total	294	180	116	4	8	52

[a] Number of isolates tested by reverse line blot (RLB) assay.

TABLE 3. Concomitant flora in *A. turicensis* infections

Infection	No. of patients in whom *A. turicensis* was found together with the following:				
	None	Aerobes	Anaerobes	Aerobes + anaerobes	Total
Genital infections					
Males	4	10	2	0	16
Females	14	19	12	4	49
Urinary tract infections	3	3	0[a]	0[a]	6
Skin-related infections:					
Pilonidal sinus	1	2	3	1	7
Perianal abscess	1	0	5	5	11
Omphalitis	2	0	1	0	3
Crural and decubital ulcer	1	0	0	4	5
Postoperative wound infection	0	0	2	1	3
Primary abscess	0	2	6	1	9
Appendicitis or cholecystitis	0	1	1	1	3
Ear, nose, and throat infection	0	0	2	0	2
Bacteremia	2	0	0	0	2
Total	28	37	34	17	116

[a] Anaerobic cultures were not routinely performed with urinary samples.

cluding 1 patient with a penile ulcus. *Chlamydia trachomatis* was also detected in this patient. In four other patients nonhemolytic streptococci were additionally found. In three instances *A. turicensis* was the only potential pathogen present. All patients except for one 2-year-old boy were of a sexually active age. Three isolates from patients with balanitis could not be identified.

Urethritis was the second most frequently occurring pathology in males. All six patients with urethritis that we investigated carried *A. turicensis*. One patient had an urethral ulcus. In the sample from this patient with an ulcus, a mixture of various bacteria was found. In two other patients *A. turicensis* was found together with *Neisseria gonorrhoeae* and *C. trachomatis*, respectively. *A. turicensis* was the only pathogen cultured from samples from the three remaining urethritis patients.

Other pathologies found in males consisted of a penile abscess and prostatitis, both of which occurred in older men (ages, 61 and 85 years, respectively). In both patients *A. turicensis* was found at a high density. Only from samples from the patient with an abscess were additional bacteria (anaerobes) cultured.

In the female genital tract, *A. turicensis* seems to cause infections at all sites: we found the organism in patients with adnexitis (two *A. turicensis* isolates among 4 ALOs), endometritis (5 among 8 ALOs), cervicitis (2 among 11 ALOs), vaginitis (7 among 17 ALOs), and vulvitis (3 among 3 ALOs). It was also found in abscesses: vulvar or perineal abscess (three *A. turicensis* isolates among three ALOs) and Bartholin's abscess (two among five ALOs). It was also present in patients with minor pathologies: leukorrhea (22 among 47 ALOs) or control cultures (e.g., after gonorrhea therapy in patients with intrauterine devices [IUDs] or in pregnant women with ruptured membranes for more than 24 h) (3 among 6 ALOs). Most women enrolled in the study were adults. The three vulvitis patients were prepubertal, as were seven of the patients with vaginitis or leukorrhea caused by *A. turicensis*. In a few instances *A. turicensis* was found in pure culture: once in a culture of a sample from the cervix of a 23-year-old woman with adnexitis, once in a culture of a sample from a 3-year-old girl with vulvitis, and in discharges from 12 females, 3 of whom were prepubertal.

(ii) Urinary tract infections. ALOs were isolated from the urine of 13 male patients and 12 female patients. In three females the same strain was found twice within a few weeks. Most patients had cystitis. Three females had urethritis. Three patients had no complaints. Two patients had malignancies (one patient with carcinoma of the bladder and one patient with carcinoma of the prostate).

Six patients were proven to harbor *A. turicensis*, and for three of the patients the organism was found as a pure culture (anaerobes were not looked for). No major extra pathogen was found: one patient with cystitis was infected with a mixed flora, one patient also had a viridans group streptococcus infection, and another patient was infected with *Aerococcus urinae*, as determined by culture of urine samples from the patients. The three patients from whom *A. turicensis* was the sole organism that was isolated were all male (two patients with cystitis and one patient with no complaints who was checked before urogenital surgery).

The two patients with *A. europaeus* infection had cystitis without concomitant flora and purulent urethritis during catheterization, respectively. *Streptococcus pyogenes* was also found in the latter patient.

All patients carrying one of the three investigated *Actinomyces* species as the only pathogen had at least some leukocytes in their urine (for *A. turicensis*-infected patients, a mean of 3 to 5 polymorphs per high-power field) but had fewer leukocytes than the numbers of leukocytes found in the urine of patients who were also carrying other flora (for *A. turicensis*-infected patients, 10 to 15 polymorphs per high-power field). All these patients except the only female patient with cystitis and infected with *A. turicensis* (age, 43 years) were ages 60 years or older.

(iii) Skin-related infections. All three *Actinomyces* species are present in patients with infections related to the skin (Table 2). All *A. radingae* strains that were isolated and most of the

A. europaeus strains that were isolated were found in patients with this type of infection, as were 38 of the 116 (33%) *A. turicensis* isolates.

A number of well-defined clinical pictures were prominent: infections of pilonidal sinuses are a striking example. For example, in 1991 we obtained 21 samples of infected pilonidal sinuses, 8 of which contained ALOs. Three of the four strains tested by reverse line blotting were *A. turicensis*. All patients in whom one of the *Actinomyces* species was found were immunocompetent and young (mean age, 24 ± 7 years).

The other microorganisms found along with the *Actinomyces* species in patients with this type of infection are indicated in Table 3. In one patient there was an indication that *A. turicensis* could cause an infection on its own. In three patients other aerobic organisms were found; in all three patients this was "*Streptococcus milleri*." The only *A. radingae* isolate causing this type of infection was accompanied only by anaerobes; the *A. europaeus* isolates were accompanied by a mixture of aerobes and anaerobes.

The type of skin infection in which these new species were most frequently found was a perianal abscess. Again, all three species were represented and in almost all patients were accompanied by anaerobes. Other aerobes were found in only 5 of the 11 *A. turicensis*-infected patients. In 1991 we received 30 samples from patients with perianal abscesses, and 4 contained ALOs. The only strain that was examined turned out to be *A. turicensis*.

The umbilicus seems to be another predilection spot for *Actinomyces* species. During the study period 10 ALOs were isolated from this region in patients equally divided between neonates and adults. All *A. turicensis* strains came from neonates; the *A. radingae* isolate was from a 72-year-old female. The *A. radingae* isolate and two of the three *A. turicensis* isolates were the only pathogen present.

Other infections of the skin can be secondary to ulcer formation (vascular, diabetic, or decubital ulcers) or secondary to surgery. Nevertheless, primary abscesses, including abscesses resulting from hidradenitis, also contained ALOs, although rarely in pure culture. Again, in all these skin infections, the majority of the ALOs turned out to be *A. turicensis*, but half of the *A. europaeus* strains were found here, as were a large group of ALOs yet to be described (Table 2).

In the nine primary abscesses containing *A. turicensis*, the latter species was the single aerobe in six of the patients, whereas in two other patients it was accompanied by "*S. milleri*" and in one patient it was accompanied by *S. aureus*. The *A. radingae* isolate was found together with *Staphylococcus aureus*, whereas *A. europaeus* was the only pathogen in one patient and was accompanied only by *Peptostreptococcus asaccharolyticus* in the other patient.

(iv) Appendicitis and cholecystitis. Intra-abdominal fluid samples were taken perioperatively from five patients with severe appendicitis, and bile was taken from a patient with cholecystitis. All strains were tested by the reverse line blotting assay. Only three were *A. turicensis* (all were from patients with appendicitis). We were unable to identify the other three ALOs.

A. turicensis was found in adult patients only (ages, 18, 31, and 54 years), and the infections were accompanied by infections with "*S. milleri*" and anaerobes, *Escherichia coli*, and anaerobes, respectively. Thus, in two patients the only aerobe found was *A. turicensis*. In both patients it was present in large quantities.

(v) Ear, nose, and throat infections. *A. turicensis* was found as the only aerobe in two patients with ear, nose, and throat infections: one 32-year-old man with a chronic middle ear infection and an 11-year-old girl with mastoiditis. In both patients it was accompanied by mixed anaerobic flora. ALOs isolated from the middle ear of an 89-year-old woman and the sinus of a 17-year-old boy with sinusitis were lost before examination.

TABLE 4. Concomitant flora per isolated strain

Concomitant flora	No. of isolates					
	Total isolated	Tested by RLB[a]	*A. turicensis*	*A. radingae*	*A. europaeus*	Other
None	84	41	28	1	2	10
Aerobes	89	58	37	0	1	20
Anaerobes	76	54	34	2	4	14
Aerobes and anaerobes	45	27	17	1	1	8
Total	294	180	116	4	8	52

[a] Number of isolates tested by reverse line blot (RLB) assay.

(vi) Bacteremias. ALOs were found in three different patients with bacteremia. In two of them they could be hybridized with the *A. turicensis* probe. Their histories were as follows. Patient 1 received an aorto-bi-ileacal bypass at the age of 57. Four months after this procedure she had recurrent febrile episodes, with *E. coli* and *Enterococcus faecium* isolated in cultures of her blood on several occasions. Therefore, she underwent cholecystectomy, but it had no influence on the fever. She later complained of pain on the scar in the left groin. Blood cultures first grew a mixture of anaerobes; 1 month later *A. turicensis* grew in pure culture, followed 3 days later by growth of a mixture of this *A. turicensis* isolate with *Bacteroides thetaiotaomicron*. An abscess became prominent in the left groin and was drained. The culture of the abscess contents grew *E. coli*, *B. thetaiotaomicron*, *Clostridium clostridiforme*, and "*S. milleri*." The *A. turicensis* organism could not be isolated from this material.

Patient 2 was a 50-year-old woman who had been suffering from multiple sclerosis for 15 years. Seven years earlier she had a vastus lateralis transplantation in order to cover a fistula over the right trochanter major. She had had fever and abdominal pain for 2 months and a rapidly exacerbating decubital ulcer over the sacrum. On the day of admission a culture of her blood was positive for *A. turicensis*. She was treated conservatively for obstipation and the ulcer, and she recovered rapidly.

In patient 1 the *Actinomyces* isolate was probably present in the abscess, together with other pathogens, and could be found as the only isolate in two of the blood cultures, probably by coincidence, since only a few days later it was accompanied by an anaerobe, which was also found in the abscess. In the second patient the probability is also high that the *A. turicensis* isolate was only one of the many germs colonizing the decubitus ulcer. In neither patient did real sepsis develop.

Concomitant flora. Table 4 summarizes the additional flora found in the different cultures. Only in cultures of samples from a minority of patients were no extra bacteria were found. In the other cultures aerobic and anaerobic bacteria were equally represented. The aerobic flora consisted mainly of gram-positive cocci, with a striking preponderance of "*S. milleri*." Among the 54 cultures in which aerobes accompanied *A. turicensis*, 12 contained "*S. milleri*" isolates, 8 contained other streptococci, 3 contained *S. aureus*, 3 contained enterococci, 1 contained *Staphylococcus epidermidis*, and 1 contained *Aerococcus urinae*. In the remaining group of cultures we found only six gram-negative rods.

TABLE 5. API Coryne and Rapid ANA II results for the *A. turicensis* strains tested

Test and code	No. of isolates	Distance[a]
API Coryne		
0010721	28	0
0010701	13	1
0010723	1	1
1010701	1	2
Rapid ANA II		
020671	19	0
022671	7	1
020071	1	2
020051	1	3

[a] Number of biochemical tests with codes different from the most frequently obtained code.

Biochemical characteristics. A number of strains of the three species were tested with two widely used biochemical identification test strips: the API Coryne and the Rapid ANA II systems. Only the *A. turicensis* isolates were available in numbers sufficient for us to be able to register their biochemical homogeneity. Thirty-eight strains were tested by the commercially available API Coryne test, and 28 of them were also tested with the Rapid ANA II system. The number codes obtained by the API Coryne test were very stable and did not correspond to any species available in the database (Table 5). The Rapid ANA II system misidentified all strains, mostly as *Actinomyces meyeri* or *Actinomyces odontolyticus*. The results for the other strains showed a much greater variability, but the strains were clearly classified as being distinct from *A. turicensis*. The major differences are described by Wüst et al. (18), Funke et al. (7), and Vandamme et al. (15).

DISCUSSION

In microbiological cultures, one detects only what one knows and expects. Therefore, it is important to know in what types of infection certain species can be expected and how they can be determined in an acceptably easy way. This article gives the first answer to both of these questions regarding three recently described *Actinomyces* species: *A. turicensis*, *A. radingae*, and *A. europaeus*.

A prudent answer is given to the question of whether the new strains are pathogenic. Each of these new *Actinomyces* species could be isolated from a limited number of patients without other bacteria being present, but a number of other bacteria, especially anaerobes, could easily have been missed due to, for example, prolonged transportation times. Moreover, the urine samples were not cultured anaerobically. Nevertheless, in contrast to the findings of Wüst et al. (17) and Funke et al. (8), a number of clearly monobacterial infections did occur: *A. turicensis* was the single microorganism seen in and cultured from samples from three young men with urethritis and discharge, 2 older men with manifest cystitis, a young woman with an infected pilonidal sinus, and another young woman with adnexitis (a cervical specimen). Furthermore, it was the only pathogen found in three neonates with inflamed umbilici. A culture of a sample from the navel of a neonate was the only place where *A. radingae* was found as a pure culture. *A. europaeus* was also found as the sole possible cause of infection in a 33-year-old female with hydradenitis and an 88-year-old man with cystitis. All these patients were clearly not immunocompromised. Nevertheless, the pathogenicity and virulence of these *Actinomyces* species still must be proven in experiments with animals.

Actinomyces-like organisms are regularly found in Papanicolaou smears from IUD users and other women (12). It seems very probable that a large number of these "*Actinomyces*" are nothing other than the organisms described here, although other gram-positive rods such as *Eubacterium* also seem to be involved (9). Their presence in healthy women and the experience that they are rather innocuous in IUD users would suggest, then, that they are harmless commensal organisms. This does not, however, exclude the possibility that they provoke infections: other bacteria such as *Streptococcus agalactiae* and "*S. milleri*" can also be found as commensal organisms in the female genital tract but are clearly known to be potential pathogens.

It is striking that whereas *A. turicensis* was found in all pathologies described in this report, the presence of *A. europaeus* was limited to urinary tract and skin-related infections, while *A. radingae* was isolated only from skin-related infections. This can be due to the limited number of *A. europaeus* and *A. radingae* isolates detected in this study. Another possible explanation could be that the growth of these two species is slightly more dependent on the presence of lipids than *A. turicensis* is. Indeed, it has been shown that the growth of all these ALO species in liquid culture is clearly promoted by supplementing the medium with lipids, e.g., in the form of rabbit or horse serum (6). This could explain their predilection for skin-related infections such as those of the tallow-filled pilonidal sinuses. Another reason for the broader clinical spectrum of *A. turicensis* could be that the sites of infection are related to the habitat where these species are usually present as commensal organisms. If this is true, then the large number of isolates from the genital tract and the perianal abscesses and the isolates from the patients with appendicitis would suggest that *A. turicensis* is normally present in the vagina and the gut. All three species would then normally be present on the skin, and because most of the skin-related infections were found below the waist, they may be commensal organisms only on the lower half of the body. However, their normal habitat is hard to define, since it is difficult to make media selective for these very susceptible bacteria.

Another relevant finding is the skewed distribution between these three species. The large majority of our isolates belonged to *A. turicensis* (91%), while *A. radingae* and *A. europaeus* accounted for only 3 and 6% of the isolates, respectively. This might also be due to differences in commensalism or to differences in the pathogenicities of the different species, but it might also be due to other factors such as our method of isolation and selection, the method and duration of culture, or the type of clinical departments we were serving. Other laboratories indeed found other ratios: Funke (6a) found, e.g., relatively more *A. radingae* isolates than we did (*A. turicensis*, 75%; *A. radingae*, 20%; and *A. europaeus*, 5%). The reason for this is unclear.

It might be worth mentioning that the species of 46 (26%) of the ALOs that we collected have still not been determined. Because this group contained clinically relevant isolates, further study of these species is required.

A last striking feature is that the most prevalent coinfectors with *A. turicensis* were isolates of the "*S. milleri*" group (12 isolates in 116 patients). This was already observed by Wüst et al. (17) and is confirmed here. This can be a coincidence or can be due to the fact that both species share the same normal habitat. It is known that "*S. milleri*" can be present on all mucous membranes. The two species, however, also share other properties: they are both slowly growing, microaerophilic

bacteria that are mostly found together with anaerobes. Another possibility is that they potentiate each other in their pyogenicities. In this series we did not find "*S. milleri*" together with *A. radingae* or *A. europaeus*.

The biochemical identification of the three *Actinomyces* species described here might be difficult. We have tested a number of strains by two widely used commercial tests. Because too few strains of *A. radingae* and *A. europaeus* have been available, it has not been possible to draw any conclusions about these organisms. *A. turicensis*, on the other hand, can clearly be discriminated with the API Coryne test strip: the code obtained is stable and unique. The Rapid ANA II strip generates a code similar to those for two well-known anaerobic *Actinomyces* species: *A. meyeri* and *A. odontolyticus*. Only the aerotolerance of a strain could then suggest that it is *A. turicensis* instead. An aerotolerant, catalase-negative, gram-positive rod should thus preferably be identified with the API Coryne strip.

We conclude that these newer *Actinomyces* species are not uncommon and that the clinical entities in which they are found suggest that they are clinically relevant, since they are also found as the only pathogen. It is suggested that each well-equipped microbiological laboratory should at least be able to discriminate *A. turicensis* from other gram-positive rods.

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