

Actinomyces species: A Danish Survey on Human Infections and Microbiological Characteristics

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Abstract: This study compared phenotypic and genotypic identification of *Actinomyces* strains, tested susceptibility to antibiotics and evaluated their clinical importance. Thirty-four *Actinomyces* strains were examined; sixteen type strains, and 18 clinical strains from different hospitals in Denmark from the period 2003-2005. Partial 16S rDNA sequencing using a stretch of 526 bases was used for genotypic identification. Susceptibility testing was done by E-test. The antibiotics examined were: benzylpenicillin, piperacillin with tazobactam, ceftriaxone, meropenem, erythromycin, clindamycin, linezolid, moxifloxacin, tetracycline and tigecycline. Clinical parameters were obtained by reviewing patient records. There was poor agreement between the phenotypic and genotypic identification. Phenotypic tests were helpful in identifying strains closely related by DNA sequences. The strains were sensitive to the examined antibiotics except for moxifloxacin to which most strains were resistant, and a few strains were resistant to meropenem and tetracycline. The clinical strains were from many different types of infections and locations. None of the patients was described as having typical actinomycetic lesions, and an apparently good outcome was obtained with different treatment regimens.

Keywords: *Actinomyces* species, Bacterial identification, phenotypic characterization, partial 16S rDNA sequencing.

INTRODUCTION

The genus *Actinomyces* consists of a heterogeneous group of anaerobic and facultative anaerobic, asporogenous, Gram-positive, non-acid-fast, rod-shaped organisms [1], many of which occur as inhabitants of mucosal surfaces, particularly the oral cavity, of humans and animals. *Actinomyces* species can cause actinomycosis, a chronic granulomatous lesion, forming suppurative abscesses and draining sinuses. Localization is most often cervicofacial (60%), thoracic (15%) or abdominal (20%) [1]. Both laboratory identification and clinical diagnosis is often troublesome. Coryneform bacteria are difficult to classify at the species or even at the genus level [2] and poses major problems for clinical microbiology laboratories in terms of labour, time, and cost, when conventional methods are used. Panels of phenotypic characteristics useful in identifying species have been described by Funke *et al.* [2] and Sarkonen *et al.* [3].

An increased recognition of the importance of coryneform bacteria as opportunistic human pathogens within the last decade [4], has occurred in a period where new diagnostic tools for identification of bacteria progressively have been introduced. These techniques, especially sequencing of genes coding for rRNA have revolutionized the insight into phylogeny and taxonomy of bacteria.

We therefore found it relevant to characterize 34 strains of *Actinomyces* species from humans, 18 recent Danish

clinical strains and 16 type strains of different species, using a recent phenotypical identification scheme, and compare the results to genotypic identification using partial 16S rDNA sequencing. In addition, susceptibility to antibiotics was determined and data on clinical manifestations of the respective patients was obtained.

MATERIALS AND METHODS

Bacterial Strains

We examined 34 *Actinomyces* strains. Sixteen type strains, received from CCUG (Culture Collection, University of Göteborg, Göteborg, Sweden), were included: *A. europaeus* CCUG 32789A^T, *A. funkei* CCUG 42773^T, *A. georgiae* CCUG 32935^T, *A. gerencseriae* CCUG 32936^T, *A. graevenitzii* CCUG 27294^T, *A. israelii* CCUG 18307^T, *A. meyeri* CCUG 21024^T, *A. naeslundii* CCUG 18310^T, *A. neuii* subsp. *neuii* CCUG 32252^T, *A. neuii* subsp. *anitratus* CCUG 32253^T, *A. odontolyticus* CCUG 20536^T, *A. radidentis* CCUG 36733^T, *A. radingae* CCUG 32394^T, *A. turicensis* CCUG 34269^T, *A. urogenitalis* CCUG 38702^T, *A. viscosus* CCUG 14476^T. Eighteen strains were clinical strains submitted from Danish Departments of Clinical Microbiology to the National Reference Laboratory for identification of bacteria, at Statens Serum Institut (SSI), in the period February 2003 - March 2005. All strains were kept as frozen (-80 °C) stocks.

Phenotypic Identification

The strains were subcultured on 10 % horse blood agar (SSI Diagnostica, Hillerød, Denmark), and the agar plates

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were incubated for five days at 35 °C in ambient air, a CO₂-enriched (5 %) and an anaerobic atmosphere, respectively. Colonial morphology and microscopic appearance in Gram-stained preparations were noted. CAMP reaction was examined on CAMP agar (SSI Diagnostica) with a beta-hemolysin-producing strain of *Staphylococcus aureus*. CAMP-plates were incubated at 35 °C in a CO₂-enriched (5 %) and an anaerobic atmosphere, respectively, and were read after 24-h and 48-h incubation. Testing for nitrate reduction was performed using a nitrate broth culture (semi-solid agar) incubated at 35 °C in an anaerobic atmosphere for a minimum of five days, prior to addition of sulfanilic acid, Cleves acid, and if necessary zinc powder [5, 6]. Rosco diagnostic tablets (Rosco A/S, Taastrup, Denmark) were used for testing hydrolysis of urea and esculin, production of α -fucosidase, α -glucosidase, β -N-acetylglucosaminidase (β -NAG) and β -galactosidase (ONPG), and acid production from L-arabinose, following the instructions of the manufacturer. *A. israelii* and *A. gerencseriae* were differentiated from each other by the presence of α -mannosidase and arginine dihydrolase (ADH). Acid production from L-arabinose, maltose, mannitol, raffinose, rhamnose, sucrose, xylose and trehalose was tested in tubes with extract-bouillon and the carbohydrate (SSI Diagnostica) [6], incubated at 35 °C in an anaerobic atmosphere for a minimum of five days. Horse citrate plasma was added to promote growth. All tests, except testing for the presence of α -mannosidase, were performed by more than one method, as the strains were also tested with the API® strips, Rapid ID 32 A and API 20 A® (bioMérieux® as, Marcy l'Etoile, France) following the instructions of the manufacturer. Rapid ID 32 A tested: ability to reduce nitrate, the presence of urease, α -fucosidase, α -glucosidase, β -NAG, ONPG and ADH, and acid production from raffinose. API 20 A® tested: presence of urease, hydrolysis of esculin, acid production from arabinose, maltose, mannitol, raffinose, rhamnose, sucrose, xylose and trehalose. Catalase was detected with 10 % H₂O₂ in a well in the API 20 A® strip with a positive reaction.

Ribosomal DNA Sequencing

For molecular biological study DNA was released by heating isolated bacteria at 95°C for 5 min. PCR amplification of part of the 16S rRNA gene was performed using the primers BSF-8 and BSR-534 as previously described [7]. DNA sequences were aligned and edited at <http://bioinfo.genotoul.fr/multalin/multalin.html> resulting in comparable sequences ranging from 324 to 351 basepairs. Furthermore, all edited sequences were compared to deposited sequences in the Bio Informatic Bacteria Identification database. Sequences obtained for clinical strains were compared to original CCUG sequences of collection strains. Results were illustrated in a phylogenetic tree using CLC workbench 4.5 software.

Susceptibility Testing

The MICs of all the strains were determined using E-test (AB Biodisk, Solna, Sweden) for the following antibiotics: benzylpenicillin, piperacillin with tazobactam, ceftriaxone, meropenem, erythromycin, clindamycin, linezolid, moxifloxacin, tetracycline, and tigecycline. All tests were per-

formed on anaerobic plates (SSI Diagnostica). Plates were inoculated with the bacterial isolates in API suspension medium (demineralised water) (bioMérieux® as, Marcy l'Etoile, France) with a turbidity equivalent of McFarland standard 4,0 [8] and incubated anaerobically at 35 °C for 48-72 hours. Results were read according to the manufacturer's guidelines. Quality controls were performed using *Bacteroides fragilis* (ATCC 25285). MIC measurements (at least triplicate) for the control strain were as follows: Penicillin: > 32 µg/ml, piperacillin/tazobactam: 0.25-0.5 µg/ml, ceftriaxone: 32 µg/ml, meropenem: 0.064 µg/ml, erythromycin: 1.5 µg/ml, clindamycin: 0.75-2.0 µg/ml, linezolid: 1.5 µg/ml.

Clinical Data

Information on predisposing factors, actual diagnosis and antibiotic treatment was recorded from patient files.

RESULTS

Phenotypic Identification

The appearance of the strains varied both in the Gram-stained preparations and with growth on agar. Microscopically many strains were without visible branching, many were small rods with an appearance similar to the coryneforms, other were coccoid or long and curved rods. On agar most strains were grey or white, colony-sizes ranged from pinpoint to three mm in diameter, and some had α -haemolysis. Colonies could be rough or smooth, elevated or flat, and some adhered to agar. The incubation atmosphere influenced appearance and growth; some strains did not grow in ambient air.

Table 1 shows results of phenotypic identification of *Actinomyces* strains following the recommendations of Sarkonen *et al.* [3]. All type strains were correctly identified. We used the same type strains as Sarkonen *et al.* except for the type strain of *A. israelii*. The clinical strains were grouped from the phenotypic identification; one strain could not be designated to a species by the performed phenotypic characterization.

Results of individual biochemical reactions sometimes differed when tested with different methods. This was for example seen when testing nitrate reduction, esculin hydrolysis and acid production from carbohydrates.

Genotypic Identification

All type strains were correctly identified. For the 16 type strains best taxon match had scores from 642 to 672 bits and differences to next best taxon matches between 22 and 260 bits. Some species were more closely related than others; *A. meyerii* and *A. odontolyticus* were most closely related, followed by *A. viscosus* and *A. naeslundii*. *A. radingae* was least connected to the other species. For 18 clinical strains scores of 480 to 670 bits were found with differences to next best taxon matches between 2 and 245 bits.

One of the clinical strains could not be allocated to a species on phenotypic characteristics, wherefore 17 clinical strains could be compared (Fig. 1).

Table 1. Results from Phenotypic Identification of Strains of Actinomyces^a

Species and Strain(s)	Pigment	Catalase	Nitrate Reduction	CAMP Test	Hydrolysis of:		Production of:				Fermentation of:							
					Urea	Esculin	α-Fuco- sidase	α-Gluco- sidase	β- NAG	β-Galac- tosidase	Arab- inose	Mal- tose	Man- nitol	Raffi- nose	Rham- nose	Suc- rose	Xy- lose	Tre- halose
<i>A. europaeus</i> CCUG 32789 A ^T	-	-	+	-	-	+	-	+	-	+	-	+	-	-	-	v	-	-
<i>A. funkei</i> CCUG 42773 ^T	-	-	+	-	-	-	+	+	-	v	v	v	-	-	+	+	+	-
<i>A. georgiae</i> CCUG 32935 ^T	-	-	v	-	-	v	-	+	-	+	-	+	-	v	+	+	+	+
<i>A. gerencseriae</i> CCUG 32936 ^T , 1 clinical strain	-	-	v, -	-	-	+	-, +	+	-	+	-, v	+	v, +	-, +	-	+	+	+
<i>A. graevenitzii</i> CCUG 27294 ^T	-	-	-	-	-	-	-	+	+	+	-	+	-	v	-	+	-	-
<i>A. israelii</i> CCUG 18307 ^T , 2 clinical strains	-	-	+, v	-	-	+	-	+	-	+	v	+	v	+	v, -	+	+	+
<i>A. meyeri</i> CCUG 21024 ^T	-	-	-	-	-	-	-	+	-	+	-	+	-	v	-	+	+	v
<i>A. naeslundii</i> CCUG 18310 ^T	-	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+
<i>A. neuii</i> subsp. <i>Neuui</i> CCUG 32252 ^T , 2 clinical strains	-	+	+, v	+	-	-	-	+	-	+	-	+	+	v	-	+	+	+, v
<i>A. neuii</i> subsp. <i>anitratus</i> CCUG 32253 ^T	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	+	+
<i>A. odontolyticus</i> CCUG 20536 ^T , 2 clinical strains	-, +	-	+	-	-	-, v	+, v	+	-	+	-	+	-	v	+, v	+	+	-
<i>A. radidentis</i> CCUG 36733 ^T	-	+	v	-	-	v	-	+	-	+	-	+	+	+	-	+	-	+
<i>A. radingae</i> CCUG 32394 ^T , 2 clinical strains	-	-	-, v	-	-	v, +	+	+	v, -	+	+, v	+	v	+, v	-	+	+, v	+, v
<i>A. turicensis</i> CCUG 34269 ^T , 5 clinical strains	-	-	-	-	-	-	+, v	+	-	-, v	-, v	v, +	-	-	-	+	+	-
<i>A. urogenitalis</i> CCUG 38702 ^T	-	-	+	-	-	+	-	+	+	+	v	+	-	+	+	+	+	+
<i>A. viscosus</i> CCUG 14476 ^T , 3 clinical strains	-	+	-, v	-	-	v	-	+	-	+	-, v	+	-	+	-	+	-, v	v
<i>A. species</i>	-	-	+	-	-	-	-	+	-	+	v	+	-	v	-	+	+	-

^a -, negative result; +, positive result; v, variable reaction (by different methods or among strains). Results for type strains are given first if different from the results obtained on clinical strains.

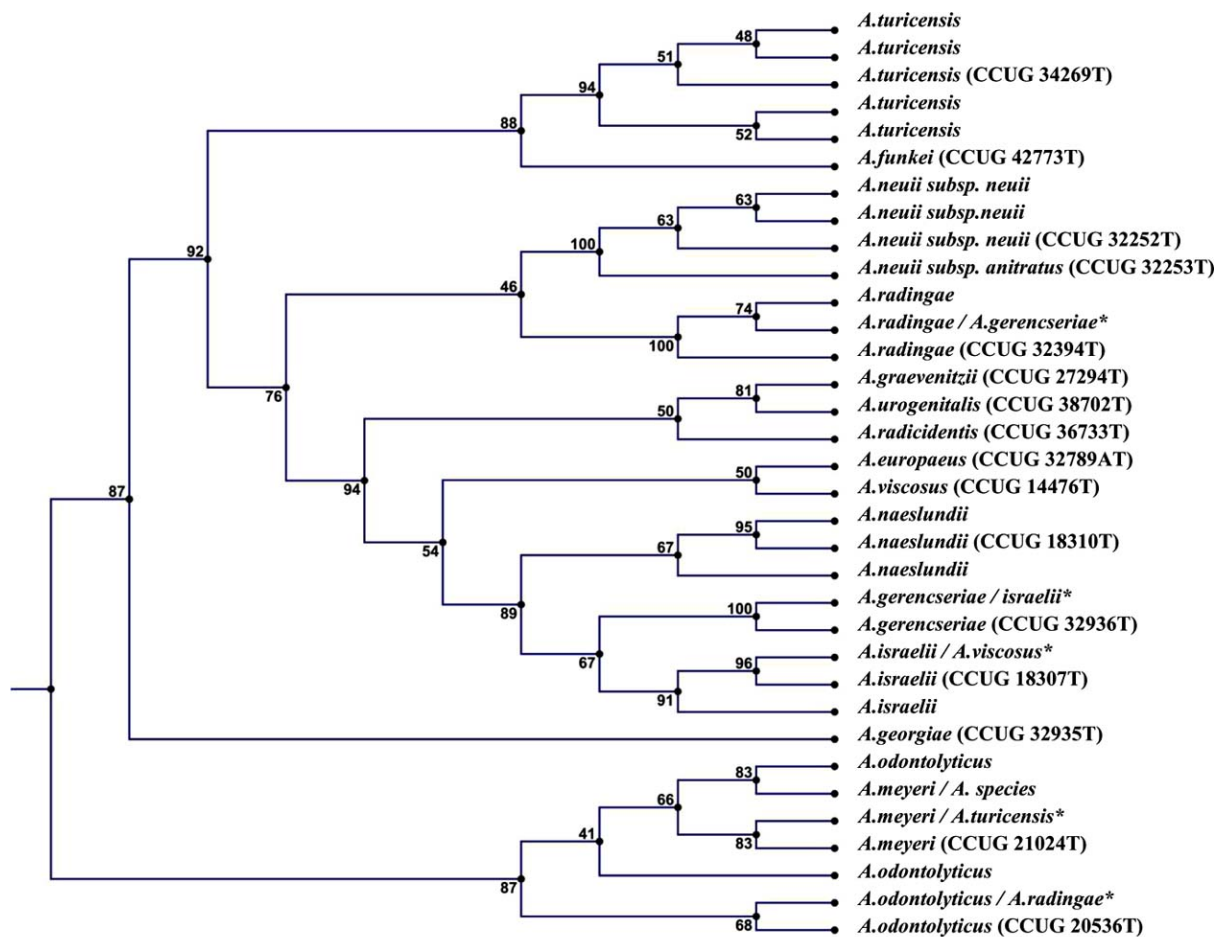


Fig. (1). Phylogenetic tree illustrating the relationship between the 16 type strains and the 18 clinical strains. Six clinical strains did not reach concordance between genotypic / phenotypic* identification (five with different species names and one with no species identification). The remaining 12 strains were allocated to the same species when comparing the two methods of identification.

Genotypic/Phenotypic Identification

Ten of the 17 clinical strains showed methodological agreement at the species level. Two additional strains showed concordance as BLAST examinations could not separate (bits score difference < 30) between *A. viscosus* and *A. naeslundii*; the latter was proposed from phenotypic characterization (and second BLAST choice) and the former from first BLAST choice. Thus, for 12 of 17 strains results from phenotypic and molecular examinations were in agreement. BLAST examinations for all strains seemed very convincing when taking all parameters into consideration (Fig. 1). Thus, for five of 17 clinical strains identifications by genotypic/phenotypic methods were in disagreement as indicated in Fig. (1) with an *. One isolate could not be designated to a species based on phenotypic characterization, which by genotypic identification belonged to the species *A. meyerii*. The isolates with disagreeing identifications were (genotypic/phenotypic identification): 1) *A. odontolyticus*/*A. radingae* (n=1), 2) *A. radingae*/*A. gerencseriae* (n=1), 3) *A. gerencseriae*/*A. israelii* (n=1), 4) *A. israelii*/*A. viscosus* and 5) *A. meyerii*/*A. turicensis*.

Susceptibility Testing

The MICs for the 34 strains are shown in Table 2. All strains were sensitive to benzylpenicillin, piperacillin/

tazobactam, ceftriaxone, meropenem, erythromycin, clindamycin, linezolid, tetracycline and tigecycline, except for meropenem and tetracycline where one strain for each antibiotic had MIC values above the upper limits for sensitivity. Notably for moxifloxacin, 21 strains had MIC's above the upper limit for being sensitive though most strains (n=16) were close to the breaking point. We experienced difficulties reading zone diameters due to hazy zone edges, which explains why it was not possible to define an MIC value for 1-5 strains for each antibiotic.

Clinical Data

Table 3 shows clinical and microbiological data on the 18 patients from whom *Actinomyces* species were isolated. The patients have been divided into groups based on species designations from the genotypic identification. The clinical strains were chosen at random during the period February 2003 - March 2005. The median age was 54 years, 10 were men and 8 women.

The strains were from soft tissue infections (5 strains), blood (4), intraabdominal abscesses (3), periodontal infections (2), and one strain each from pus from bladder, empyema of the pleural space, spinal fluid and a swab from cornea.

Table 2. Minimal Inhibitory Concentrations of 10 Antimicrobial Agents Against *Actinomyces species*. (n=34), Including Type Strains of 16 Different Species

Antimicrobial Agent	MIC (mg/L)													**	MIC Range (mg/L)
	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	8	16	32		
Benzylpenicillin [#]	2*	1		1	9	6	6	4						5	0,004-0,5
Piperacillin/tazobactam [#]			1	1	2	2	7	7	4	7				3	<0,016-2
Ceftriaxone			1	1	2	6	8	5	6					4	0,016-32
Meropenem			5	5	4	8	5	2					1	4	0,012-32
Erythromycin [#]			21	8		1								4	<0,016-0,094
Clindamycin [#]				4	13	3	5	4	1	1				3	0,016-1,5
Linezolid						2	9	18	1					4	0,125-0,75
Moxifloxacin						1	3	8	10	6	4		1	1	0,064-32
Tetracycline [#]						1	11	11	5	1		1		4	0,094-16
Tigecycline			1	10	12	8								3	0,016-0,125

1)* Number of strains with adequate growth for reading MIC

2)** Number of strains with undefined MIC due to poor growth.

3) Grey shadings mark the upper limit for sensitivity according to international guidelines. Values above indicates resistance. (Pharmacological breakpoints 2007 from SRGA are used for antibiotics marked[#]. Pk/pd breakpoints from EUCAST are used for the other antibiotics).

Nine patients received both surgical intervention and antibiotic treatment and, respectively, 5 and 4 patients received either antibiotic treatment or surgical intervention. When antibiotics were given, the drug(s) and duration varied, but always included β -lactam antibiotics. The following β -lactam antibiotics were used: penicillin, ampicillin, dicloxacillin, mecillinam, cefuroxime, ceftriaxone and meropenem. In some cases ciprofloxacin, gentamicin and metronidazole were added. The patient with keratitis was treated with topical agents containing chloramphenicol, tetracycline, polymyxin and gentamicin. All patients recovered, except one who died from other causes. Two patients had sequelae; one each with neurologic sequelae following meningitis and reduced vision following keratitis.

DISCUSSION

When describing the species *A. europaeus* in 1997 [2], Funke *et al.* used 15 phenotypic characteristics to differentiate the species from other human-derived, aerobically growing *Actinomyces* and *Arcanobacterium* species. In the scheme by Sarkonen *et al.* [3] using 18 characteristics, which also is used in this study, the same characteristics were used except that pyrazinamidase production and acid production from glucose plus glycogen was not tested for. However, additionally the scheme by Sarkonen *et al.* [3] takes into consideration pigmentation, production of α -fucosidase and acid production from arabinose, raffinose, rhamnose and trehalose. The number of *Actinomyces species* has increased since these schemes were made. Often new species have been created from subgroups of existing species, complicating the distinction between species based on phenotypic characteristics as inclusion of new tests have been needed for differentiation between species [1]. The fact that the

same reaction done with different methods, as also seen in this study, may result in diverse results complicates phenotypic identification even more. Recently, former genospecies 1 and 2 of *A. naeslundii* have been validly published as *A. oris sp. nov.* and *A. johnsonii sp. nov.*; these new species cannot be readily differentiated using conventional phenotypic testing [9]. All these developments stresses the need for applying molecular methods for identification of *Actinomyces*.

Actinomyces species has a varied appearance in the microscope. It may be the typical Gram-positive branching bacilli, which often present with a beaded appearance, but not seldom more coccoid or variable forms occurs. Likewise, the typical molar tooth appearance of *A. israelii* may be absent. The appearance may be similar to common relatively avirulent pathogens, which often may be present together with *Actinomyces species*. It is therefore likely that *Actinomyces* sometimes is overlooked in samples. Misidentifications or non-identifications are presumed to occur in the routine microbiology laboratory. 16S rRNA gene sequence analysis has shown to be a powerful test in confirming or identifying suspected or non-identified *Actinomyces species*.

Three of the examined strains had very low differences in scores between best and next best taxon matches. When examining phylogenetic trees based on a comparison of approximately 1,320 nucleotides, *A. meyerii* and *A. odontolyticus*, and *A. viscosus* and *A. naeslundii*, are closely related explaining the low bits-differences [2]. Results of phenotypic reaction may be helpful in species identification in such cases. All in all, 16S rDNA sequencing of a 526 base-pair stretch gave a good separation into proposed species.

Table 3. Clinical and Microbiological Data on 18 Patients from whom *Actinomyces* species were Isolated

Species/ no. of Isolates	Age Land Sex	Isolated from	Underlying Illness	Major Clinical Manifestations	Coisolates	Treatment	Outcome
<i>A. odontolyticus</i> / 1	56 F	blood	cirrhosis	bloody vomiting and diarrhoea	- ^a	antibiotics (m, mt)	death from other causes
<i>A. turicensis</i> / 4	24 F	pus (nates)	- ^a	soft tissue abscess on nates	-	surgery	recovery
	56 M	tissue (necrotic)	extraction of infected tooth	fasciitis of face and neck	<i>H. parainfluenzae</i> , NHS, CNS, <i>Prevotella</i> spp.	surgery, 17 and later 15 days of antibiotics (p/cu + mt, d)	recovery
	63 F	pus (bladder)	chronic dialysis	cystitis	<i>B. fragilis</i> , CNS	rinsing of the bladder, 25 days of antibiotics (d, ci + mt)	recovery
	46 F	pus (rectum)	-	rectum abscess	-	drainage, 6 weeks of antibiotics (p, g, mt, followed by p alone)	recovery
<i>A. neuüi</i> / 2	91 M	blood	chronic nephropathy	cystitis	-	9 days of antibiotics (cu, mc)	recovery
	67 M	blood	surgery of ureter-stenosis	perirenal abscess	CNS, coryneforms	drainage, min. 37 days of antibiotics (a, followed by p + ci)	recovery
<i>A. meyeri</i> / 4	34 M	pus (cheek)	extraction of infected tooth	soft tissue abscess on cheek	-	surgery and antibiotics (d)	*does not show for check-up
	33 M	root of a tooth	-	periodontal infection	throat-flora	dental treatment and antibiotics (p)	recovery
	36 M	pus (pleura)	cerebral palsy, epilepsy	empyema following episode of pneumonia	microscopy also showed Gram-pos. cocci in chains	drainage, decortication, 2-3 weeks of antibiotics (cu, mt)	*does not show for check-up
	66 M	pus (abdomen)	long episode of intermittent diarrhoea	periappendical abscess	<i>E. coli</i>	drainage (twice)	recovery
<i>A. israelii</i> / 2	38 M	spinal fluid	alcoholism	meningitis	-	antibiotics (initially high doses of p, a, ct)	neurologic sequelae
	35 F	pus (abdomen)	-	periappendical abscess	-	surgery, 4 days of antibiotics (cu, mt)	recovery
<i>A. radingae</i> / 2	52 M	pus (scrotum)	-	soft tissue abscess of scrotum	anaerobic Gram-pos. cocci and Gram-neg. rods	surgery, a prior to hospitalization	recovery
	74 F	pus (back)	-	soft tissue abscess on the back	-	surgery	recovery
<i>A. viscosus</i> / 1	87 F	blood	COLD, Mb. cordis	source unknown	-	9 days of antibiotics (cu, p, mc)	recovery
<i>A. naeslundii</i> / 1	52 F	swab (cornea)	treated thyroid disease	keratitis followed injury of the cornea	<i>Lactobacillus</i> sp. found in other samples	topical antibiotics	reduced vision
<i>A. gerencseriae</i> / 1	66 M	swab (oral cavity)	-	periodontal infection	-	surgery, 4 weeks of antibiotics (p)	recovery

F, female, M, male, ^a, none, ^{*}, assumed recovery*H.*, *Haemophilus*, *B.*, *Bacteroides*, NHS, non-hemolytic streptococci, CNS, coagulase-negative staphylococci, pos., positive, neg., negative

a, ampicillin/amoxicillin, ci, ciprofloxacin, ct, ceftriaxone, cu, cefuroxime, d, dicloxacillin, g, gentamicin, m, meropenem, mc, mecillinam, mt, metronidazole, p, penicillin.

Sequence analysis is especially useful when a rapid identification is desired due to for example clinical circumstances. Thus, molecular bacterial identification is a powerful tool for national reference laboratories enhancing both the speed and validity of performed examinations. Using BLAST examinations may also be helpful in giving up to date taxonomic considerations.

In vitro antimicrobial susceptibility of *Actinomyces species* is quite predictable [10]. Strains are generally sensitive to β -lactams. However, some may exhibit higher MIC values as also shown by McNeil and Schaal. The medium we used for susceptibility testing contains L-cystein, which reduces the activity of β -lactam antibiotics [11]; therefore, underestimated susceptibility for these antimicrobial agents can be expected. Erythromycin was the most active antimicrobial agent *in vitro*, followed by tigecycline. Clindamycin, linezolid and tetracycline also showed good *in-vitro* activity with MICs within the therapeutic range for all strains, except one strain, that was resistant to tetracycline. It is important knowing that fluorquinolones generally have poor activity against *Actinomyces species* [10]. In agreement with this moxifloxacin for most strains in this study had MIC values above the upper limit for sensitivity. Susceptibility testing of *Actinomyces species* can be problematic; therefore, it would be relevant to elaborate standard guidelines for susceptibility testing procedure and to determine species-specific breakpoints.

In the article by Clarridge III and Zhang [12] a large number of clinical *Actinomyces species* were examined. Seventy-three strains were identified by genotypic identification. *A. turicensis* was the most frequently isolated species. With genotypic identification the most frequently identified species in our study were *A. turicensis* and *A. meyeri*. The normal niche of *A. turicensis* has been assessed [12] to be gastrointestinal, genital or skin in that area. Some strains were also isolated from abscesses of the face, neck, breast, chest and back. Our findings are in accordance with this, see Table 3. The normal niche of *A. meyeri*, *A. israelii* and *A. odontolyticus* was assessed to be oropharynx, and this is in accordance with three of our *A. meyeri* strains being from abscess of cheek, root of tooth and empyema of pleural space, respectively. Finally, *A. radingae* was associated with chronic soft tissue abscesses of the breast, chest and back, and the normal niche was assessed to be skin of the upper body. One of our two strains was from an abscess on the back.

Actinomyces is often difficult to diagnose and eradicate, needing surgery and a long course of antibiotics [13, 14]. Typical actinomycetic lesions contain between 1 and 10 bacterial species in addition to the pathogenic *Actinomycetes* [10]. We do not have information of typical actinomycotic lesions in our patients. Several of the patients did not receive antibiotics at all or only short courses of treatment with recovery. These cases were especially soft tissue infections, but also abscesses in the abdomen and positive blood cultures. Thus, it is important to be aware of that many infections involving *Actinomyces species* may not present as typical actinomycetic lesions. The great recovery rate may be

associated to the wide range of antibiotic sensitivity (except quinolones) among *Actinomyces species* and that infections may have a milder/different course than the classic severe *A. israelii* infections and therefore not necessitating the very long periods of high doses of for instance penicillin as otherwise recommended.

In conclusion, we find poor agreement between the phenotypic and genotypic identification of *Actinomyces species*. Phenotypic tests were helpful in identifying strains closely related by DNA sequences. The examined *Actinomyces* strains were *in-vitro* sensitive to many antibiotics including β -lactams, but not quinolones, and two strains were resistant to meropenem and tetracycline, respectively. Standard guidelines for susceptibility testing of *Actinomyces* are lacking. With a varied appearance, both microscopically and on agar, often looking like common relatively avirulent bacteria, *Actinomyces* may easily be overlooked in samples. *Actinomyces* can be present in infections without typical actinomycetic lesions, and some of these infections probably do not need the same aggressive and long treatment as actinomycosis.

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CONFLICT OF INTEREST DECLARATION

There are no relationships constituting dual or conflicting interests.

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