Propionibacterium avidum – a virulent pathogen causing hip periprosthetic joint infection

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Short title: *Propionibacterium avidum* hip PJI

Summary

We describe 13 periprosthetic joint infections caused by *Propionibacterium avidum*. The majority were hip-associated and occurred after hip arthroplasty surgery using an anterior surgical approach. Upon phylogenetic analysis, isolated strains clustered with *P. avidum* PJI strains from Sweden.

Abstract

Background. Propionibacteria are important members of the human skin microbiota, but are also opportunistic pathogens associated with periprosthetic joint infections (PJI). While the role of *Propionibacterium acnes* in PJI has been widely described, insight into the capacity of *Propionibacterium avidum* to cause PJI is limited.

Methods. An unusual cluster of four hip PJIs caused by *P. avidum* in one orthopedic center in 2015 prompted us to retrospectively identify and analyze clinical data related to previous *P. avidum* PJI cases (1997-2015). We also characterized the hemolytic and biofilm-producing capacity of our four clinical *P. avidum* strains isolated in 2015, and investigated their phylogenetic relationships by whole genome sequencing.

Results. We retrospectively identified 13 *P. avidum* PJIs, with the majority being hip-related infections (n=11). Preoperative synovial fluid cultures were *P. avidum* positive in 63.6% of cases. Six out of 12 patients (50%) with available case histories were treated with an exchange of the prosthesis. In all but one of the six patients treated with debridement-retention of the prosthesis, treatment failed thus requiring a two-stage revision. The isolated *P. avidum* strains showed a more pronounced hemolytic activity, but a similar biofilm-forming ability when compared to *P. acnes*. Whole genome sequencing identified two phylogenetic clusters highly related to *P. avidum* PJI strains isolated in Sweden.

Conclusions. We describe the largest series of *P. avidum* PJI predominantly located in the hip.

Phylogenetic similarity of our *P. avidum* strains to PJI strains isolated elsewhere suggests these invasive lineages may be common.

Keywords: *Propionibacterium avidum*, *Cutibacterium avidum*, periprosthetic joint infection (PJI), hip, biofilm, whole genome sequencing

Introduction

Periprosthetic joint infections (PJI) following prosthesis implantation result in high morbidity [1]. The incidence is rising due to the increasing life span of our population resulting in high numbers of degenerative disorders requiring joint replacements. The most commonly isolated microorganisms in PJI are staphylococci., followed by streptococci., enterococci., Gram-negative bacteria and anaerobes [2].

Propionibacteria are Gram-positive anaerobic bacteria and integral components of the normal human skin microbiota, but also cause opportunistic infections including PJI [3]. Of the three members of the cutaneous group of human propionibacteria, *Propionibacterium acnes* is by far the most frequent cause of PJI. Individual case reports of soft tissue and medical device-related infections due to *Propionibacterium avidum (*recently proposed as *Cutibacterium avidum)* and, even less commonly, *Propionibacterium granulosum* have been described [7-14].

In 2015, we identified and treated a cluster of four patients with *P. avidum* PJIs occurring within a single orthopedic center. Due to this unusual observation, we decided to conduct a wider, and much needed, epidemiological and clinical assessment of patients with *P. avidum* PJI for potential risk factors and treatment outcomes. We also investigated *P. avidum* PJI strains for key virulence properties, and performed whole genome analysis to examine their phylogenetic relationship to one another, as well as a small number of previously sequenced strains isolated from PJIs.

Materials and Methods

Patients and Study design

The Department of Orthopedics of the University Hospital Balgrist is a specialized tertiary care hospital with 120 beds. In 2015, approximately 5,000 surgical procedures were performed, of which 326 procedures were primary hip arthroplasties. In 2015, we prospectively identified four patients with a hip *P. avidum* PJI. Clinical and epidemiological patient's history was retrieved from the prospectively managed database on all infections from the Infectious Diseases Consulting Service, and from the hospital clinical information system. We also conducted a retrospective analysis of the microbiological laboratory database of the Institute of Medical Microbiology, University of Zurich, to identify further *P. avidum* infections at the University Hospital Balgrist (1997-2014). The clinical presentation of the patients with *P. avidum* isolated in tissue, synovial or sonicated fluid was reviewed. Infection was differentiated from contamination when *P. avidum* grew in at least two biopsy or sonication fluid samples [4]. We calculated the in-hospital incidence of *P. avidum* PJI as the number of *P. avidum* PJI divided by number of total surgeries per year performed at the clinic Balgrist, taking into account the increasing number of primary implantation of arthroplasties or revision surgeries in recent years. Basic characteristics, clinical presentation at the time of diagnosis, diagnostic steps according to MSIS criteria [5], surgical and antibiotic treatment, and outcome of PJI were analyzed.

Potential risk factors such as an association with a particular surgeon, changing the surgical incision approach, antibiotic prophylaxis or patient BMI were studied. The surgical incision approach used in hip arthroplasty surgeries was changed from lateral to anterior in 2006. As a result, infection rate before and after introduction of this change was investigated to identify whether the surgical approach

used was a potential risk factor. Perioperative intravenous antibiotic prophylaxis with cefuroxime 1.5g was routinely administered 30-60 minutes prior to skin incision, followed by two additional doses. Skin was disinfected three times with a povidone-iodine solution (Betaseptic^{TM}) throughout the entire study period.

The cantonal ethic authority of Zurich, Switzerland approved the study protocol (KEK Nr. 2016-00145 and Nr. 2015-0357).

Bacteriology

Microbiological techniques and standard biochemical methods for the detection and identification of *Propionibacterium* species were performed as previously described [6]. In short, incubation time for synovial fluid and sonication fluid was seven days and 10 days for tissue biopsies. Diagnosis of *P. avidum* included a positive reaction for catalase, CAMP factor, and esculin, and a negative test for indole to distinguish from other *Propionibacterium* spp. [7] From 2012, strains were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry spectrometry (MALDI-TOF MS) using a Bruker MALDI Biotyper in combination with research-use-only (RUO) versions of the MALDI Biotyper software package (version 3.0) and the reference database V.3.3.1.0 (4613 entries) or later database versions. When ordered by the Infectious Diseases specialist, antibiotic susceptibility testing was performed using E-test strips (Bio-Mérieux) on Brucella agar plates (McFarland 0.5) cultivated for 48 hours.

Bacterial strains

A total of five strains isolated from four patients in 2015 [clinical isolate (CI) 828, 853, 878, 882, 855] were analyzed. We also examined four randomly selected clinical *P. acnes* strains (CI803, CI805, CI806, CI820) from our bacterial biobank for comparison of hemolytic activity. To compare *P. avidum* with *P. acnes* for biofilm formation we used *P. acnes* strain ATCC 11827, which is known to adhere to foreign materials and produce biofilms *in vivo* [8, 9].

Hemolysis and biofilm formation.

Zones of hemolysis were measured (in mm) on Brucella agar plates. Biofilm formation was measured *in vitro* using a microtiter plate assay [10]. The results were compared to those obtained with the *P. acnes* strain ATCC 11827. The ratio of biofilm mass over total biomass of *P. acnes* and *P. avidum,* respectively were calculated.

Whole genome sequencing and phylogenetic analyses

Genomic DNA was extracted from *P. avidum* cultures using a QIAamp DNA Micro Kit (Qiagen) according to the manufacturer's instructions. Sequencing libraries were prepared using a NexteraXT library kit (Illumina), pooled and then sequenced on an Illumina MiSeq. Paired-end sequencing reads of 300-bp were assembled using MIRA v4.0.2 [11], and the assemblies were manually refined using Consed and Gap5 [12]. Core genomic regions shared by all analyzed *P. avidum* strains were compared as described [13] using Nucmer [14]. The core SNP sites were concatenated and imported into MEGA5 [15] for phylogenetic tree analysis.

Comparison of the exopolysaccharide (EPS) encoding locus in *P. avidum* strains was performed using Nucmer; the 35-kb EPS region previously described in the strain T13 [16] was aligned against all *P. avidum* genomes. Homologous regions identified in each strain were manually assembled with Gap5 [12] using the T13 region as reference, and the resulting scaffolds were then aligned with MAFFT [17] for phylogenetic analysis in MEGA7*.*

Statistical analysis

Wilcoxon rank-sum tests were used to compare continuous variables and Fisher's exact test to compare categorical variables.

Results

Cohort of P. avidum PJI cases

In 2015, we identified a cluster of four *P. avidum* PJIs that occurred after primary hip arthroplasty surgery, which was conducted via an anterior approach. Retrospective analysis of our microbiological database identified an additional nine patients with a PJI due to *P. avidum*, and two patients with osteomyelitis and a soft-tissue infection due to *P. avidum* (all between January 1997 and December 2014). Of the 13 PJIs (four prospectively, nine retrospectively), 11 were hip (84.6%) and two shoulder-related (Figure 1).

Arthroplasty surgery was performed by 13 surgical teams in six operating theatres excluding an association between post-surgical infection and a specific surgeon or team. Only eight patients had surgery on the same joint preceding infection at the University Hospital Balgrist –labeled as ‘in-house acquired infection’.

Among them, hip arthroplasties were more affected than shoulder (seven hip infections of a total of 6,860 surgeries [0.10%]) versus one shoulder infection of a total of 1963 surgeries [0.04%] between 1997 and 2015. For hip arthroplasty, the standard surgical incision approach changed from lateral to anterior in 2006 within our clinic. We observed a lower ratio of postoperative infections compared to the number of total surgeries of 0.04% (1/2262) during the time period before changing the incision approach (1997-2005), as compared to 0.13% (6/4598) after the new approach was adopted (2006-2015). The risk for a postsurgical infection was 2.95 times higher in the latter time period (RR = 2.95, 95%CI 0.36 – 24.5, p =0.44). Except for the additional routine use of tranexamic acid since 2005, no hospital hygiene procedures (type of irrigation, skin cleansing, ventilation system) had been changed during the observed period. All patients routinely received perioperative antibiotic prophylaxis with cefuroxime.

One of the patients had a *P. avidum* PJI in 1997. Since the medical history records of this patient no longer exist, this patient was excluded from further analysis. Thus, we describe in detail a cohort of 12 patients treated for a *P. avidum* PJI at the same orthopedic center.

Clinical characteristics of patients with P. avidum PJI

The median age of our 12 patients (seven females) was 61 years at the time of diagnosis. Nine out of 12 patients (75%) with a *P. avidum* PJI were obese with a BMI higher than 30 kg/m^2 (Table 1 and Supplementary Table 1). All patients presented with pain, six with wound secretion or sinus tract formation, and four with local signs of inflammation, such as skin erythema and swelling. Fever was reported in four

patients. In four patients with a preoperative MRI, periprosthetic soft-tissue abscess and joint effusion were observed, with communication between the abscess and the joint effusion (Figure 2, Supplementary table 1); a further two cases (number 2 and 4) showed osteomyelitis of the acetabulum. The majority of infections (75%) was delayed (i.e. presenting one month and later after joint surgery).

Diagnosis was confirmed preoperatively in five patients with ≥ three minor criteria according to MSIS PJI definition criteria [5, 18]. Among the 11 cases in which a pre-operative puncture of synovial fluid had been performed, *P. avidum* was cultivated from seven (63.6%) of the patients. Five of these patients (71.4%) showed elevated leucocytes > 3,000 cells/µl (range 8,000-308,000 cells/µl) in the synovial fluid cell, and six (85.7%) ≥ 80% neutrophil granulocytes (Table 2). PJI was found to be monomicrobial in eight and polymicrobial in four patients (Table 2).

All but one of the 12 *P. avidum* strains were susceptible to clindamycin, levofloxacin, and rifampin (Table 3). In one patient, we found that the initially isolated strain was resistant to clindamycin and six months later, after levofloxacin and rifampin treatment, also resistant to ciprofloxacin and levofloxacin. Following surgical debridement or exchange of the prosthesis, all patients were treated intravenously for approximately two weeks with a beta-lactam (or vancomycin in case of allergies), followed by an oral therapy. Oral treatment and its duration was chosen according to MIC values and the surgical approach adopted, respectively. Thus antibiotics were given for a total of three months for Debridement-Antibiotics-Irrigation-Retention (DAIR) as well as one-stage exchange and for six weeks for two-stage exchange.

Six out of 12 patients were primarily treated with a complete one- or two-stage exchange of the prosthesis, five with a DAIR procedure, and one with antibiotics

alone. In all but one of the six patients treated with either an initial DAIR or antibiotic alone, treatment failed necessitating a two-stage revision of the prosthesis with a good clinical outcome.

Phenotypic analysis of Propionibacterium avidum strains from a cluster of PJI

Hemolysis and biofilm production. The *P. avidum* strains isolated from a cluster of PJIs in our clinic in 2015 showed a strong hemolytic reaction, which was significantly greater than that of the *P. acnes* strains isolated from other hip PJIs (Figure 3A, p = 0.01). The ratio of biofilm to total mass of the *P. avidum* strains was, however, not significantly different from the *P. acnes* strain ATCC 11827 [8] (Figure 3B).

Whole genome sequencing and phylogenetic analysis. In one of the four patients presenting with a PJI in 2015, we detected two phenotypically different *P. avidum* strains (CI853 and CI855). To characterize the genetic diversity of our five PJI *P. avidum* strains, and therefore determine their relatedness, we performed whole genome sequencing followed by phylogenetic analysis; publically available *P. avidum* genomes from two PJIs (T13, T14, T15) and five various non-PJI sources (MJR7694, 44067, ATCC25577, TM16, UCD/PD2) were also included for comparison. Illumina sequencing generated draft genome sequences which consisted of 69-106 contigs depending on the strain considered (Table 4). All five strains had almost identical G+C content (63.4-63.5%) and genome sizes ranging from 2.48-2.54 Mbp (Table 4). A total of 172 genomic regions totaling 2.1 Mbp were found to be shared among the 13 *P. avidum* strains.

Comparison of the core genomic regions showed that CI853 and CI855, which were isolated from the same patient, differed only by a single SNP, and CI828, CI882 and CI878, which were isolated from three different patients, similarly displayed a very high degree of relatedness to each other with 99.6%-99.8% identity at SNP sites (Figure 4). CI828, CI878 and CI882 also clustered together with the recently described *P. avidum* T13 and T15 strains recovered from patients with a hip PJI in Sweden. CI853 and CI855 were found to be similar to a further PJI strain also isolated in Sweden (T14) (89.9% SNP identity). Compared with an average identity of 55.1% among the five non-PJI strains at these sites, this high degree of similarity suggests that the isolates within each group may be clonal. All the sequenced strains harbored a gene cluster encoding exopolysaccharide synthesis (EPS) as previously described (Figure 5) [14], as well as genes involved in survival, fitness and defense.

Discussion

This is the first clinical study describing a large cohort of patients with PJI due to the skin commensal *P. avidum*. We observed that *P. avidum* was predominantly diagnosed in association with hip arthroplasty (85%). This is in contrast to published data on *P. acnes*, which mainly causes shoulder PJI infections (prosthesis and post arthroscopy-related) but rarely hip infections [6]. We interpreted these results as a consequence of preferential skin colonization by *P. avidum* of sweat glands in the moist groin and perianal regions [19] and contrast to relatively low abundance on the face [20]. As we found *P. avidum* PJI predominantly in the hip of obese patients, we hypothesize that this association results from *P. avidum* overgrowth in the moist skin folds typically found in the groin region of obese individuals. This may facilitate their

entry into the surgical wound, even after pre-operative skin antisepsis, leading to downstream infection.

Synovial fluid analysis showed a high number of leukocytes and a positive culture for *P. avidum* in 67% of cases. Thus in contrast to *P. acnes*, pre-operative differentiation of septic from aseptic-loosening of the prosthesis was much easier [21]. Debridement in conjunction with antibiotics was not sufficient to treat the infections. All five infections with a delayed presentation, and treated with debridement or antibiotics alone, required a subsequent two-stage exchange of the prosthesis. This indicates that the surgical treatment approach in *P. avidum* PJI should be the same as that described for other bacterial species [1], i.e. when duration of symptoms is $>$ 3 weeks, a sinus tract is present, or the implant is already loose, one- or two-stage revision of the arthroplasty is required for a successful treatment outcome [22].

We observed obesity as a potential risk factor for *P. avidum* PJI after hip arthroplasty. A high BMI was noted in all but one patient, which is in line with a recent publication describing hip PJIs due to *P. avidum* [16]. Overall, obesity with a BMI > 35/kg^2 or > 100kg is a risk for orthopedic infections in general [23]. Most of our infections were associated with hip arthroplasties. We did not observe a significant increase in infection when the incision approach was changed, in line with the study of Clauss *et al*. [24]; however, since the combined numbers of both studies are small, it is yet unclear whether the incision approach for hip arthroplasty represents a risk factor for PJI. In general, better characterization between *P. acnes* and *P. avidum* strains may be an important factor, however at our institution except for the introduction of MALDI in 2012 diagnostic methods remained the same.

While the pathogenicity of *P. avidum* infections is poorly understood, we found a more pronounced hemolytic activity by *P. avidum* strains as compared to *P. acnes*, in line with reports in the literature [22]. Underlying its potential importance, hemolysis was recently described as a "clinical marker" to better distinguish orthopedic infections with *P. acnes* versus a contaminated culture, although this association is controversial [39-40]. A potentially key virulence trait of *P. avidum* in relation to PJIs is its ability to form biofilms on medical implants. The extracellular polymeric matrix of such biofilms is different from that produced by *P. acnes* biofilms [25]. In addition, *P. avidum* produces a capsule that is unique and has not been described for *P. acnes* nor *P. granulosum* [25]. This capsule may protect against phagocytosis. In the genomes of all five *P. avidum* isolates described in this study, we identified homologs of an EPS-encoding island previously found to be present in *P. avidum*, but not other cutaneous propionibacteria, which may be potentially important in adherence [14]. This island is flanked by tRNA genes, suggesting acquisition by horizontal gene transfer. One PJI isolate, T14, contains an EPS island that clusters with that from the prostate-derived isolate TM16 [46] upon phylogenetic analysis. Thus, expression of this locus may be a general virulence determinant not just related to PJI.

Phylogenetic analysis showed that our sequenced strains formed two distinct clusters along with strains isolated from patients with hip PJIs in Sweden [16], and non-PJI strains isolated from the vaginal microbiota [26] and a skin abscess [44]. Currently a detailed understanding of the population genetic structure of *P. avidum* is lacking. We speculate that major phylogenetic divisions and clonal lineages of *P. avidum* with varying disease potential or ecological specialization occur, similar to that observed with *P. acnes* phylogroups I, II and III recently proposed as distinct

bacterial subspecies [27]. This is tentatively supported by the observation of at least two distinct serotypes of *P. avidum* with a cell wall composition that mirrors *P. acnes* types I and II [28, 29].

In conclusion, this is the first description of a large series of PJI caused by *P. avidum*. We show that the skin commensal *P. avidum* predominantly caused delayed PJI which were only resolved by two-stage revisions. We did not identify a specific risk factor for the increasing number of *P. avidum* PJI's in recent years. Further studies evaluating skin colonization with *P. avidum* might help to select patients at higher risk for invasive *P. avidum* infections.

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Tables and Figures

Table 1. Clinical characteristics of 12 patients with PJI caused by *P. avidum*, either as a monomicrobial (n=8) or part of a polymicrobial (n=4) infection, at the time of initial surgical treatment of infection at the University Hospital Balgrist.

Characteristics	**Number (%)**
Median age (range), years	61 (range 45-81)
Female	7 (58.3)
BMI median (range)	34.0 (27.9-40.6)
Obesity grade 1 (>30)	3 (25)
Obesity grade 2 (>35)	5 (41.7)
Obesity grade 3 (>40)	1 (8.3)
Underlying joint disorder for arthroplasty	
Degenerative	9 (75)
Trauma	2 (16.7)
“Head necrosis”	1 (8.3)

Place of last arthroplasty surgery before infection	
University Hospital of Balgrist	7 (58.3)
Other Hospital	5 (41.7)
Localisation of infection	12
Hip PJI	10 (83.3)
Shoulder PJI	2 (16.7)
Signs and symptoms	
Pain	12 (100)
Wound secretion or sinus tract	6 (50)
Swelling, skin erythema	4 (33.3)
Fever	4 (33.3)
Time to diagnosis of infection* after last surgical revision of arthroplasty*	
Median (range), weeks	13.1 (2.3-63.2)
Time to initial septic surgery after last surgical revision of arthroplasty	

Median (range), weeks	
	30.2 (2.9-100.3)
Surgical treatment	
DAIR including partial exchange of prosthesis	5 (41.6%)
One-stage exchange	2 (16.7%)
Two-stage exchange	4 (33.3%)
Antibiotic treatment only	1 (8.3%)
Peroral antibiotic treatment after initial i.v treatment	
Clindamycin	4 (33.3)
Levofloxacin/rifampin	4 (33.3)
Ciprofloxacin/rifampin	2 (16.7)
Clindamycin/rifampin	2 (16.7)

* Time to first microbiological diagnosis of *P. avidum* (either preoperative synovial puncture or intraoperative tissue samples) with confirmation of infection due to MSIS criteria [5]. DAIR, Debridement-Antibiotics-Irrigation-Retention.

Table 2. Diagnostic characteristics of 12 patients with PJI caused by *P. avidum* at time of septic surgery at the University Hospital Balgrist

Nr	Age, sex	PJI site	Preoperative			Intraoperative		*P. avidum* strains	Polymicrobial infection [other pathogen]
			Blood	Synovial fluid		Tissue biopsies	Sonication fluid		
			CRP, ESR	*P. avidum* growth	Lc [% neutrophils]	*P. avidum* positive/total taken	Positive *P. avidum* [CFU/ml] in		
Prospective (cluster in 2015)									
1	59, f	Hip	4.9, 31	Negative	ND [80%]	4/6	≥ 100	CI878	No
2	53, f	Hip	200, 103	Positive	308,000 [80%]	2/5	≥ 100	CI882	No
3	64, f	Hip	74, 82	Positive	48,800 [80%]	5/5	60	CI853, 855	No
4	81, m	Hip	62.7, 82	Positive	248,000 [80%]	4/6	In broth	CI828	Yes [*S. aureus*]
Retrospective (1997-2014)									
5	57, f	Hip	7.6, 52	Positive	8,800 [80%]	2/8	20	NA	No

Nr	Age, sex	PJI site	Preoperative			Intraoperative		*P. avidum* strains	Polymicrobial infection [other pathogen]
			Blood	Synovial fluid		Tissue biopsies	Sonication fluid		
			CRP, ESR	*P. avidum* growth	Lc [% neutrophils]	*P. avidum* positive/total taken	Positive *P. avidum* [CFU/ml] in		
6	72, f	Hip	176, ND	Positive	ND	3/4	NA	NA	Yes [*F. magna*]
7	45, f	Hip	17, 21	Positive	38,300 [80%]	2/3	Negative	NA	No
8	56, m	Hip	15.2, 54	Negative	2,500 [ND]	2/4	Negative	NA	Yes [*F. magna*]
9	65, f	Hip	14, 37	Negative	ND	1/5	≥ 100	NA	No
10	69, m	Shoulder	119, ND	Positive	ND	3/3	NA	NA	No
11	56, m	Shoulder	38/ND	Negative	ND	1/4	≥ 100	NA	No
12	63, f	Hip	95/ND	ND	ND	0/7*	20 and 27	NA	Yes [*S. epidermidis]*

f, female; m, male; *S. aureus, Staphylococcus aureus*; *S. epidermidis, Staphylococcus epidermidis; F. magna, Finegoldia magna;* ND, not done (possible reasons include dry aspirate or hemolytic sample); NA, not available; Lc, leucocytes, ESR, erythrocyte sedimentation rate

* Diagnosis of *P. avidum* PJI was based on clinical presentation of sinus tract as well as previous growth of *P. avidum* in another hospital.

Table 3. MIC_{50} and resistance pattern of 12 *P. avidum* strains from PJIs using EUCAST breakpoints for Gram-positive anaerobes.

Antibiotic	**Samples (Nr)**	**MIC50 (range) (mg/l)**	**MIC breakpoint (mg/l)**		
			EUCAST S ≤	**R >**	**Resistant (%)**
Penicillin[a]	5	0.064 (0.03 - 0.125)	0.25	0.5	0 (0%)
Clindamycin[a]	12	0.032 (<0.016 - >256)	4	4	1 (8.3%)
Ciprofloxacin[b]	12	0.25 (0.19 - >32)	0.5	1	1 (8.3%)
Levofloxacin[b]	10	0.125 (0.094 - >32)	1	2	1 (10%)
Rifampin	12	0.004 (0.003 - 0.008)	-	-	
Cefuroximeb	4	0.38 (0.38 – 0.5)	4	8	0 (0%)

MIC50, minimal inhibition concentration (median); EUCAST clinical breakpoints in mg/l (Table v. 6.0, valid from 2016-01-01) are shown for Gram-positive anaerobes[a]. If no clinical breakpoints for Gram-positive anaerobes exist, breakpoints from pharmacokinetic/pharmacodynamic (PK/PD) data (non-species related)[b] were taken [30].

Table 4. Genome assembly characteristics of *P. avidum* PJI isolates compared in this study.

Strain	Accession*	Assembly Coverage	Size (Mb)	Contigs	Genes	Proteins	GC%
ATCC 25577	AGBA01	29×	2.55	7	2,290	2,200	63.3
44067	CP005287.1	375×	2.53	1	2,297	2,184	63.5
CI 853	NBIS00000000	114×	2.53	105	2,394	2,224	63.5
CI 855	NBIR00000000	219×	2.54	106	2,406	2,227	63.5
CI 828	NBIQ00000000	249×	2.48	76	2,313	2,152	63.4
CI 882	NBIP00000000	327×	2.50	69	2,360	2,182	63.4
CI 878	NBIO00000000	190×	2.54	89	2,422	2,251	63.4
MJR7694	LRVD01	76×	2.47	16	2,235	2,118	63.4
TM16	AOUA01	34×	2.54	420	2,441	2,133	63.4
UCD-PD2	LYSN01	76×	2.67	51	2,442	2,304	63.4
T13	LLJH01	50×	2.46	15	2,223	2,109	63.4
T15	LLJJ01	50×	2.46	14	2,223	2,110	63.4
T14	LLJI01	50×	2.52	9	2,290	2,184	63.4

*NCBI WGS accession number. For 44067 the NCBI Nucleotide accession number is shown.

Figure legends

Figure 1. Incidence and localization of *P. avidum* PJI among all patients treated at the University Hospital Balgrist at the time of diagnosis (1997 and 2015).

Figure 2. Spectrum of MRI findings of four patients with *P. avidum* PJIs. A and B: A 81-year old male patient (patient number 4, see Figure 1) two years after total hip arthroplasty of the right hip. Coronal image demonstrates joint effusion (thin arrow) adjacent to the neck of the prosthesis and osteomyelitis (outline arrowhead) of the acetabulum. Axial image in the same patient shows large soft-tissue abscess (outline arrows) anterior to the hip joint breaking through the superficial muscle fascia (black arrowhead). C and D: 53-year old female patient (patient number 2) 6 months after THA of the left hip. Coronal image demonstrates joint effusion (thin arrow), and extensive bone marrow edema in the acetabulum consistent with osteomyelitis (outline arrowhead). Further, extension of the infection into the soft tissue of the pelvis is seen (#). Axial image after intravenous gadolinium administration at the level of the middle third of the femoral shaft shows anterior intramuscular abscess (broad solid arrow) and epifascial abscess (outline arrows) extending to the dermis, with typical peripheral contrast enhancement. E: 59-year old female patient 2 years after THA of the right hip with soft tissue abscess (outline arrow) along the anterior surgical approach, extending to the femoral neck. F: 64-year old female patient (patient number 1) 19 days after THA of the right hip with soft-tissue abscess (outline arrow) along the anterior surgical approach, extending to the femoral neck.

Note – Asterisk, femoral component of THA; STIR, Short Tau Inversion Recovery; SEMAC, slice-encoding for metal artifact correction; WARP, optimized inversion pulse; T1, T1-weighted; fs, fat-saturated; hiBW, high readout bandwidth.

Figure 3. Phenotypic characterization of *P. avidum* strains isolated in 2015 from hip PJIs (CI 828, CI 853, CI 878, CI 882). Panel A) Hemolysis activity of four *P. avidum* strains compared to four *P. acnes* strains on Brucella agar zone diameter (mm) measured (unpaired t-test, Mann-Whitney test) using serial dilution from a starting inoculum of 2-5 x 108 CFU/ml. Panel B) Biofilm formation of four *P. avidum* PJI strains compared to the *P. acnes* biofilm strain ATCC 11827 using a static biofilm assay (unpaired t-test, Mann-Whitney test). Data shown are from three experiments done in technical triplicates.

Figure 4. A neighbor-joining phylogenetic tree constructed on the SNPs in the core genome regions of the *P. avidum* strains, including five *P. avidum* strains isolated from four patients with PJI described in this study (CI828, CI853, CI855, CI878, CI882), and previously sequenced strains (T13, T14, T15, ATCC25577, UCD-PD2, MJR7694, 44067, and TM16). Horizontal bar represents p-distances based on substitutions at the SNP sites. Bootstrap values are based on 500 replicates.

Figure 5. A neighbor-joining phylogenetic tree constructed based on nucleotide differences within the *P. avidum*-specific EPS-encoding genomic island of 13 sequenced *P. avidum* strains. Horizontal bar represents p-distances based on substitutions at 16,217 positions aligned across all strains. Bootstrap values are based on 500 replicates.

Figure 1

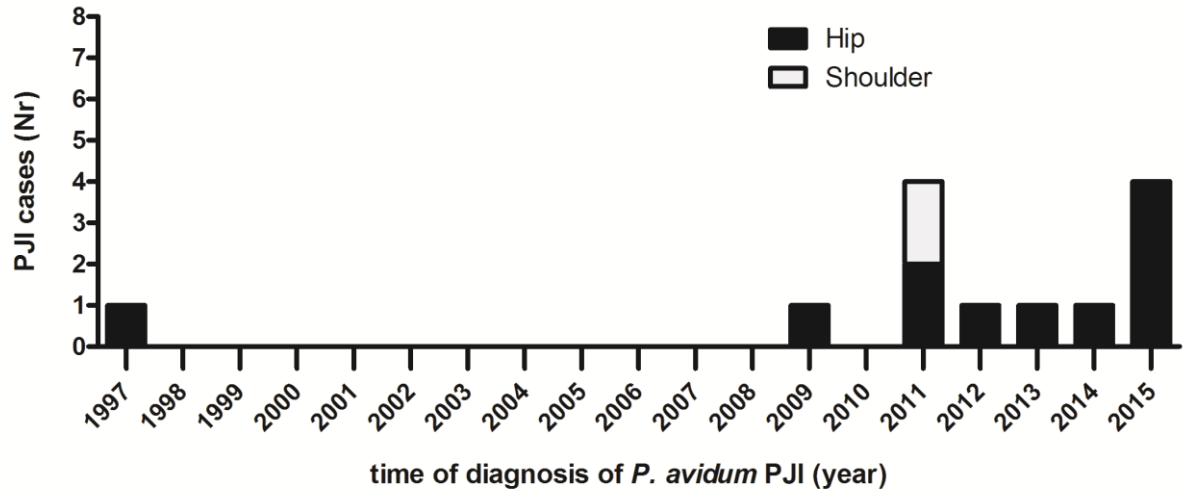

Hip
Shoulder
PJI cases (Nr)
0
1
2
3
4
5
6
7
8
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
time of diagnosis of *P. avidum* PJI (year)

Figure 2

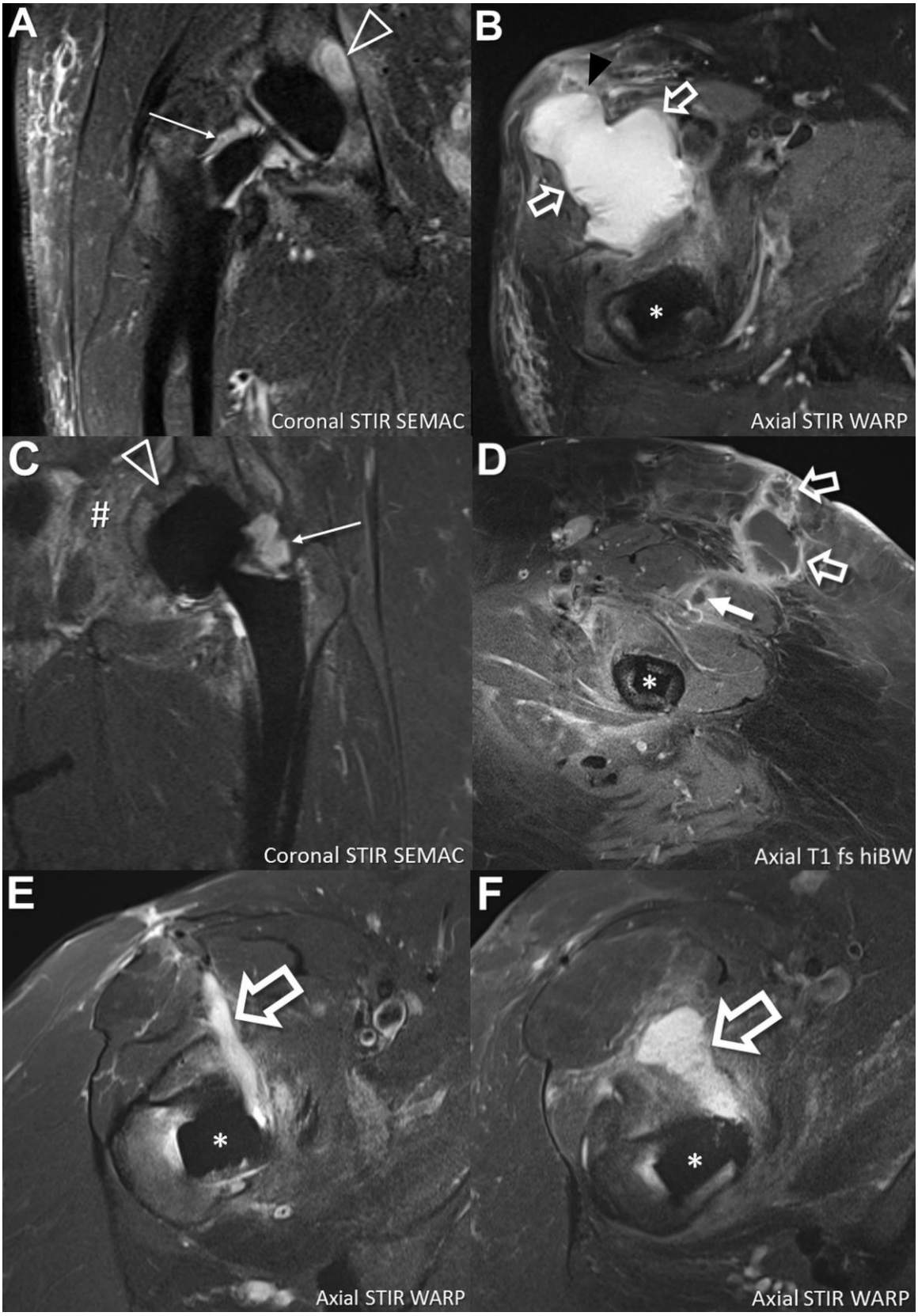
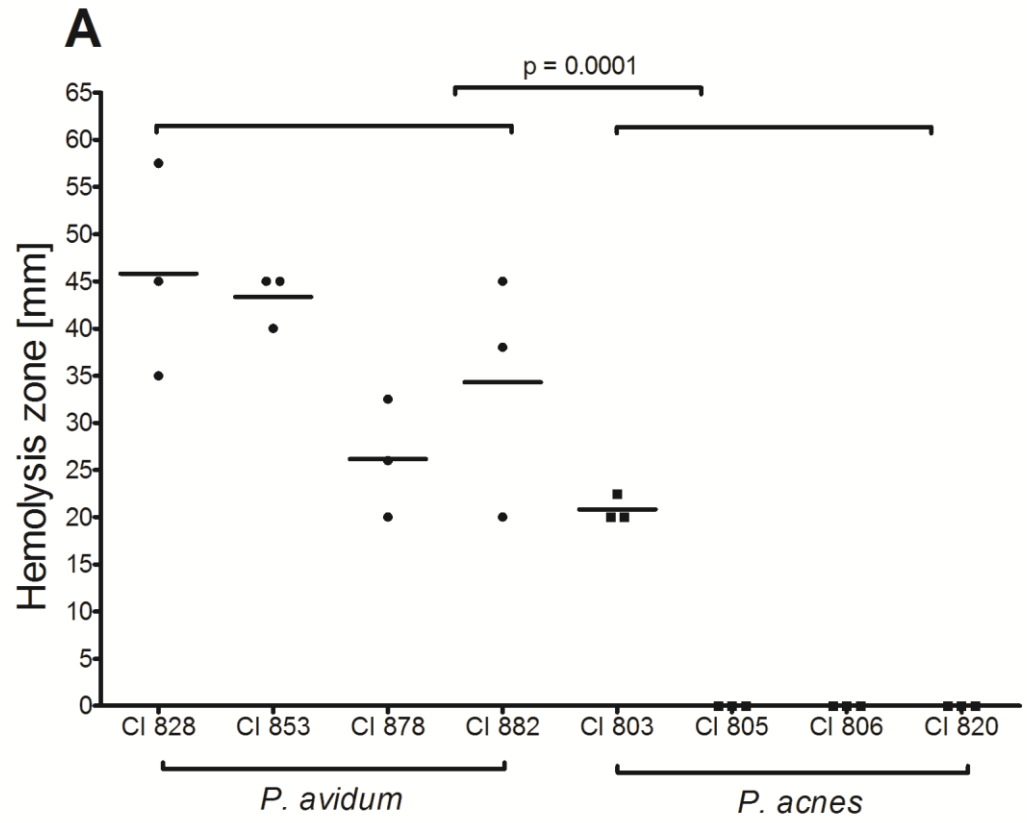

A
Coronal STIR SEMAC
B
Axial STIR WARP
C
#
Coronal STIR SEMAC
D
Axial T1 fs hiBW
E
Axial STIR WARP
F
Axial STIR WARP

Figure 3


A
p = 0.0001
Hemolysis zone [mm]
65
60
55
50
45
40
35
30
25
20
15
10
5
0
CI 828
CI 853
CI 878
CI 882
CI 803
CI 805
CI 806
CI 820
P. avidum
P. acnes

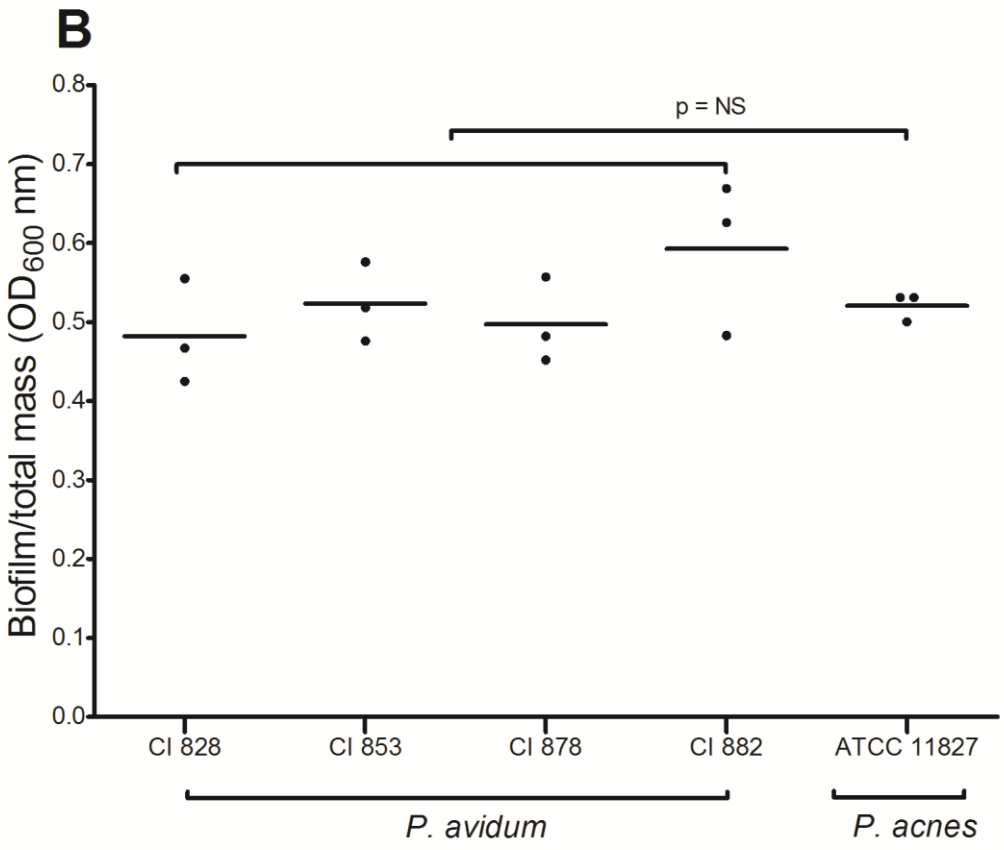

B
p = NS
Biofilm/total mass (OD600 nm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CI 828
CI 853
CI 878
CI 882
ATCC 11827
P. avidum
P. acnes

Figure 4

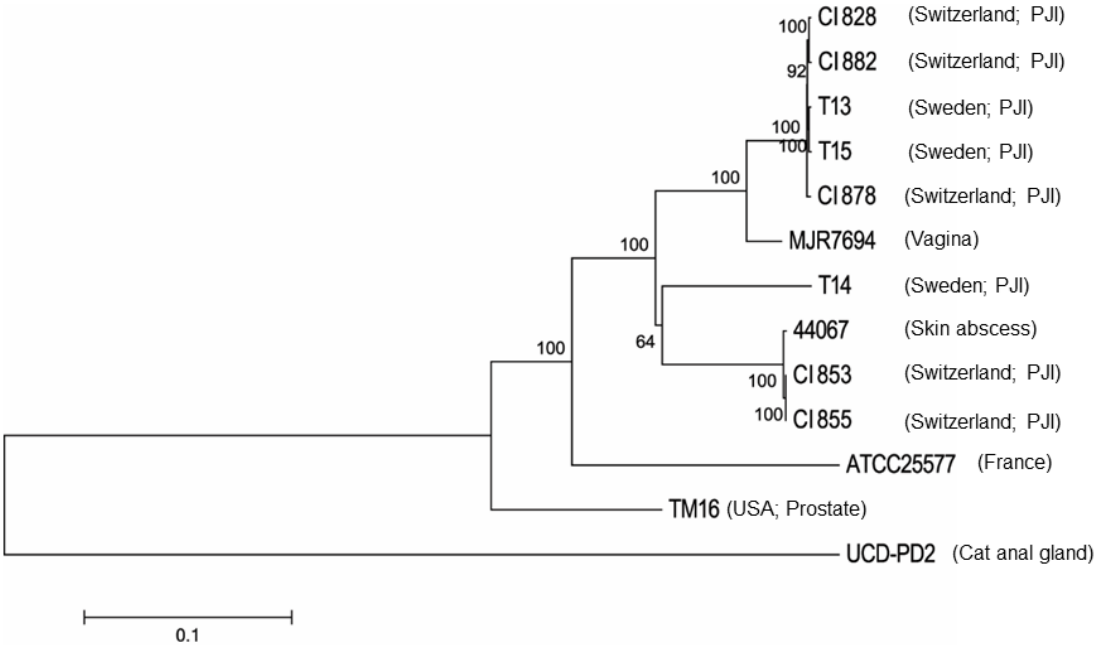
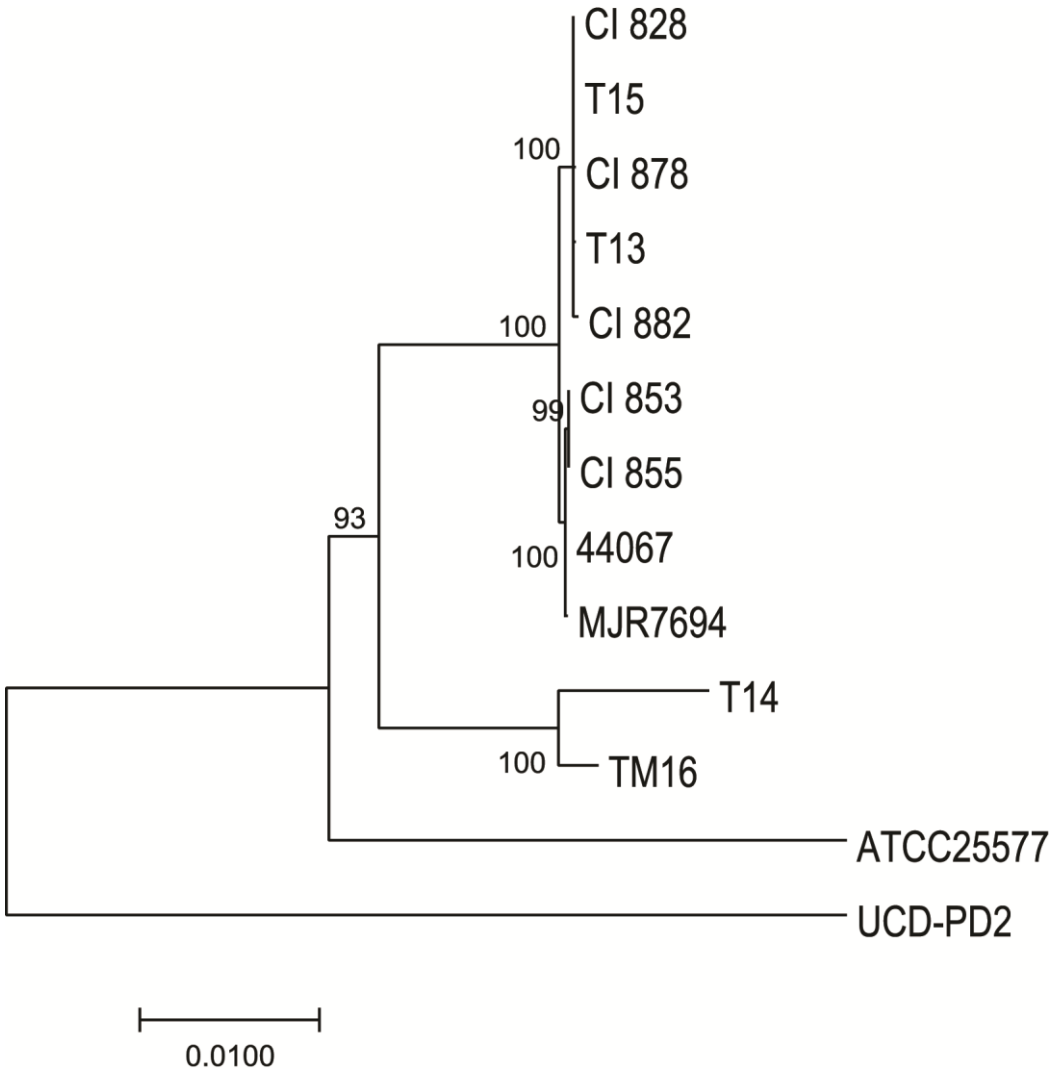

100
CI828 (Switzerland; PJI)
92
CI882 (Switzerland; PJI)
T13 (Sweden; PJI)
100
100
T15 (Sweden; PJI)
100
CI878 (Switzerland; PJI)
100
MJR7694 (Vagina)
T14 (Sweden; PJI)
64
44067 (Skin abscess)
100
100
CI853 (Switzerland; PJI)
100
CI855 (Switzerland; PJI)
ATCC25577 (France)
TM16 (USA; Prostate)
UCD-PD2 (Cat anal gland)
0.1

Figure 5


CI 828
T15
100
CI 878
T13
100
CI 882
99
CI 853
CI 855
93
100
44067
MJR7694
T14
100
TM16
ATCC25577
UCD-PD2
0.0100