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Actinobaculum schaalii Causing Fournier's Gangrene[▽]

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***Actinobaculum schaalii*, which belongs to the group of Gram-positive rods, is difficult to culture. Using molecular genetics, *Actinobaculum schaalii* could be identified as a causing microorganism in a case of Fournier's gangrene.**

CASE REPORT

A 33-year-old man was presented at the emergency ward with fever (37.8°C) and pain and swelling in the left groin area. He was an obese smoker (body mass index [BMI], 36) suffering from diabetes mellitus. Radiological examination revealed an important infiltration of the cutis and subcutis in both the left and right groin area up to the scrotum. Air bubbles were present. Blood analysis showed a C-reactive protein level of 33.6 mg/dl and a leukocyte count of 20.3×10^9/liter (85.9% neutrophils). Blood cultures were negative. A type of necrotizing fasciitis, Fournier's gangrene, was suspected, and a surgical procedure was promptly performed: aggressive debridement and cleansing of all involved tissue. Empirical treatment was initiated with amoxicillin-clavulanate (6 g every 24 h intravenously). During surgery, samples were taken from the involved tissue and cultured. After 48 h of incubation, tiny gray colonies, referred to as isolate 34317, were observed. It was the only isolate recovered from the surgical specimens. Colonies grew similarly under aerobic and anaerobic conditions (sheep blood agar, 37°C, 5% CO_2). Another 48 h later, these colonies showed weak beta hemolysis. Gram staining of the cells revealed small, nonmotile, non-spore-forming, Gram-positive coccoid rods with rudimentary branching. Biochemical test results are presented in Table 1. *In vitro* susceptibility to benzylpenicillin, amoxicillin, ciprofloxacin, ceftazidime, ceftriaxone, metronidazole, imipenem, amikacin, gentamicin, clindamycin, and vancomycin was investigated using the Etest (AB Biodisk, Solna, Sweden). An inoculum suspension at a McFarland standard of 0.5 was applied to Mueller-Hinton blood agar (Becton Dickinson, Heidelberg, Germany). MICs were read after 48 h of anaerobic incubation (Table 2). Analysis with the BD Phoenix automated microbiology system (Phoenix 100; BD, Erembodegem, Belgium) identified the bacterium as *Arcanobacterium haemolyticum*. Using the API Coryne system (bioMérieux, Craponne, France), no identification could be obtained. Subsequently, complete 16S rRNA gene sequencing and phylogenetic analysis were performed. DNA was extracted according to the protocol of Niemann et al. (16). The 16S rRNA gene was amplified by PCR using conserved primers (3) and subsequently purified using the NucleoFast 96 PCR cleanup kit (Macherey-Nagel, Düren, Germany). Sequence analysis was performed as described previously (15). Sequence assembly and phylogenetic analysis were performed using the software package BioNumerics (Applied Maths, Belgium). A consensus sequence (1,500 nucleotides) was obtained, and the highest percentage of 16S rRNA gene sequence similarity was found with *Actinobaculum schaalii* (98.4%), followed by *Actinobaculum urinale* (95.5%), *Actinobaculum massiliense* (94.6%), and *Actinobaculum suis* (93.9%). A similarity of less than 94% was obtained with other validly described species of the family *Actinomycetaceae*. The DNA-DNA relatedness of isolate 34317 to the type strain of *Actinobaculum schaalii* (LMG 18293^T), taken from the BCCM/LMG Bacteria Collection, was investigated. Genomic DNA was extracted according to a modification of the procedure of Gevers et al. (8). Hybridizations were performed in the presence of 50% formamide at 46°C according to a modification of the method described by Ezaki et al. (6). The mean percentage of DNA-DNA relatedness based on 8 hybridizations was 78%. The difference between the reciprocal values was 19%. With this technique, the average standard deviation was 14 units (9). Since 70% DNA-DNA relatedness has been generally accepted as the limit for species delineation (21), it can be concluded that the Gram-positive coccoid rods found in our case of Fournier's gangrene belong to the species *Actinobaculum schaalii*.

In the days following surgery, samples were taken from the wounds in the perineal area. Culture showed secondary infection with *Escherichia coli*, *Proteus mirabilis*, anaerobic bacteria (*Bacteroides fragilis*), and *Candida albicans*. Antibiotic treatment was switched to ciprofloxacin (500 mg, twice a day *per os*) and metronidazole (1,500 mg every 24 h intravenously). Eventually, aggressive debridement and cleansing, together with antibiotic treatment, were successful, and the patient was dismissed after 1 month.

DISCUSSION

Fournier's gangrene is a type of necrotizing fasciitis typically affecting the perineal and genital regions. Most likely, it begins as a local infection next to a portal of entry, such as a perianal or perirectal abscess. It is a rare but serious condition, with

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TABLE 1. Biochemical characteristics of *Actinobaculum schaalii* isolated from a patient with Fournier's gangrene

Characteristic	Result
Manual testing for:	
Catalase	−
Oxidase	−
Hippurate	+
API Coryne system	
Nitrate reduction	−
Pyrazinamidase	+
Pyrrolidonyl arylamidase	−
Alkaline phosphatase	−
β-Glucuronidase	−
β-Galactosidase	+
α-Glucosidase	+
N-Acetyl-β-glucosaminidase	−
Esculin hydrolysis	+
Urease activity	−
Gelatin hydrolysis	−
Acid from:	
Glucose	+
Ribose	+
Xylose	+
Mannitol	−
Maltose	+
Lactose	−
Sucrose	+
Glycogen	−

TABLE 2. Antimicrobial susceptibilities of *Actinobaculum schaalii* determined with the Etest

Antimicrobial agent	MIC (μg/ml)
Benzylpenicillin	<0.016
Amoxicillin	0.094
Ceftazidime	1.5
Ceftriaxone	0.064
Imipenem	0.032
Amikacin	1.5
Metronidazole	>256
Ciprofloxacin	2
Gentamicin	0.50
Clindamycin	<0.016
Vancomycin	0.094

mortality rates ranging from 7.5 to 18%. Several risk factors have been identified, including diabetes mellitus, obesity, alcohol abuse, smoking, and immunosuppression (4–7, 9, 18, 21). In our case, no portal of entry could be identified, but 3 predisposing conditions were present: deregulated diabetes mellitus, a BMI of 36, and cigarette smoking. While Fournier's gangrene is believed to be caused by polymicrobial infections, we identified *Actinobaculum schaalii* as a causing microorganism. Indeed, only *Actinobaculum schaalii* was identified in samples taken from necrotic tissue during surgery. Polymicrobial infection with *Escherichia coli*, *Proteus mirabilis*, and anaerobic bacteria was observed later, in samples taken from the wounds after debridement. *Actinobaculum schaalii* belongs to the group of Gram-positive rods, many of which have been considered harmless. This scenario has changed with the introduction of molecular testing, in particular, the use of 16S rRNA gene sequencing. This tool made it possible to identify bacteria more easily (tentatively to the species level). As a result, Gram-positive bacteria are being increasingly identified as a cause of infection, including complicated skin and soft tissue infections (CSSI) (2). The genus *Actinobaculum* was first described in 1997 and is closely related to the genera *Actinomyces* and *Arcanobacterium*. To date, 4 species have been validly described: *Actinobaculum suis*, *Actinobaculum massiliense*, *Actinobaculum urinale*, and *Actinobaculum schaalii* (14) *Actinobaculum suis* is known to cause urinary tract infections (UTIs) and abortions in sows and has long been assigned to a variety of genera, including *Corynebacterium*, *Eubacterium*, and *Actinomyces* (22) *Actinobaculum massiliense* and *Actinobaculum urinale* have been identified more recently as a putative cause of chronic cystitis in elderly women (10, 11). *Actinobaculum massiliense* might also be involved in superficial skin infections (20). *Actinobaculum schaalii* is increasingly identified as a cause of UTIs in elderly patients with underlying urologic conditions (1, 17). The invasive potential of *Actinobaculum schaalii* has long been considered limited, although more recent studies describe the implication of *Actinobaculum schaalii* in urosepsis, osteomyelitis, and endocarditis (12, 13, 19). Unfortunately, this bacterium is difficult to culture and requires long incubation. Indeed, in our case, colonies of *Actinobaculum schaalii* grew only after at least 48 h of incubation. As such, it could easily be missed. Biochemically, we were not able to identify *Actinobaculum schaalii*, not even with more advanced biochemical systems. Only 16S rRNA gene sequencing and DNA-DNA hybridization allowed us to identify the causing microorganism in our case of Fournier's gangrene, but this technique is not available in most laboratories due to its high cost and the need for trained personnel. Interestingly, Bank et al. recently developed a specific real-time quantitative PCR assay for identifying *Actinobaculum schaalii* based on the gyrase B (*gyrB*) gene (1). Using PCR, they found *Actinobaculum schaalii* in 22% of urine samples obtained from patients above 60. This result was much higher than the previous 0.4% they reported by urine culture of the same age group. Their results show that bacterial species, especially slow-growing species, are more common than what culture results indicate and may highlight the importance of such species in causing human infection. After recent reports on invasive infection by *Actinobaculum schaalii*, including osteomyelitis, urosepsis, and endocarditis (12, 13, 19), we add to the literature a case of necrotizing fasciitis caused by this bacterium. Further studies and the use of PCR techniques may shed light on the true incidence and invasive potential of *Actinobaculum schaalii*.

Nucleotide sequence accession number. The sequence of our *Actinobaculum schaalii* isolate has been deposited in GenBank/EMBL/DDBJ under accession number HQ992948.

REFERENCES

1. **Bank, S., A. Jensen, T. M. Hansen, K. M. Soby, and J. Prag.** 2010. *Actinobaculum schaalii*, a common uropathogen in elderly patients, Denmark. Emerg. Infect. Dis. **16:**76–80.
2. **Clarke, T. M., D. M. Citron, and S. Towfigh.** 2010. The conundrum of the gram-positive rod: are we missing important pathogens in complicated skin and soft-tissue infections? A case report and review of the literature. Surg. Infect. **11:**65–72.
3. **Coenye, T., et al.** 1999. Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. Int. J. Syst. Bacteriol. **49:**405–413.
4. **Czymek, R., et al.** 2009. New insights into the epidemiology and etiology of Fournier's gangrene: a review of 33 patients. Infection **37:**306–312.

5. **Eke, N.** 2000. Fournier's gangrene: a review of 1726 cases. Br. J. Surg. **87:**718–728.
6. **Ezaki, T., Y. Hashimoto, and E. Yabuuchi.** 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int. J. Syst. Bacteriol. **39:**224–229.
7. **Fournier, J. A.** 1883. Gangrene foudroyante de la verge. Med. Pract. **4:**589–597.
8. **Gevers, D., G. Huys, and J. Swings.** 2001. Application of rep-PCR fingerprinting for identification of Lactobacillus species. FEMS Microbiol. Lett. **205:**31–36.
9. **Goris, J., K. Suzuki, P. De Vos, T. Nakase, and K. Kersters.** 1998. Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. Can. J. Microbiol. **44:**1148–1153.
10. **Greub, G., and D. Raoult.** 2002. "*Actinobaculum massiliae*," a new species causing chronic urinary tract infection. J. Clin. Microbiol. **40:**3938–3941.
11. **Hall, V., et al.** 2003. *Actinobaculum urinale* sp. nov., from human urine. Int. J. Syst. Evol. Microbiol. **53:**679–682.
12. **Haller, P., et al.** 2007. Vertebral osteomyelitis caused by *Actinobaculum schaalii*: a difficult-to-diagnose and potentially invasive uropathogen. Eur. J. Clin. Microbiol. Infect. Dis. **26:**667–670.
13. **Hoenigl, M., et al.** 2010. Endocarditis caused by *Actinobaculum schaalii*, Austria. Emerg. Infect. Dis. **16:**1171–1173.
14. **Lawson, P. A., E. Falsen, E. Akervall, P. Vandamme, and M. D. Collins.** 1997. Characterization of some *Actinomyces*-like isolates from human clinical specimens: reclassification of *Actinomyces suis* (Soltys and Spratling) as *Actinobaculum suis* comb. nov. and description of *Actinobaculum schaalii* sp. nov. Int. J. Syst. Bacteriol. **47:**899–903.
15. **Moretti, C., et al.** *Erwinia oleae* sp. nov., isolated from olive knots caused by *Pseudomonas savastanoi* pv. Savastanoi. Int. J. Syst. Evol. Microbiol., in press. doi:10.1099/ijs.0.026336-0.
16. **Niemann, S., A. Puehler, H.-V. Tichy, R. Simon, and W. Selbitschka.** 1997. Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. J. Appl. Microbiol. **82:**477–484.
17. **Reinhard, M., et al.** 2005. Ten cases of *Actinobaculum schaalii* infection: clinical relevance, bacterial identification, and antibiotic susceptibility. J. Clin. Microbiol. **53:**5305–5308.
18. **Sorensen, M. D., et al.** 2009. Fournier's gangrene: population based epidemiology and outcomes. J. Urol. **181:**2120–2126.
19. **Sturm, P. D. J., J. Van Eijk, S. Veltman, E. Meuleman, and T. Schülin.** 2006. Urosepsis with *Actinobaculum schaalii* and *Aerococcus urinae*. J. Clin. Microbiol. **44:**652–654.
20. **Waghorn, D. J.** 2004. Actinobaculum massiliae: a new cause of superficial skin infection. J. Infect. **48:**276–277.
21. **Wayne, L. G., et al.** 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. **37:**463–464.
22. **Woldemeskel, M., W. Drommer, and M. Wendt.** 2002. Microscopic and ultrastructural lesions of the ureter and renal pelvis in sows with regard to Actinobaculum suis infection. J. Vet. Med. Ser. A **49:**348–352.