Case Report

A case of diabetic foot ulcers complicated by severe infection and sepsis with *Trueperella bernardiae*

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Introduction: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows us to identify bacteria directly from positive blood cultures with detailed taxonomy naming of the bacterial strains. Knowing the name of the infective bacteria makes it possible to adjust the antibiotic therapy early on. However, it also gives rise to detection of a number of unusual pathogens, which raises new questions on the pathogenicity and medical importance of a number of rarely isolated bacteria such as *Trueperella bernardiae*.

Case presentation: We describe a case of diabetic foot ulcers in a 45-year-old male, which led to below-the-knee amputation due to widespread infection and sepsis with *T. bernardiae* and co-infection with *Peptoniphilus lacrimalis*. The patient was treated initially with surgical debridement in combination with piperacillin/tazobactam and thereafter with amoxicillin for a total period of 1 month.

Conclusion: Based on this case, as well as previous case reports in the literature, we conclude that *T. bernardiae* is a rarely isolated low-pathogenic bacterium in patients with severe co-morbidities. Often co-infection with other low-pathogenic bacteria is observed. *T. bernardiae* is susceptible to and readily treated with β -lactam antibiotics.

Keywords: diabetic foot ulcers, necrotic gangrene, otitis, soft tissue infection, sepsis, *Trueperella bernardiae*, *Peptoniphilus lacrimalis*, piperacillin/tazobactam, amoxicillin.

Received 31 October 2014

Accepted 16 December 2014

Introduction

Since the original description of *Trueperella bernardiae* in 1987 as a coryneform group 2 bacterium by the Centers for Disease Control and Prevention (Na'was *et al.*, 1987), a total of 15 human pathogen strains have been reported in the literature. In 1995; Sanger sequencing of the *rrs* gene from seven clinical isolated strains, including one isolate from the coryneform group 2 bacteria, led to the identification of a distinct species named *Actinomyces bernardiae* (Funke *et al.*, 1995). *Actinomyces bernardiae* was later reclassified as *Arcanobacterium bernardiae* (Ramos *et al.*, 1997) and most recently as *T. bernardiae* (Yassin *et al.*, 2011). In 2012; the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) main spectrum for *T. bernardiae* was identified

and added to the MALDI-TOF MS database (Hijazin *et al.*, 2012). *T. bernardiae* is a non-motile, facultatively anaerobic, Gram-positive, short rod.

T. bernardiae has been described as a pathogen in urinary tract infections (Ieven *et al.*, 1996; Lepargnear *et al.*, 1998), joint infections (Adderson *et al.*, 1998; Berner *et al.*, 2009; Lo'ez *et al.*, 2009), skin and soft-tissue infections (Weitzel *et al.*, 2011; Otto *et al.*, 2013), necrotizing fasciitis (Clarke *et al.*, 2010) and a case of eye infection (Funke *et al.*, 1995). Six strains of *T. bernardiae* have been cultured from blood specimens, but no deaths due to *T. bernardiae* have been reported.

Here, we report a case of diabetic foot infection and sepsis with *T. bernardiae*, which led to crus amputation due to spreading of the infection from the necrotic toes up to the talocrural joint and otitis.

Case report

A 45-year-old male was admitted to the Department of Orthopaedic Surgery at Bispebjerg University Hospital, Copenhagen, Denmark, in July 2014 due to infected diabetic

Abbreviations: MALDI-TOF MS - matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *T. bernardiae* - *Trueperella bernardiae*, *P. lacrimalis* - *Peptoniphilus lacrimalis*.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are KM624631 (*Trueperella bernardiae*) and KM624632 (*Peptoniphilus lacrimalis*)

foot ulcers. The patient's medical history included obesity and diabetes mellitus type II with associated atherosclerosis, neuropathy and diabetic foot ulcers. Partial amputation of two toes on the right foot had been performed previously. Furthermore, the patient suffered from depression. Before admission, the patient had not been in contact with the healthcare system for several months. He had refrained from taking his antidepressants and left the bandages, covering the diabetic foot ulcers, unchanged.

Upon hospitalization, clinical examination of the patient revealed a characteristic neglected diabetic foot with gangrene on his left side (Fig. 1), while his right foot had several pressure ulcers with necrosis. There were no visual signs of ongoing bacterial infections or necrotizing fasciitis. Removal of necrotic tissue by partial amputation of the toes on the left foot and ulcer revision of the right foot were indicated and therefore planned, but were not considered acute. No antibiotic treatment was initiated at this point.

Two days after hospitalization, and the day before the scheduled amputation surgery, the patient developed fever (temperature 38.2 °C) and tachycardia (heart rate 136). Laboratory samples revealed leucocytosis (white blood cell count $12.5 \times 10^9 \text{ l}^{-1}$ with 74 % neutrophils, normal range $3.5\text{--}8.8 \times 10^9 \text{ l}^{-1}$) and a C-reactive protein level of 261 mg l^{-1} (normal range $<10 \text{ mg l}^{-1}$). As the patient was showing clinical signs of sepsis, blood cultures were performed and intravenously administered piperacillin/tazobactam 4 g every 8 h, ciprofloxacin 400 mg every 12 h and gentamicin 240 mg once daily were started.

The planned surgical debridement was effected with amputation of the gangrenous toes on the left foot. The metatarsal bones showed signs of osteitis and a foul-smelling liquid was elicited. The infection was found to be widespread and expanded into the talocrural joint. Below-the-knee amputation was performed. During surgery, a pus specimen was taken for microbiological analysis. Following surgery, the patient's

Fig. 1. Picture of left foot three days prior to amputation.

condition improved, and laboratory data showed decreasing levels of both white blood cell count and C-reactive protein.

After 48 h of incubation on 5 % blood agar plates in ambient air, the pus specimen yielded growth of *Staphylococcus simulans* (which was considered a contaminant) and *T. bernardiae*. Both were identified by MALDI-TOF MS. Blood cultures (growth in one of two anaerobic bottles and no growth in the two corresponding aerobic bottles at 35 °C in the BacT/ALERT 3D system; BioMerieux) were also positive after 48 h, and the Gram-stained slides showed small Gram-positive coccoid bacilli. After another 48 h of incubation on 5 % blood agar plates in 5 % CO₂, growth of two different types of unpigmented, pinpoint colonies was seen, which were identified as *T. bernardiae* and *Peptostreptococcus* sp. by MALDI-TOF MS. The *Peptostreptococcus* sp. was later identified as *Peptoniphilus lacrimalis* by 16S rRNA gene PCR and Sanger sequencing.

We found that *T. bernardiae* grew on 5 % blood agar incubated aerobically in 5 % CO₂ and anaerobically, as well as on chocolate agar (defibrinated lysed horse blood) with or without vitamin K and cysteine (all media produced by Statens Serums Institut, SSI Diagnostica). However, the growth of *T. bernardiae* was improved anaerobically. Furthermore, identification of both *T. bernardiae* and *P. lacrimalis* isolated from the blood culture was confirmed by Sanger sequencing of a PCR product targeting the *rrs* gene. Bacterial nucleic acids were isolated on a MagNA Pure 96 system (Roche Diagnostics). PCR was done utilizing AmpliTaq Gold DNA polymerase in PCR Buffer II with 1.5 mM MgCl₂ and 0.2 mM dNTPs (Applied Biosystems) with 200 nM each primer (BSF6/22m: 5'-GRAGAGTTTGATCMTGGCTCAG-3', and BSR1541/20m: 5'-AAGGAGGTGATCCARCCGCA-3') in a skirted Twin-Tec PCR Plate 96 (Eppendorf) and sealed with MicroAmp optical adhesive film (Applied Biosystems). PCR cycling was done on an Eppendorf Mastercycler and the conditions were: Hot-Start polymerase activation for 15 min at 95 °C, 40 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C in the initial five cycles followed by 58 °C in the remaining 35 cycles for 30 s, and elongation at 72 °C for 60 s, with a final elongation at 72 °C for 5 min. PCR product (5 µl) was mixed with 5 µl of each PCR primer or one of four other sequencing primers (10 µM BSF339/19m: 5'-CTCCTACGGAGGCAGCAG-3', BSF917/19m: 5'-GAATTGACGGGGRCCCGCA-3', BSR534/18m: 5'-ATTACCGCGGCTGCTGGC-3' or BSR1114/18m: 5'-GGGTTGCGCTCGTTTCGG-3') (Holler *et al.*, 2011). The PCR product and primer mixes were sent for purification and Sanger sequencing by MacroGen Europe. Raw sequences were aligned in the Geneious Pro software and identified using BLAST.

The antimicrobial susceptibility of the isolates was determined by disk diffusion testing according to EUCAST guidelines (<http://www.eucast.org/>). *T. bernardiae* was found to be susceptible to penicillin (34 mm), piperacillin/tazobactam (34 mm), linezolid (29 mm), moxifloxacin

(31 mm) and rifampicin (34 mm). The MIC of penicillin was 0.25 mg l^{-1} , determined by E-test (bioMérieux). Like *T. bernardiae*, *P. lacrimalis* was susceptible to penicillin. Based on the antimicrobial susceptibility tests, the antimicrobial therapy was changed to orally administered amoxicillin 1 g every 6 h for another 14 days, prolonging the antimicrobial treatment to a total of 1 month.

When the patient was discharged from the hospital after 16 days, the laboratory data showed a normal white blood cell count of $7.2 \times 10^9 \text{ l}^{-1}$ and a near-normal C-reactive protein of 12 mg l^{-1} . The left leg stump showed no signs of infection or necrosis, and rehabilitation of the patient was arranged. The patient was followed in the outpatient clinic and no signs of reoccurrence have been observed for 3 months.

Discussion

The present case describes a presumably slowly progressing *T. bernardiae* infection in the left foot of a patient with advanced diabetes mellitus. Although necrotic gangrene, otitis and extensive soft-tissue infection may have persisted for a long period of time and finally were complicated by sepsis, the patient did not develop necrotizing fasciitis and was rapidly cured by surgical debridement and a relatively short period of antibiotic treatment.

This is in concordance with previous case reports on *T. bernardiae* infections. In general, patients have severe comorbidities, co-infections with other bacteria are often observed and the infections progress slowly. In the most severe case reported to date, *T. bernardiae* together with *Morganella morganii* were isolated from an abdominal necrotizing fasciitis in the lower left abdominal quadrant in a patient with several co-morbidities including advanced diabetic mellitus type II, morbid obesity and chronic obstructive pulmonary disease (Clarke *et al.*, 2010). After several surgical debridements down to the necrotic fascia, the patient was left with a $30 \times 45 \text{ cm}$ defect, which resolved. In another case report, a left hip prosthesis inserted 27 years earlier had loosened due to *T. bernardiae* infection. The bacterium was isolated from biopsies taken during surgical revision (Loïez *et al.*, 2009).

Including this case report on *T. bernardiae*, a total of nine reports can be found in the literature. In all cases, cultures of *T. bernardiae* from the original sample materials took 2–5 days, and the bacteria were readily cultured on blood or chocolate agar incubated aerobically in 5 % CO_2 or anaerobically at 35–37 °C. All isolates tested were susceptible to cephalosporins, carbapenems, vancomycin, rifampicin and clindamycin. One isolate was considered to be resistant to penicillin G with a MIC of 0.5 mg l^{-1} but was susceptible to all other penicillins (Loïez *et al.*, 2009), whereas all other tested isolates were considered susceptible to all penicillins.

This case adds additional data to our understanding of *T. bernardiae* and demonstrates together with the previous

case reports in the literature that the pathogenicity of this rare opportunistic pathogen is low, and that *T. bernardiae* is usually encountered in co-infections in patients with severe co-morbidity.

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