Case report

Detection of *Robinsoniella peoriensis* in multiple bone samples of a trauma patient

Percy Schröttert a, *, Kathleen Hartwich b, c, Boyke Bunk d, Isabel Schober d, Sina Helbig e, Wolfram W. Rudolph f, Florian Gunzen a

a Institut für Medizinische Mikrobiologie und Hygiene, Medizinische Fakultät Carl Gustav Carus, TU Dresden, Fetscherstr. 74, 01307, Dresden, Germany

b Elblandzentrums für Orthopädie und Unfallchirurgie, Universitätsklinikum Dresden Carl Gustav Carus, Fetscherstr. 74, 01307, Dresden, Germany

c Zentralbereich Klinische Infektiologie, Universitätsklinikum Dresden Carl Gustav Carus, Fetscherstr. 74, 01307, Dresden, Germany

d Leibniz-Institut DSMZ-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7 B, 38124, Braunschweig, Germany

e Institut für Medizinische Mikrobiologie und Hygiene, Medizinische Fakultät Carl Gustav Carus, TU Dresden, Fetscherstr. 74, 01307, Dresden, Germany

* Corresponding author. Institute of Medical Microbiology and Hygiene, Faculty of Medicine, TU Dresden, Fetscherstr. 74, 01307, Dresden, Germany.

E-mail address: percy.schroettner@tu-dresden.de (P. Schröttert).

1. Introduction

The bacterium *R. peoriensis* was first described by Cotta et al. (2009) [1]. *R. peoriensis* is a Gram-positive bacterium with short rods and the ability to form spores. *R. peoriensis* grows only under anaerobic conditions [1]. Type strains were isolated from samples from swine manure storage pits [1]. *R. peoriensis* has also been identified from faecal material of other animals (e.g. wood turtles and porpoises) and humans [2–4]. Therefore, it is likely that *R. peoriensis* is part of the regular intestinal flora of both animals and humans. Consequently, it may also be found in environmental samples, such as soil [1,5].

To date, 12 cases have been published in which *R. peoriensis* was identified as the origin of infection [1,5–12]. In most of these cases, the patients had undergone previous surgery and had conditions that were associated with immunosuppression (e.g. diabetes or cancer) [1,5–12].

We describe the case of a 58-year-old male trauma patient with an open medial fracture of the right ankle joint. Our case is the 5th case of *R. peoriensis* infection which is associated with a preceding trauma.

1.1. Case report

In April 2016, a 58-year-old male fell from a ladder sustaining an open medial luxation type III B fracture of the right ankle joint according to the classification proposed by Gustilo and Anderson in 1976 [13]. The fracture was accompanied by complete tibiolateral luxation. A contamination of the open wound with soil was likely. The patient was taken to a hospital in Saxony, which was close to his home. The first surgical stabilization included debridement, placement of an external fixator and transfusion of the medial malleolus using a Kirschner wire. Subsequently, multiple revision operations were required in the same hospital and eventually a
sural flap was performed. Details about microbiologic testing and antibiotic treatment during that stay were not provided. Although there was evidence of bone healing, load stability was not achieved.

The patient presented himself to the Department of Orthopaedics and Trauma Surgery of the University Hospital Dresden (Saxony, Germany) for the first time in November 2016. He had sought a consultation due to an emerging fistulous puncture wound at the inside of the right ankle joint. On admission, the patient reported load-dependent pain. At that time, the patient had been taking amoxicillin-clavulanic acid [1.000 mg three times a day (t.i.d)] orally for several weeks; the antibiotic was discontinued at the time of consultation. Computer tomography revealed progressing osteonecrosis, accompanied by osteolysis of both the talus and distal tibia and failure of the osteosynthesis. Thus, an explantation of all material and a radical debridement of the necrotic parts were carried out after an antibiotic-free interval of four weeks. It should be noted that the fistula had already reached the distal tibia with notable destruction. Bone samples were sent to the Department of Pathology of the University Hospital Dresden for a histopathological examination and to the Institute of Medical Microbiology and Hygiene of the TU Dresden for microbiological diagnostics. The pathological examination showed extensive mediulary fibrosis and signs of florid osteomyelitis, including purulent inflammation. R. peoriensis was detected in 11 samples of bone tissue taken from the distal tibia, the ankle joint and talus (Table 1). No antibiotic treatments were started at that time, due to uncertainty about the pathogenicity of the discovered bacteria. One week later, another debridement with a cement spacer implantation took place. One bone tissue culture again grew R. peoriensis and Staphylococcus epidermidis (Table 1). Thereafter, levofloxacin [750 mg one time a day (q.d.)] was started as an empirical antibiotic therapy by the primary service. The patient returned to the hospital for additional debridement of the distal tibia and a change of spacer 5 weeks later in January 2017 while continuing to take levofloxacin.

Microbiological examinations derived from samples of the talus and the distal part of the tibia again revealed R. peoriensis and S. epidermidis (Table 1). The patient was hospitalized again for revision surgery in April 2017 due to ongoing signs of infection. At that time levofloxacin was discontinued and antibiotic treatment was started with daptomycin (6 mg/kg q.d.) and piperacillin-tazobactam (4.5 g t.i.d.) according to the previously isolated pathogens. On day 7 of the new antibiotic regime, joint fusion surgery of the right ankle joint was performed. Intraoperative biopsies of bone taken from the talus and distal tibia once again showed growth of R. peoriensis (Table 1). On the thirteenth day of treatment, piperacillin-tazobactam was deescalated to ampicillin-sulbactam 3 g t.i.d. Five days later the Infectious Diseases consulting service was called. A change to oral treatment with metronidazole (400 mg t.i.d.) was decided to administer for at least 4 more weeks was recommended since a osteomyelitis caused by R. peoriensis was suggested. The patient eventually received 6 weeks of metronidazole and presented for follow-up in September 2017. At this time point, the foot could be fully loaded. A radiological examination revealed complete bony consolidation of the arthrosis. An overview of the samples analyzed at the Institute for Medical Microbiology and Hygiene is provided in Table 1. An overview of the antibiotics administered is given in Table 2.

1.2. Laboratory findings

Both smears and samples from tissues were collected (Table 1). Swab swabs containing Amies Medium (Sarstedt, Nümbrecht, Germany) were used. Swabs were plated on Columbia Blood Agar containing 5% sheep blood (Oxoid Deutschland, Wesel, Germany), Bile Chrysoideine Glycerol Agar (Sifin Diagnostics, Berlin, Germany), Yeast Cysteine Blood Agar for detection of anaerobic bacteria (Oxoid Deutschland, Wesel, Germany), Sabouraud Glucose Agar (BD, Heidelberg, Germany), liquid Schaedler Broth, bioMérieux, Nürtingen, Germany) and Brain- Heart Infusion (BD, Heidelberg, Germany) [14]. Columbia Blood Agar, Bile Chrysoideine Glycerol Agar, Sabouraud Glucose Agar plates and Brain- Heart Infusion were incubated at 37 °C under aerobic conditions for 24 h and (in case no growth was detected) for another 24 h. The liquid Schaedler Broth was incubated at 37 °C and 5% CO2 under aerobic conditions for 7 d. Yeast Cysteine Blood Agar was used to detect anaerobic bacteria. These plates were transferred to anaerobic jars. AnoGen generating sachets (Thermo Scientific, Dreieich, Germany) were used to create anaerobic conditions. The plates were incubated at 37 °C as well. Additionally, Gram-staining was carried out revealing Gram-positive rod-shaped strains.

Tissue samples were collected in sterile sample tubes (Greiner Bio One, Frickenhausen, Germany). The samples were directly transferred to liquid Schaedler Broth and incubated at 37 °C for 7 d. On day 7, the cultures showed turbidity indicating microbial growth. Subsequently, 50 μl of the broth dilution were plated on Columbia Blood Agar containing 5% sheep blood, and another 50 μl were plated on Bile Chrysoideine Glycerol Agar. Both plates were incubated under aerobic conditions at 37 °C. In addition, another 50 μl were plated on Yeast Cysteine Blood Agar to detect anaerobic bacteria.

With one exception (sample #8, Table 1), the aerobic agar plates were negative 24 and 48 h later. However, under anaerobic growth conditions, growth was detected after 48 h of incubation. Small grey to white colonies were detected with variable sizes, showing no haemolysis. Gram-staining showed Gram-positive rods. Subsequently, the bacteria were identified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) using whole cell analysis without applying formic acid. The current MALDI biotype database (# 1829023 MBT Compass Library, rev E, Version 8) was applied which contains 7854 reference spectra and covers 2748 species. MALDI biotype software flexContol Version 3.4 was used. The strain was analyzed three times showing the following results: 1) Clostridium thermopalmarium (score: 1.242), 2) Bacteroides slyersiae (score: 1.291) and 3) R. peoriensis (score: 1.521). Although the score for R. peoriensis was clearly the highest of the three results, a MALDI-TOF MS score below 1.7 is not reliable and therefore these results could not be regarded as secure identification [15].

Thus, sequencing of the 16S RNA gene was performed using TPU1 5'-AGA GTT TGA TCM TGG CTC AG - 3' as the forward primer and RTU4 (5'-TAC CAG GGT ATC TAA TCC TGT T-3') as the reverse primer [15]. 16S RNA sequencing revealed 99% identity to the 16S RNA gene of R. peoriensis PPC44 deposited in GenBank (accession number: AF445283.2).

Antimicrobial susceptibility testing was performed using the gradient-diffusion method [16]. In brief, a McFarland standard of 0.5 was created using NaCl and a DensiChek Densitometer (bioMérieux, Nürtingen, Germany). The bacteria were plated on Bru cella Agar (BD, Heidelberg, Germany) using a cotton swab. Test strips to determine the minimal inhibitory concentration (MIC) were purchased from Liofilchem (obtained from bestbion, Köln, Germany). Strips for each antibiotic were then placed on agar plates. The following antibiotics were tested: penicillin G, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, ertapenem, imipenem, meropenem, clindamycin, vancomycin, metronidazole, chloramphenicol, levofloxacin and daptomycin. All agar plates were incubated at anaerobic conditions as described above. Clinical categorization was assessed from MIC results according to EUCAST clinical breakpoints v 8.1 (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/8.1_Breakpoint_Tables).
described, but no results were obtained regardless of threshold resistance genes was performed using ResFinder 2.1 as recently tentatively responsible for resistance mechanisms were, therefore, carried out on genes pbpA_1, pbpA_3, pbpE, and pbpX. Continuing further genome investigations on automated annotation applying Prokka 1.8 [18]. Screening for molecular basis for resistance against penicillin G and levo

1.3. Genomic basis of antibiotic susceptibility

Whole genome sequencing was conducted to reveal the molecular basis for resistance against penicillin G and levofloxacin. Sequencing of a Nextera XT DNA Library (Nextera XT DNA Library Prep Kit; Illumina, San Diego, CA, USA) was performed on an Illumina NextSeq 550 instrument (Illumina, San Diego, CA, USA) followed by genome assembly using Velvet 1.2.10 [17] and an automated annotation applying Prokka 1.8 [18]. Screening for resistance genes was performed using ResFinder 2.1 as recently described [19], but no results were obtained regardless of threshold values chosen. Continuing further genome investigations on resistance mechanisms were, therefore, carried out on genes putatively responsible for \( \beta \)-lactam resistance. As a result the following resistance genes were detected, probably explaining resistance to penicillin: \( \text{pbpA}_1, \text{pbpA}_2, \text{mrcA}, \text{pbpX}, \text{pbpA}_3, \text{pbpE}, \text{pbp} \) and \( \text{blaR1} \). Fluoroquinolone resistance, i.e. to levofloxacin, usually determined by a point mutation within the \( \text{gyrA} \) gene could not be resolved as no sequence from a susceptible isolate could be retrieved from the non-redundant nucleotide database. The genome including annotation was deposited in NCBI GenBank under accession number QGQD00000000.

1.4. Literature search

A literature search was conducted in PubMed (https://www.ncbi.nlm.nih.gov/pubmed). The terms “Robinsoniella peoriensis” (21 results) and “Robinsoniella peoriensis infection human” (8 results) were used. The available literature was evaluated regarding the content of clinical and laboratory information. Only English literature titles were considered. In addition, the species description was included [1].

2. Discussion

In total, twelve cases dealing with infections caused by \( R. \text{peoriensis} \) were identified. Seven of these twelve reported cases regarding infections associated with \( R. \text{peoriensis} \) were surgery-related. In four of these seven cases a surgery became necessary due to a preceding trauma [5,6,8,9,11]. Of these four cases, one patient suffered from complications after a total hip replacement due to a femoral neck fracture [9]. Another patient had open pelvic and femur fractures and in this case contamination of the open wound with soil was probable [5]. Of these four cases, one patient described in a case study by Gomez et al. was admitted to the hospital with femoral and tibial fractures after a motor vehicle accident [5]. The fourth patient underwent surgery due to a fracture of the right femur [6]. This illustrates, that only a limited number of case reports dealing with \( R. \text{peoriensis} \) as the cause of an infection have
This close relationship between *R. peoriensis* belongs to the clostridium rRNA XIVa subcluster [4].

Comparison of identification strategies for *R. peoriensis* (reported cases and present report).

<table>
<thead>
<tr>
<th>Number</th>
<th>Age</th>
<th>Gender</th>
<th>Specimen</th>
<th>16S rRNA gene sequencing (sequence similarity to <em>R. peoriensis</em> in %)</th>
<th>Biochemical identification</th>
<th>MALDI-TOF MS</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>79</td>
<td>female</td>
<td>deep wound on the heel</td>
<td>–</td>
<td>–</td>
<td>not performed</td>
<td>[1]</td>
</tr>
<tr>
<td>#2</td>
<td>42</td>
<td>male</td>
<td>blood culture (peripheral vein)</td>
<td>99%</td>
<td>C. clostridioforme (88.4%; API 20 A)</td>
<td>not performed</td>
<td>[12]</td>
</tr>
<tr>
<td>#3</td>
<td>50</td>
<td>male</td>
<td>seven cultures</td>
<td>six out of seven samples positive for <em>R. peoriensis</em> (identity in % not given)</td>
<td>C. clostridioforme (96%; API Rapid ID 32 A)</td>
<td>not performed</td>
<td>[6]</td>
</tr>
<tr>
<td>#4</td>
<td>61</td>
<td>female</td>
<td>bone tissue (femur)</td>
<td>99.7%</td>
<td>–</td>
<td>not performed</td>
<td>[5]</td>
</tr>
<tr>
<td>#5</td>
<td>68</td>
<td>female</td>
<td>wound debriment (surgical tissue)</td>
<td>99.7%</td>
<td>–</td>
<td>not performed</td>
<td>[5]</td>
</tr>
<tr>
<td>#6</td>
<td>45</td>
<td>male</td>
<td>wound drainage</td>
<td>99.4%</td>
<td>C. tertium (99.9%; RapID ANA II)</td>
<td>not performed</td>
<td>[5]</td>
</tr>
<tr>
<td>#7</td>
<td>79</td>
<td>female</td>
<td>blood culture</td>
<td>99.4%</td>
<td>–</td>
<td>not performed</td>
<td>[5]</td>
</tr>
<tr>
<td>#8</td>
<td>76</td>
<td>male</td>
<td>blood culture</td>
<td>99.7% and 99.58%</td>
<td>C. beijerinckii/butyricum (91.6%; API 20 A)</td>
<td>not performed</td>
<td>[10]</td>
</tr>
<tr>
<td>#9</td>
<td>45</td>
<td>female</td>
<td>wound fluid from deep areas/ samples from bone graft components of the implant/ tissue</td>
<td>99.3%</td>
<td>–</td>
<td>not performed</td>
<td>[11]</td>
</tr>
<tr>
<td>#10</td>
<td>74</td>
<td>female</td>
<td>–</td>
<td>99.5%</td>
<td>–</td>
<td>not performed</td>
<td>[9]</td>
</tr>
<tr>
<td>#11</td>
<td>70</td>
<td>male</td>
<td>tissue samples (clot and excised homograft)</td>
<td>100%</td>
<td>–</td>
<td>not performed</td>
<td>[8]</td>
</tr>
<tr>
<td>#12</td>
<td>63</td>
<td>male</td>
<td>blood culture</td>
<td>99.17%</td>
<td>C. clostridioforme (92%; Vitek 2)</td>
<td>not performed</td>
<td>[7]</td>
</tr>
<tr>
<td>#13</td>
<td>54</td>
<td>male</td>
<td>tissue samples (bone and subcutaneous)</td>
<td>99%</td>
<td>–</td>
<td>not performed</td>
<td>present report</td>
</tr>
</tbody>
</table>
together with a beta-lactamase gene (EC 3.5.2.6), both giving hints to the strains genetic potential for antimicrobial resistance. However, functional analysis of the identified potential resistance mechanisms has not been performed yet. The Nitrocefin test was positive, which indicates the activity of a beta-lactamase. However, further studies are needed to explain if the resistance against penicillin G is due to this enzyme alone or if penicillin-binding proteins may play an additional role.

3. Conclusion

We demonstrate the case of a 58-year-old trauma patient with prolonged infection of bone tissue caused by *R. peoriensis*. In the course of his treatment, multiple surgical revisions became necessary. We assume, that *R. peoriensis* was acquired directly when the accident took place, but initially was not recognized as the causative agent for impaired wound healing. The reason may be that is not a common bacterial pathogen routinely dealt with in diagnostic laboratories. The prolonged infection caused by *R. peoriensis* may be explained by two facts: First, a secure identification of *R. peoriensis* is currently only possible applying molecular techniques such as sequencing of the 16S rRNA gene. Not all laboratories and hospitals, however, have access to molecular analyses. Second, *R. peoriensis* was not recognized as the potential cause of infection. The species was just recently described and because of that only limited data are available underlining its role as a pathogen. The failure to initially recognize the pathogen as causative for the infection resulted in insufficient antimicrobial treatment, which likely resulted in the prolonged infection.

All MIC values were interpreted according to the EUCAST guidelines (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1. http://www.eucast.org). The breakpoints for Gram-positive anaerobes were applied for penicillin G, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, ertapenem, imipenem, meropenem, clindamycin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-

References


