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First pediatric case of osteomyelitis caused by *Robinsoniella peoriensis*

NEŞE İNAL^{1*} , ALPER KARAGÖZ², EGEMEN TURHAN³ and
GÜLŞEN HAZIROLAN¹

¹ Department of Medical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

² Department of Molecular Biology and Genetics, USA University, Turkey

³ Department of Orthopedics and Traumatology, Hacettepe University Faculty of Medicine, Ankara, Turkey

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ORIGINAL ARTICLE



ABSTRACT

Robinsoniella peoriensis is a gram-positive, spore-forming, anaerobic rod. In our study, we isolated *R. peoriensis* from an open fracture of the left distal tibia of a three-year-old male patient. Tissue anaerobic culture was positive for *R. peoriensis*. It was identified with both matrix-assisted laser desorption ionization time-of-flight mass spectrometry and confirmed via 16S rRNA gene sequencing. The patient responded to ampicillin-sulbactam and amikacin antibiotic therapy. Antimicrobial susceptibility testing should be performed to guide the choice of treatment. To the best of our knowledge, this is the first report of *R. peoriensis* osteomyelitis in a pediatric patient and first report from Turkey.

KEYWORDS

Robinsoniella peoriensis, anaerobe infections, MALDI-TOF-MS

INTRODUCTION

Robinsoniella peoriensis is a gram-positive, spore-forming, anaerobic rod originally isolated from swine manure storage pits. The bacterium belongs to phylum Firmicutes within the family Lachnospiraceae. Phylogenetic analysis showed that this organism belonged to the clostridial ribosomal ribonucleic acid rRNA cluster XIVa subgroup, with the closest related genus being *Ruminococcus*. The organism was named *R. peoriensis* [1]. *R. peoriensis* strains have been initially isolated from environmental sources, however, this pathogen has been also isolated from clinical samples, namely, human blood, abdominal fluid, wound, muscle hematoma puncture, necrotic tissue and has been shown to cause serious infections such as bacteremia, soft tissue infections, prosthetic joint infections and subperiosteal abscesses [1–9].

We describe the case of a three-year-old male trauma patient with an open fracture of the left distal tibia. To our knowledge, this is the first case of osteomyelitis caused by *R. peoriensis* in a pediatric patient.

CASE REPORT

A three-year-old male patient was admitted to the Hacettepe University Faculty of Medicine Pediatric Emergency Department with the diagnosis of tibia open fracture. The patient had purulent discharge at the wound site and he was admitted to the Orthopedics Service with the plan of debridement, open reduction and internal fixation. His initial vital signs on admission were normal and initial laboratory test results were as follows: Hb, 10.9 gr/dl; white blood cell

*Corresponding author.

Tel.: +90 0312 305 1560;

fax: +90 0312 310 0580.

E-mail: nese-inal-108@hotmail.com

count, $10.3 \times 10^3/\mu\text{L}$ (neutrophils, 82.6 %); and platelets, $16 \times 10^3/\mu\text{L}$. The serum C-reactive protein (CRP) level was elevated to 1.11 mg/dL, erythrocyte sedimentation rate was 22. There was no underlying disease. Magnetic resonance imaging revealed osteomyelitis-compatible signal changes at the fractured ends of the left distal tibia (Fig. 1). An intra-medullary abscess (1 cm), paratenon inflammation, periosteal reaction at fracture line level and subperiosteal contrast, granulation tissue was observed in the distal left tibia.

The wound was debrided and tissue anaerobic culture was sent to Hacettepe University Faculty of Medicine Clinical Bacteriology Laboratory. Tissue anaerobic culture was inoculated on Schaedler agar supplemented with vitamin K and hemin and fluid thioglycollate medium. AneroGen gas-generating sachets (Thermo Scientific, Dreieich, Germany) were used to create anaerobic conditions. A chocolate blood agar plate was streaked and incubated in 5% CO_2 at 37 °C to assess aerotolerance. Small, smooth, nonhemolytic colonies were seen after 48 h of incubation under anaerobic conditions on Schaedler agar (Fig. 2). Gram staining from the purified colony was revealed gram-positive spore-forming bacilli (Fig. 3). Catalase test was found to be negative.

The isolate was identified as *R. peoriensis* that yielded a higher identification score value of 2.23 with matrix-assisted laser desorption ionization time-of-flight mass spectrometry [MALDI-TOF MS (Bruker, Germany)]. The strain was also confirmed with 16S rRNA sequence analysis and the molecular 16S rRNA gene sequence was found to be a similarity >99% compared to the reference sequence of the type strain *R. peoriensis*.

Genomic DNA was isolated using the UltraClean Microbial DNA Isolation Kit (Mo Bio, Solana Beach, CA) according to manufacturer's instructions. The 16S rRNA sequence for the type strain of *R. peoriensis*, PPC31T, was used for determining PCR primers (GenBank accession number AF445285). One primer set (RP16S1 shown in Table 1) was initially tested against genomic DNA from various strains. Each PCR reaction contained 2.5U AmpliTaq 360 DNA Polymerase (Life Technologies, Grand Island, NY), 1X 360 Buffer, 1 μL genomic DNA and primers in 50 μL final volume and annealing temperature of 64 °C and a MgCl_2 concentration of 1 mM was used. Final conditions for the PCR assay were: 94 °C for 5 min, followed by 25 cycles of 94 °C for 45 s, 63 °C for 30 s, 72 °C for 2 min, and a final

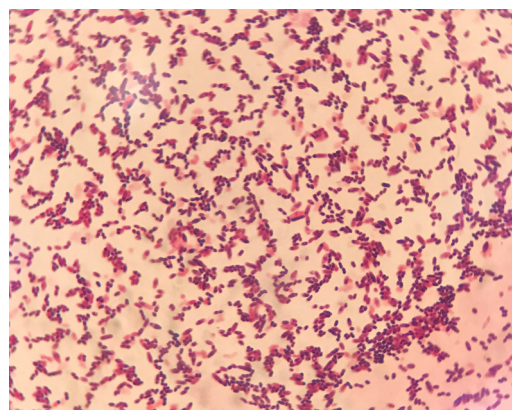


Figure 2. Gram stain of *R. peoriensis* (x100)

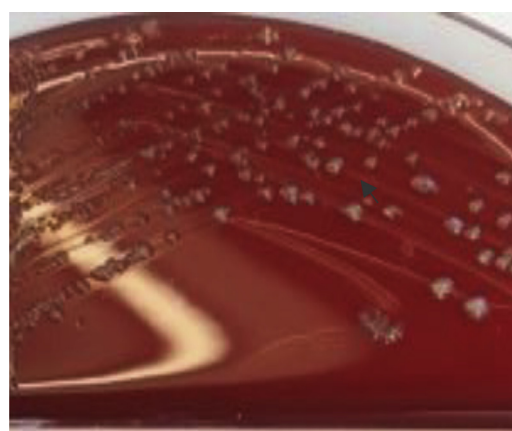


Figure 3. Colony morphology of *R. peoriensis* on Schaedler agar

extension step of 72 °C for 10 min. An aliquot of each reaction was analyzed using agarose-gel electrophoresis to identify the PCR product [2].

According to the result of wound culture, the patient's antibiotic therapy was administered as ampicillin-sulbactam 200 mg/kg daily and amikacin 15 mg/kg/dose. Negative pressure wound therapy was used to allow open wound secondary healing. After the treatment, the patient was discharged due to the lack of microbial growth on control of

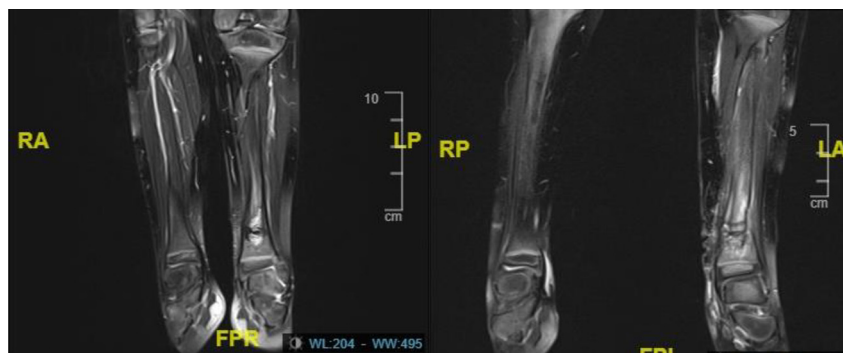


Figure 1. MR imaging is showed osteomyelitis-compatible signal changes in the left distal tibia

Table 1. Sequences of PCR primers used in this study

Name	Sequence (5'-3')	Position of primer	Size of Product (bp)
RP16S1-FW	GTCTCTCCAGAGTGCCCAAC	385–404	876
RP16S1-RV	AGGTAACGGCTTACCAAGGC	1260–1241	

tissue cultures and his stable vitals and good general condition.

DISCUSSION

Most anaerobic infections originate from the patient's microflora or exogenous environmental source. Immunosuppressed patients with malignancies or undergoing hematopoietic stem cell transplantation are more susceptible to these anaerobic infections, which often arise from the translocation of endogenous flora[3]. In addition to the importance of anaerobic microorganisms in human normal

flora, infections due to anaerobic organisms can occur in both children and adults; these may be the result of traumatic injuries and wounds. Traditional methods for the identification of clinically relevant anaerobes are slow, time-consuming, and expensive. Anaerobic bacteria cannot be easily identified by conventional phenotypic methods. It has been demonstrated that delayed or inaccurate identification of anaerobic organisms during infection can lead to increased morbidity and mortality in these patients. Thus, rapid and accurate methods for the identification of anaerobic organisms are necessary for a clinical laboratory [4]. MALDI-TOF MS appears to be more accurate and faster than routine biochemical identification methods when 16S rRNA sequencing is used as the gold standard for verifying

Table 2. Case reports of *R. peoriensis* infections

Patient	Age and sex	Sample	Identification	Treatment	References
Case1	79-woman	Deep wound on the heel	–	Not known	1
Case 2	42-man	Blood culture (peripheral vein)	16S rRNA gene sequencing	Metronidazole	5
Case 3	61-woman	Abdominal fluid	16S rRNA gene sequencing	Percutaneous drainage vancomycin, piperacillin-tazobactam	6
Case 4	68-woman	Surgical wound debridement	16S rRNA gene sequencing	Surgical wound debridement broad-spectrum antibiotics	6
Case 5	45-man	Wound drainage	16S rRNA gene sequencing	Wound debridement clindamycin, linezolid	6
Case 6	79-woman	Blood culture	16S rRNA gene sequencing	Piperacillin/tazobactam, levofloxacin and metronidazole	6
Case 7	76-man	Blood culture	16S rRNA gene sequencing	Piperacillin/tazobactam, and levofloxacin was empirically and antibiotics were replaced with vancomycin, meropenem, sulfamethoxazole - trimethoprim	7
Case 8	45-woman	Wound fluid from deep areas/ samples from the bone graft	16S rRNA gene sequencing	Debridement of necrotic tissue and removal of the bone graft clindamycin and rifampicin	8
Case 9	74-woman	Components of the implant/tissue	16S rRNA gene sequencing	Ampicillin-sulbactam, rifampicin	9
Case 10	70-man	Tissue sample	16S rRNA gene sequencing	Ceftriaxone and metronidazole	10
Case 11	63-man	Blood culture	16S rRNA gene sequencing	Piperacillin/tazobactam and imipenem	11
Case 12	67-woman	Tissue samples (bone and subcutaneous)	16S rRNA gene sequencing	Daptomycin/doxycycline	12
Case 13	58-man	Tissue samples (bone and subcutaneous)	16S rRNA gene sequencing	Ertapenem	13
Case 14	3-man	Tissue samples	16S rRNA gene sequencing	Ampicillin-sulbactam and amikacin	Present report

the identity of the organism. In this report, *R. peoriensis* was identified by MALDI-TOF-MS and confirmed by 16S rRNA sequence analysis as in other studies [5].

In literature, 13 cases have been published in which *R. peoriensis* was identified as the cause of infection. The first report of an isolate of *R. peoriensis* was in 2009 from the deep heel wound of a 79-years old woman [1]. *R. peoriensis* was isolated from a patient with pancreatic cancer in 2010 [6]. Gomez et al. reported the isolation of *R. peoriensis* from four human specimens in 2011 [7]. *R. peoriensis* was isolated in a 76 years old man who developed bacteremia in 2012 [8]. *R. peoriensis* was isolated in 45 years female after the posterior instrumentation of idiopathic thoracolumbar scoliosis [9]. Cases of periprosthetic infection and aortic cross homograft myographic pseudoaneurysm, aspiration pneumonia and hardware-associated soft tissue infection caused by *R. peoriensis* have been reported [10–13]. *R. peoriensis* was detected in multiple bone samples from the right talus and tibia in 2019 [14]. In most *R. peoriensis* infections an association with trauma or surgery has been demonstrated. [7, 13, 14]. Summary of *R. peoriensis* cases reported in Table 2.

To our knowledge, *R. peoriensis* has not been reported as a causative agent in a pediatric patient. We report the first case of osteomyelitis due to *R. peoriensis* in a child. An antimicrobial susceptibility test of the isolate could not be performed. Therefore, *R. peoriensis* antibiotic susceptibility profile reviewed from literature. *R. peoriensis* appears to have variable susceptibility patterns. Some cases reported low MICs to ampicillin, piperacillin/tazobactam, ertapenem, metronidazole and vancomycin [7, 9]. Other studies have reported elevated MICs to penicillin, clindamycin, and moxifloxacin [7, 9, 10]. Our patient was successfully treated with intravenous ampicillin-sulbactam and amikacin.

CONCLUSION

R. peoriensis is an emerging human pathogen diagnosed more frequently in recent years due to the utilization of MALDI-TOF MS in the clinical microbiology laboratory.

In most cases, there was no clear epidemiological link to explain where patients acquired this organism, which is typically found in swine manure. The patient had no animal contact. *R. peoriensis* was identified by MALDI-TOF-MS and verified by 16S rRNA sequence analysis. This report is the first case of osteomyelitis caused by *R. peoriensis* in a child. It was faster and correctly identified with MALDI-TOF-MS. The patient responded to ampicillin-sulbactam and amikacin antibiotic therapy. A successful clinical outcome was achieved for the patient. However, more studies are needed to understand this organism's virulence, transmission, and antimicrobial susceptibility pattern.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

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