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Case Report

Molecular diagnosis and enrichment culture identified a septic pseudoarthrosis due to an infection with *Erysipelatoclostridium ramosum*

Fathiah Zakham[a], Trestan Pillonel[a], Anne-Sophie Brunel[b], Pierre-Yves Zambelli[c], Gilbert Greub[a], Antony Croxatto[a], Claire Bertelli[a,*]

[a] Institute of Microbiology, Lausanne University Hospital and Lausanne University, Switzerland
[b] Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and Lausanne University, Switzerland
[c] Unité Pédiatrique de Chirurgie Orthopédique et Traumatologique UPCOT, Hôpital de l'Enfance, Lausanne University Hospital, Switzerland

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ABSTRACT

We describe here a rare case of septic pseudarthrosis due to *Erysipelatoclostridium ramosum* in a female young patient. The patient, currently in remission from Ewing's sarcoma treated by a bone resection and allograft combined with chemotherapy, suffered from a chronic femoral pseudarthrosis in a context of bone insufficiency and graft resorption. A broad range 16S PCR followed by sequencing, as well as an enrichment culture of a bone biopsy revealed the presence of *E. ramosum,* an anaerobic firmicute with a low Gram-positive affinity staining and low GC content, that was further characterized by whole genome sequencing (WGS).

Introduction

Erysipelatoclostridium ramosum is a Gram-positive anaerobic enteric bacterium that is part of the normal human gut microbiota. Originally named *Bacillus ramosum* (Forrester Joseph and Spain David, 2014; Yutin and Galperin, 2013), and then most widely known as *Clostridium ramosum,* this species has been renamed as *Erysipelatoclostridium ramosum* in 2013 (Yutin and Galperin, 2013). The *Erysipelatoclostridium* genus belongs to the new family *Erysipelatoclostridiaceae* with other members of the XVIII clostridial cluster (Yutin and Galperin, 2013) and was recently confirmed as a monophyletic group in a standardized revision of the taxonomy (Parks et al., 2018).

E. ramosum infections are rare and were mostly reported in young patients (<5 y.o.) suffering from inner ear infections or in immunocompromised elderly people (Forrester Joseph and Spain David, 2014; Dahya et al., 2015). Clinical presentations encompass a variety of cases: gas gangrene, pseudomembranous colitis, acute otitis media, non-autoimmune hemolytic anemia, pyelonephritis, brain or lung abscess, peritonitis, spondylodiscitis, osteomyelitis, arthritis, bacteremia and septicemia (Dahya et al., 2015; Lavigne et al., 2003; Alcalde-Vargas et al., 2012; García-Jiménez et al., 2016; van der Vorm et al., 1999; Gerber et al., 2018; Mohandas et al., 2001; Al-kali et al., 2008).

Bone infection was first reported in 2001, for a 91-year-old diabetic woman with a history of hypertension (Mohandas et al., 2001). The first case of spondylodiscitis due to *E. ramosum* in an elderly immunocompetent patient without a specific history of bone infections was reported in 2003 (Lavigne et al., 2003). Recently, *E. ramosum* was detected in a tibia of a young immunocompetent individual after a traumatic fracture and an amputation above knee was performed after treatment failure (Dahya et al., 2015). Here, we present a new case of septic pseudarthrosis due to *E. ramosum* in a young adult patient presenting complications to plate osteosynthesis stabilization following a pathological fracture. Microbiological and genomic analyses of the *E. ramosum* strain isolated from a bone fragment confirmed the antibiotic susceptibility and the taxonomical assignment of the isolate.

* Corresponding author at: Institute of Microbiology, Rue du Bugnon 48, 1011 Lausanne, Switzerland.
E-mail address: Claire.Bertelli@chuv.ch (C. Bertelli).

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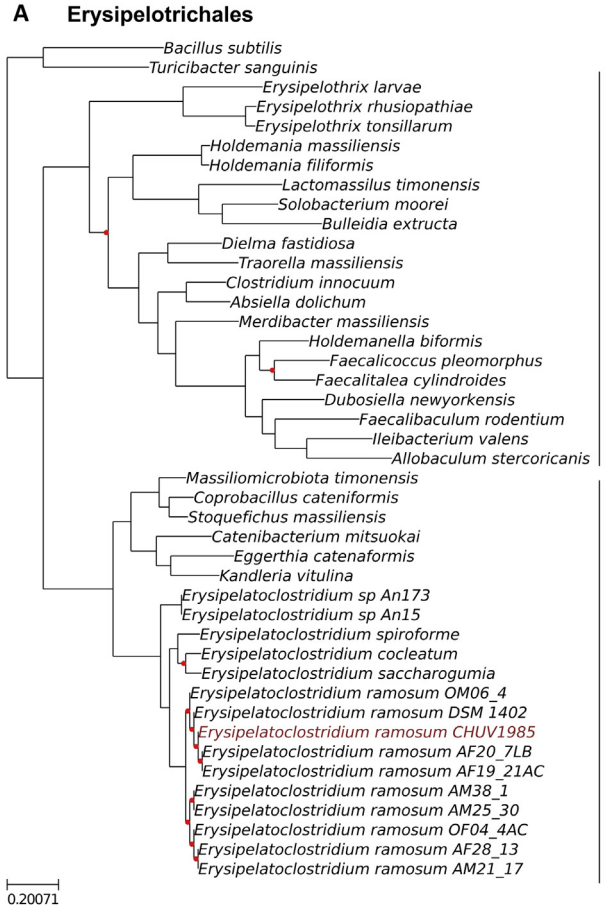
Case report

A 26-year-old female patient, with a history of metastatic Ewing's sarcoma treated by chemotherapy and left index amputation, underwent a large tumor resection and allograft on the right distal femur for recurrence in 2001. In remission since then, she presented in 2010 a pathological fracture on the same site that was stabilized by osteosynthesis. Two years later, a femoral pseudoarthrosis with rupture of distal screws was treated by a complex surgery associating the change of osteosynthesis material, in presence of a demineralized bone matrix, and a condylar periosteal flap. Culture of bone biopsies and sonication were sterile.

Since January 2016, the patient presented a pain at the right distal femur in a context of recurrent bone insufficiency and progressive resorption of the allograft. The patient was admitted to the hospital in May for a replacement of the screw, the partial removal of the allograft and reconstruction of the implant with injectable bone cement. Although the first culture was sterile, the strain was recovered after 5 days by culture from bone and allograft biopsies following a 2-day enrichment in thioglycollate medium and subculture on Schaedler blood agar incubated in anaerobic conditions, a standard operating procedure for bone biopsies in our laboratory. The strain was identified by MALDI-TOF (Croxatto et al., 2012) as C. ramosum (reclassified as E. ramosum). PCR amplification of the 16S rRNA confirmed the identification of E. ramosum in one of the peroperative bone biopsies. Antibiotic susceptibility testing performed according to EUCAST guidelines revealed that the microorganism was susceptible to clindamycin. Oral clindamycin (600 mg 3x/d) was prescribed and the patient was discharged. After six weeks, a new bone allograft was performed after the ablation of the spacer. The antibiotic initially planned for at least one year was stopped in October 2016 after 8-month treatment because of gastrointestinal side effects. Two years later, the patient presents no recurrence of femoral pain.

Bacterial genomics

Genomic DNA was extracted with the Wizard Genomic DNA Purification kit (Promega, USA), libraries were prepared using Nextera XT (Illumina, San Diego, USA) and sequencing was performed on a MiSeq sequencer (Illumina). Raw sequence data was trimmed by Trimmomatic v0.36 (parameters: SLIDINGWINDOW:5:20, LEADING:28, TRAILING:28, MINLEN:50), assembled by SPAdes v3.11.1, and contigs larger than 500 bp harboring over 5-fold mean coverage were annotated using PROKKA v1.13. The draft genome of *E. ramosum* strain CHUV1985 comprises 3,458,191 bp and exhibits a G + C content of 33%. The strain was predicted to encode 3259 genes, 7 rRNAS, 56 tRNAs and one tmRNA. One representative genome for each 32 species of the order Erysipelotrichales as well as all *E. ramosum* genomes available in NCBI's RefSeq as of October 2018 (Supplementary Table S1) were used to reconstruct groups of orthologous proteins (orthogroups) using OrthoFinder 2.0.2. *Bacillus subtilis* and



B Frequency of *E. ramosum* isolation in Lausanne University Hospital since 1997

Sample type	Number of cases	Percentage (%) of cases
Abscess	8	7.5
Stool	3	2.8
Anal/genital swab	1	0.9
Blood culture	43	40.6
Liquid (ascites, peritoneal, pleural, dialysis)	13	12.3
Drain	1	0.9
Superficial smear	7	6.6
Deep wounds	9	8.5
Fournier gangrene	2	1.9
Placenta smear	5	4.7
Tissue fragment	4	3.8
Bone	3	2.8
Urine	4	3.8
Ureter	2	1.9
Prosthetic material	1	0.9
Total	106	100

Figure 1. Phylogeny of the order Erysipelotrichales and frequency of *E. ramosum* identification.
(A) The maximum likelihood tree, based on the alignment of 146 core genes, divided the order *Erysipelotrichales* into two clades corresponding to families *Erysipelotrichaceae* and *Erysipelatoclostridiaceae*. *E. ramosum* strain CHUV1985 (highlighted in red) clusters with other *E. ramosum* strains, hence forming a monophyletic clade in the genus *Erysipelatoclostridium*. The genomes of *Bacillus subtilis* and *Turicibacter sanguinis* were used here as outgroups. (B) *E. ramosum* strains were only infrequently isolated in the clinical microbiology laboratory of the University Hospital of Lausanne. Over 20 years, only 3 isolates were obtained from bones and one isolate, reported in this manuscript, from prosthetic material.

Turicibacter sanguinis which are other members of the *Firmicutes* were used as an outgroup. The protein sequences of the 146 orthogroups present as single copy in each genome were aligned with MAFFT v7.310 and concatenated to construct a maximum-likelihood phylogeny with Fasttree 2.1.9, double precision. The phylogenetic reconstruction clustered CHUV1985 with other *E. ramosum* strains (Figure 1A). In agreement with the recent revision of the bacterial taxonomy (Parks et al., 2018), the branch supporting all *Erysipelatoclostridium* species was monophyletic, which confirmed the common ancestry of these species and supported their classification in the same genus. The average nucleotide identity (ANIb), calculated with pyani v0.2.7 between *E. ramosum* strain CHUV1985 and the 9 other strains of the same species was higher than 99% (Supplementary Table S2), thereby corroborating its taxonomic affiliation. References to all bioinformatics tools used for this analysis are available in the supplementary material.

Discussion

The isolation of *E. ramosum* from a bone and tissue fragment of this young patient suggested the presence of a chronic infection at the site of bone resection and graft, likely negatively impacting bone regeneration at the site of the allograft. While allografts are a known source of infectious complications and non-fusion with the adjacent bone, the patient's graft suffered a massive resorption within 18 months, after several years of stability. No blood culture was documented positive to *E. ramosum* for this patient in the past years. Hence, we can only speculate whether the infection was blood-borne, but remained undetected, or linked to surgery. Infections to *E. ramosum* are generally rare, with only 106 cases diagnosed in the University Hospital of Lausanne since 1997 (Figure 1B). Most *E. ramosum* isolates were obtained from blood culture, and *E. ramosum* was isolated from bones in only three cases (2.8%) and from prosthetic material in a single case reported here (0.9%). To our knowledge, this is the first case of *E. ramosum* infection after an orthopedic procedure with an allograft.

16S-targeted PCR and sequencing could rapidly confirm the etiologic agent of the infectionthat had been first isolated by enrichment and culture in anaerobic conditions. Therefore, the use of both molecular diagnostic and enrichment media is recommended in case of culture-negative osteomyelitis. Genomic analyses enabled to confirm the taxonomic classification of the strain and the monophyly of the newly formed *Erysipelatoclostridium* genus. Rapid whole genome sequencing can be used routinely to support classic microbiology diagnostic methods such as culture or molecular diagnostics by PCR in case of rare infections or dubious taxonomic affiliations.

Data availability

The genome sequence of *E. ramosum* strain CHUV1985 has been deposited in the European Nucleotide Archive under the project number PRJEB30816 with chromosome accession numbers CAACVM010000001-CAACVM010000065.

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

The present case report did not have to be approved by the ethical committee, in compliance with the guidelines for case reports of less than 5 cases.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.001.

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