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CASE REPORT

Unusual encounter: Isolation of *Carnobacterium divergens* in an immunocompetent patient with a meat grinder Injury. Case report

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Abstract

The genus *Carnobacterium* belongs to lactic acid bacteria, this group are composed by 12 species. These microorganisms are associated with mucosal surface, present in food-related habitats including plant, wine, milk, and meat environments. In humans, evidence of *Carnobacterium spp.,* about the disease spectrum remains unknown. Current work is about a 25-year-old male, was admitted to the emergency room for transmetacarpal traumatic amputation of his left hand caused by a meat grinder. In deep soft tissue samples obtained from surgery, *Carnobacterium divergens* was isolated. He was treated with piperacillin-tazobactam and was switched to amoxicillin-clavulanate for five more days with clinical success. Written informed consent was obtained from the participating patient for publication of this case report and any accompanying images. Therefore, the pathogenic role of *Carnobacterium* spp., remains unknown and classically is not considered pathogenic to humans. However, in previous cases where was associated with infection, the described outcomes were good. The misidentification showed that conventional identification methods are not accurate.

Keywords: *Carnobacterium*; meat grinder; *C. divergens*; case reported.

Hallazgo inusual: Aislamiento de *Carnobacterium divergens* en un paciente inmunocompetente con una lesión producida por un molino de carne. Reporte de caso.

Resumen

El género *Carnobacterium* pertenece a las bacterias ácido lácticas, este grupo está compuesto por 12 especies. Estos microorganismos están asociados a la superficie de las mucosas, presentes en hábitats relacionados con los alimentos, incluidos los entornos de las plantas, vino, leche y carne. En humanos, la evidencia de *Carnobacterium spp*, sobre el espectro de la enfermedad sigue siendo desconocida. El presente trabajo se trata de un varón de 25 años ingresó en urgencias por amputación traumática transmetacarpiana de la mano izquierda causada por una picadora de carne. En muestras profundas de tejidos blandos obtenidas en la cirugía, se aisló *Carnobacterium divergens*. Fue tratado con piperacilina-tazobactam y se le cambió a amoxicilina-clavulánico durante cinco días más con éxito clínico. Se obtuvo el consentimiento informado por escrito del paciente participante para la publicación de este informe de caso y de las imágenes que lo acompañan. Por lo tanto, el rol de *Carnobacterium spp.,* permanece incierto y clásicamente no se considera patógeno para el ser humano. Sin embargo, en casos anteriores en los que se asoció a infección, los decensales clinicos no se asociaron a morbilidad o mortalidad. La identificación errónea demostró que los métodos convencionales de identificación no son precisos.

Palabras clave: *Carnobacterium*; picadora de carne; *C. divergens*; reporte de caso

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Introduction

Carnobacterium are gram-positive rods within the phylum *Bacillota,* class *Bacilli,* order *Lactobacillales* and family *Carnobacteriaceae*[1]. The genus was isolated from vacuum-packed meat, chicken, and fish, and two ecological groups have been suggested: group I, associated with animals and their products; and group II, associated with Arctic ice lakes or Pleistocene ice[2]. Twelve species have been described, members of which are fermentative and able to grow mainly facultatively, although some species can grow aerobically or even microaerophilically[2]. Two species, *Carnobacterium divergens* and *C. maltaromaticum*, are commonly found in dairy products. They are often utilized as probiotics or biopreservatives because they can synthesize bacteriocins that restrict or suppress the development of foodborne pathogens[3]. In humans, there is no evidence that *Carnobacterium* belongs to the gut microbiota; therefore, it is not considered as pathogen[4]; however, there are few case reports of local infection (e.g., abscesses) and systemic infection (e.g., bacteremia). Here, we report a case of *C. divergens* isolated from a traumatic hand wound caused by a meat grinder. Written informed consent was obtained from the participating patient for the publication of this case report and any accompanying images.

Case presentation

A 25-year-old male, without no previous medical history was admitted to the emergency room due to traumatic transmetacarpal amputation of his left hand caused by a meat grinder. On admission, the patient had a temperature of 36.4 °C, blood pressure of 100/91 mmHg, and beat rate of 96 beats/min; he denied having fever or taking any antibiotics previously. Laboratory studies revealed a hemoglobin of 17.4 g/dL, platelets count 269 $\times 10^9$/L, and white cells count of 14.1$\times 10^9$/L and medical staff decided to start on empiric piperacillin-tazobactam (TZP) 4.5 grams intravenously every 6 hours as monotherapy to supply coverage against anaerobic and gram-negative rods. The patient was transferred to the operating room where debridement with radiocarpal disarticulation was performed, deep soft tissue samples were taken and sent to the Clinical Microbiology Laboratory, and then a vacuum-assisted closure system was set.

The tissue specimens were processed. One day after incubation on 5% sheep blood agar at 37 °C, grayish-white colonies (1-2 mm diameter) were obtained. Gram staining was performed on direct colonies observing gram-positive rods. Initially, was identified with Vitek MS (bioMérieux, Marcy L´Etoile, France) with 99 % identity, however, as this bacterium is uncommon, second identification using VITEK® 2 compact GP card was performed having *Enterococcus faecalis* (89% of identity), due the poor identification level, a second identification with VITEK® 2 compact was performed and was identified as *Lactococcus lactis subsp. lactis* (91% of identity). We decided to perform biochemical tests according to the literature[2], the colonies were catalase-negative and non-hemolytic, Vogues-Proskauer negative, motility negative, and esculin-positive, and were able to grow from 0.5-5% NaCl. To confirm identification, the strain was sequenced with the 16S rRNA gene (27F 5-AGA GTT TGA TYM TGG CTC AG-3′ and 338R 5′-ACT CCT ACG GGA GGC AGC-3) in duplicate[5]. Each PCR product was re-amplified and labeled using the BigDye® Direct Cycle Sequencing Kit (Applied Biosystems). Sequences were aligned using GenBank and compared. A sequence was identified, such as *Carnobacterium divergens,* with 99.9 % identity.

Broth microdilution susceptibility tests were carried out in Mueller-Hinton broth supplemented with cations and 5% sheep lysed blood using the CLSI (Clinical & Laboratory Standards Institute) standardized methods[6]; the minimum inhibitory concentrations (MICs) were: ceftriaxone 32 µg/mL, ceftazidime > 64 µg/mL, imipenem 0.25 µg/mL, meropenem 0.062 µg/mL, amoxicillin/clavulanic acid 0.25/0.125 µg/mL. The high concentrations of cephalosporins, but lower concentrations of carbapenem and ß-lactam with ß-lactam inhibitors, suggest the presence of a ß-lactamase. Ciprofloxacin 0.25 µg/mL, vancomycin 0.5 µg/mL. The highest concentrations were erythromycin (1 µg/mL), clindamycin (2 µg/mL), linezolid (4 µg/mL), and amikacin (8 µg/mL) (Table 1).

Resistance to benzylpenicillin, ampicillin, amoxicillin, ticarcillin, and piperacillin has been reported in *C. divergens* and proved susceptible to amoxicillin-clavulanic acid and TZP,

Table 1. MICs of antibiotics for *C. divergens*

Antibiotics	MIC (µg/mL)
Penicillin	0.125 µg/mL
Oxacillin	4 µg/mL
Amoxicillin/clavulanic acid	0.25/0.125 µg/mL
Ceftriaxone	32 µg/mL
Ceftazidime	> 64 µg/mL
Imipenem	0.25 µg/mL
Meropenem	0.062 µg/mL
Amikacin	8 µg/mL
Ciprofloxacin	0.25 µg/mL
Clindamycin	2 µg/mL
Erythromycin	1 µg/mL
Vancomycin	0.5 µg/mL
Linezolid	4 µg/mL

strains hydrolyzed nitrocefin due a ß-lactamase as resistance mechanism. Sequencing revealed a 912 bp coding sequence encoding a class A β-lactamase named CAD-1[7].

Our patient was treated with TZP 4.5 grams intravenously every 6h, for five days and after MICs values patient was switched to amoxicillin-clavulanate (500mg/125 mg) every 12 h for five more days with clinical success[7,8].

Lactic acid bacteria are widely associated with mucosal surfaces and are present in food-related habitats (plants, wine, milk, and meat environments)[7]. *C. divergens* and *C. maltaromaticum* are able to grow in meat products at temperatures as low as 1.5 or 2 °C and they are the most frequent members (up to 50% *C. divergens* and up to 26% *C. maltaromaticum*) isolated from the microbial community of raw meat (beef, pork, lamb, and poultry)[4], detected also in a variety of processed meat products, including the cured pork product bacon, ham, a Danish processed pork product, various processed meat products, and cooked poultry meat and these organisms have not been isolated from the gastrointestinal system or skin of chicken, cattle, pigs, or sheep. Transmission routes into food-manufacturing factories and meat products are not well defined[4], besides, lactic acid bacteria are the most commonly used probiotics in food[9].

The most recent literature describes six cases of human infection with *Carnobacterium spp*.. Carson et al. performed a search for all English-written articles published on human infections with *Carnobacterium spp*. Two cases of *Carnobacterium spp*. were identified in traumatic wounds associated with exposure to water, without any other associated risk factors. In these cases, diagnosis was made using a wound swab. Three cases were isolated from blood cultures, two of which were diagnosed as cancer (prostate and central nervous system lymphoma) and chronic steroid use. One patient had diabetic ketoacidosis and was receiving enteral nutrition. Another case was reported to be a suspected gastrointestinal source of infection in an immunocompetent individual with a history of handling and consuming fish[8].

Our case report describes an immunocompetent patient with traumatic amputation due to grinder meat. The isolation of the bacteria at the site where the sample was taken correlates with a sufficient inoculum for the development of an infectious process according to the microbial physiology and sufficient inoculum to be isolated in microbiological media.

Conventional identification in the laboratory is complex; thus, in five of six cases, the final identification was by using 16S rRNA gene sequence analysis and in one case reported by Jeong I-H *et al., Carnobacterium* was misidentified as *Enterococcus gallinarum* with VITEK® 2 GP card and Vitek MS identified *Carnobacterium divergens*[10]. In our case, VITEK 2 was misidentified as *Enterococcus faecalis* and *Lactococcus lactis subsp. lactis,* and the identification was performed using Vitek MS. VITEK® 2 resulted in misidentification because of the absence of this microorganism in its database.

These similarities in the identification process showed that conventional identification methods are not accurate; thus, the use of molecular biology and mass spectrometry techniques offers many advantages over conventional microbiological and molecular techniques, including reliability and rapidness, as it takes only a few minutes for the identification of microbes, simplicity, cost-effectiveness, and the use of MALDI-ToF routine application in clinical microbiology laboratories has allowed the identification of underestimated environmental bacteria[11]. Regarding antimicrobial susceptibility, there are no recommended interpretative criteria or breakpoints established by the CLSI for susceptibility testing of antimicrobial agents against *Carnobacterium spp*. causing human infections[10]. We reported MICs using a broth microdilution susceptibility assay.

Although *Carnobacterium* is not considered pathogenic and the disease spectrum remains unknown, in previous cases, outcomes were good despite patients having serious risk factors such as cancer or neutropenia. We describe the case of an immunocompetent man with traumatic amputation and grinder meat with good clinical outcomes.

Our study has limitations, such as the true role of Carnobacterium as a pathogen. We consider that the recovery of microorganisms after surgical debridement was related to a sufficient inoculum to be recovered by the culture media considered and in combination with the administration of antibiotics in a timely manner, and selection based on the minimum inhibitory concentrations was a determinant in the prevention or continuity of the infectious process.

Ethical considerations

Ethics approval and consent to participate. Ethical approval was obtained from Members of the Research Ethics Committee of the Luis Guillermo Ibarra Ibarra National Rehabilitation Institute. Written informed consent was obtained from the patient.

Protection of persons. No experimental or patient-directed interventions were performed. Informed consent is provided.

Protection of vulnerable populations. Patient does not present vulnerability criteria, signed informed consent.

Confidentiality. Written informed consent was obtained from the participating patient for publication of this case report and any accompanying images.

Privacy. Name and sensitive patient data are not published or used. Security of privacy is guaranteed at all times. Informed consent has been attached.

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Conflict of interests. The authors have no conflict of interest to declare

Authors´ contribution. CBF: Conceptualization, investigation, methodology, writing – original draft and writing – review & editing; MLGH: Methodology; MHD: Methodology; GCG: Methodology; CACC: Methodology, formal analysis, writing – original draft; RFC: Methodology, supervision, writing – original draft and writing – review & editing.; LELJ: Conceptualization, investigation, methodology, writing – original draft and writing – review & editing. All authors contributed to read and approved the version of the submitted manuscript.

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