Case Report

Isolation of *Carnobacterium* sp. from a human blood culture

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Received 16 October 2009
Accepted 12 January 2010

Carnobacterium species have been isolated from the environment and are not regarded as human pathogens, although they are known to cause disease in fish. Only two reports describing isolation of *Carnobacterium* species from human pus were found in the literature. We report what we believe to be the first isolation of *Carnobacterium* sp. from a human blood culture.

Introduction

Carnobacterium species are Gram-positive rods belonging to the family *Lactobacillaceae* and are not regarded as human pathogens. The genus *Carnobacterium* comprises 11 species, but only two of these, *Carnobacterium divergens* and *Carnobacterium maltaromaticum* (formerly *Carnobacterium piscicola*), are isolated frequently from the environment and food (Leisner *et al.*, 2007). *Carnobacterium mobile* and *Carnobacterium funditum* have been isolated rarely from mostly fish and shrimp (Leisner *et al.*, 2007; Chenoll *et al.*, 2007; Laursen *et al.*, 2005; Ringo & Olsen, 1999; Ringo *et al.*, 2006).

Carnobacterium species have been isolated previously from human pus (Chmelar *et al.*, 2002; Xu *et al.*, 1997). We report what we believe to be the first isolation of *Carnobacterium* sp. from a human blood culture.

Case report

A 43-year-old white male was admitted with acute pain between his scapulae, neck and back of the head, fever and malaise. The symptoms had started less than 1 day previously. He was an otherwise healthy individual, without any relevant past medical history. The patient turned out to be a fish devotee with extensive contact with seafood: he prepared and ate frozen and modified atmosphere-packed shrimp a week before, ate various raw and cooked fish and shrimp 2 days before, as well as prepared and ate fresh and unfrozen catfish 1 day before

onset of symptoms. The physical examination was unremarkable. The patient's body temperature was 39.1 °C, the heart rate was 115 beats min⁻¹ and the respiratory rate was 21 min⁻¹. Results of the laboratory testing showed a white blood cell count of 13 550 µl⁻¹, with 77 % neutrophils, 13 % lymphocytes and 9 % monocytes. The serum basic chemistry was normal except for a serum creatinine level of 119 µmol l⁻¹ (normal range 30–97) and a lactate dehydrogenase level of 335 U l⁻¹. The C-reactive protein level was 27.3 mg l⁻¹ (normal range <8). One set of blood cultures (aerobic/anaerobic) was taken. Considering meningococcal meningitis as a differential, empirical therapy with intravenous ceftriaxone 2 g q.12 h was initiated. The patient refused lumbar puncture at that time because of the absence of neck stiffness. Septicaemia was suspected and the patient was transferred to the intensive care unit (ICU) under the direction of the attending ICU physician.

C-reactive protein levels increased subsequently to 227.9 mg l⁻¹ with an unremarkable procalcitonin level of 0.21 ng ml⁻¹ (normal range <0.50). Chest X-ray, a computed tomography scan of the neurocranium and a magnetic resonance tomography scan of the neurocranium and spine were all unremarkable. As the patient developed neck stiffness, a lumbar puncture was performed. The cerebrospinal fluid (CSF) was clear in colour and analysis was unremarkable with a cell count of 1 µl⁻¹. Rapid diagnostic tests for meningococci and pneumococci as well as Gram and Ziehl–Neelsen staining and bacterial cultures of the CSF were negative. Real-time PCR of the CSF was slightly positive only for varicella-zoster virus (VZV) DNA (viral load 0.04 genome equivalents µl⁻¹). Because of the lack of significance of this low viral load, real-time PCR for VZV was repeated and was negative. Therefore, a diagnosis

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Abbreviation: CSF, cerebrospinal fluid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the cultivated isolate is GQ281028.

of VZV meningitis was ruled out. Subsequent comprehensive serological tests for viral infections were unremarkable.

Bacterial growth was detected within 48 h in the one set of blood cultures collected on admission, revealing Gram-positive rods. Consequently, additional antibiotic therapy with intravenous ampicillin 4 g q.8 h was initiated to cover *Listeria monocytogenes*, although infection with this organism is rare in immunocompetent individuals. The patient's condition improved rapidly and his C-reactive protein levels and other blood test results normalized. The antibiotic regimen was changed to oral moxifloxacin 400 mg q.24 h. One week after admission, the patient was discharged in good clinical condition.

Microbiological studies

Blood cultures collected on admission were placed in the BACTEC 9050 System (Becton Dickinson) and turned positive after 2 days of incubation. A Gram stain of the pellet showed Gram-positive, lactobacillus-like rods. Within 24–

48 h, the isolate yielded growth on blood, chocolate and Schaedler agar, with 1–2 mm diameter, grey, β -haemolytic colonies (Fig. 1). Surprisingly, a Gram stain of these colonies exhibited Gram-positive cocci; the catalase reaction was negative. Due to divergent Gram staining, 16S rRNA gene analysis was carried out by using eubacterial universal primers. A BLAST search of the obtained partial 16S rRNA gene sequence (1450 bp) was performed using the taxonomy browser of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>), and retrieved from GenBank. Homology of 100% was achieved for *Carnobacterium* species, 99% for *C. mobile* and 97% for *C. funditum*. The GenBank accession number for the 16S rRNA gene sequence of the cultivated isolate is GQ281028.

MICs were determined by Etest (AB Biodisk) by using an inoculum corresponding to McFarland standard 0.5, applied on Mueller–Hinton agar plates with 5% (v/v) sheep blood (Becton Dickinson Microbiology Systems) and incubated for a 48 h period. MICs obtained were: penicillin, 0.25 mg l⁻¹; ampicillin, 0.064 mg l⁻¹; amoxicillin–clavulanic acid, 0.125 mg l⁻¹; piperacillin–tazobac-

Fig. 1. *Carnobacterium* sp. on blood (a) and chocolate (b) agar after incubation at 37 °C for 24–48 h. The isolate yielded growth of 1–2 mm diameter, grey, β -haemolytic colonies on blood agar containing 5% sheep blood. (c) Gram staining of *Carnobacterium* sp. after incubation at 37 °C exhibiting mainly Gram-positive cocci (like streptococci); (d) Gram staining of *Carnobacterium* sp. after incubation at 25 °C exhibiting mainly Gram-positive lactobacillus-like rods.

tam, 0.25 mg l⁻¹; cefuroxime, 16 mg l⁻¹; ceftriaxone, 128 mg l⁻¹; imipenem, 0.5 mg l⁻¹; gentamicin, 0.25 mg l⁻¹; and vancomycin, 0.5 mg l⁻¹.

To evaluate the different Gram-staining results for the same organism, *Carnobacterium* sp. was recultured at 37 °C and 25 °C. While Gram staining of *Carnobacterium* sp. cultured at 37 °C revealed mainly Gram-positive cocci, Gram staining of colonies cultured at 25 °C demonstrated mainly Gram-positive rods (Fig. 1).

Discussion

C. mobile has been isolated rarely, from cooked and vacuum packed turkey (Chenoll *et al.*, 2007), cooked modified atmosphere-packed shrimp in brine (Laursen *et al.*, 2005), and live Arctic charr (Ringo & Olsen, 1999) and Atlantic cod (Ringo *et al.*, 2006). *C. funditum* has been isolated with extremely low frequency from polar lakes/sea, the intestine of live fish and marine sponge, but never from food (Leisner *et al.*, 2007). Carnobacteria are not known to be members of the human gastrointestinal microbial community (Leisner *et al.*, 2007). The presence of virulence factors in carnobacteria is not well documented. *Carnobacterium viridans*, however, shows β -haemolytic activity on sheep blood agar (Holley *et al.*, 2002) and *C. maltaromaticum* can be a fish pathogen.

To date, two cases reporting the isolation of *Carnobacterium* spp. from human pus have been described in the literature (Chmelar *et al.*, 2002; Xu *et al.*, 1997).

To our knowledge, this is the first report of *Carnobacterium* sp. isolated from a human blood culture. However, whether or not *Carnobacterium* sp. was accountable for the patient's clinical course remains unclear. Three main arguments suggest a causal association: first, the isolation of *Carnobacterium* sp. from the blood cultures taken at admission; second, the patient's exposure to fish and other seafood; third, a clinical response to ampicillin and clinical deterioration on the initial treatment with ceftriaxone to which the isolate was resistant (MIC 128 mg l⁻¹). However, some facts question a causal association. As the isolate was obtained in one set of blood cultures only, contamination was not effectively ruled out. Furthermore, the patient's most prominent symptom was severe pain between the scapulae, neck and back of the head with development of neck stiffness, and the CSF was marginally positive for VZV by PCR. Therefore, zoster sine herpete, an uncommon variant of zoster, may be a possible differential diagnosis. Also, antibiotic therapy was changed, based on the results of the Gram staining, to cover infection with *L. monocytogenes* with a corresponding clinical response. As reported by Leisner *et al.* (2007), growth of carnobacteria

may inhibit *L. monocytogenes* by bacteriocin production and glucose depletion. Therefore, infection by *L. monocytogenes* cannot be ruled out. The fact that growth of *Carnobacterium* sp. may have an influence on the identification of *L. monocytogenes* infection may be an interesting matter for future cases.

Discussing the possible route of infection, the patient may have become infected through a microlesion in his hand while preparing fish or through oral/pharyngeal lesions (the patient's family suffered from respiratory disease for 2 months) while consuming raw or cooked fish.

To conclude, we report the first isolation of *Carnobacterium* sp. from a human blood culture. No further focus for infection or pathogen was found despite extensive diagnostic procedures. The causal association with the clinical symptoms in this case, however, remains uncertain.

Acknowledgements

We thank Katharina Seeber, MSc, for editorial assistance.

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