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First isolation of *Clostridium indolis* in a patient with chronic osteitis: a case report and literature review of human infections related to *Clostridium saccharolyticum* group species

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Clostridium indolis is an anaerobic spore-forming Gram-positive bacillus belonging to the *Clostridium saccharolyticum* group. Its clinical significance in human remains poorly known. We describe the first case of osteitis related to *C. indolis*, identified by MALDI-TOF mass spectrometry and provide a literature review of human infections related to *C. saccharolyticum* group species.

In august 2014, a 37 years-old woman was admitted to the surgical intensive care unit of our tertiary care center, because of an open Cauchoi III fracture of left tibia and fibula with skin damages after a motorcycle injury. On admission, the patient was intubated and ventilated. Body temperature was 36°C. Heart rate and blood pressure were normal. Ionogram, blood cells count and C-reactive protein (CRP) were all within normal range whereas serum creatinine phosphokinase (CPK) level was increased to 697 UI/L secondary to muscular lysis. She underwent an urgent surgery consisting in a tibial-fibular osteosynthesis performed by a multidisciplinary surgical critical care team involving orthopedic, vascular and plastic surgeon. In early 2015, she presented a subcutaneous collection located close to orthopedic screws. This process has grown chronically and was well tolerated during 4 months. On 15 June 2015, she was readmitted because of an acute purulent discharge from this collection that started 4 days before. Her body temperature was normal. Laboratory investigations revealed inflammation markers such as slightly elevated CRP (7.5 mg/L) and moderate neutrophil polynucleosis (7.9 G/L). The orthopedic treatment consisted in the removal of two screws and washing. Five bacteriological specimens (screws and collection) were sampled as recommended by the *Société de Pathologie Infectieuse de Langue Française* (SPILF) and sent to our microbiology laboratory for analysis. All samples grew with *Enterobacter cloacae* (on all media seeded: 5 out of 5) displaying wild type antibiotic susceptibility pattern to beta-lactams. On the basis of these results and according to the recommendations of SPILF for osteo-articular infections on osteosynthesis material, a six-weeks antibiotic course of Intra-

48 Venous (IV) cefepime (2g t.i.d) associated with ciprofloxacin IV (400 mg b.i.d) was initiated.
49 One week later, a new intervention was performed to remove the intramedullary nail materiel.
50 Four surgical samples (3 soft tissues and 1 bone) were sent to our laboratory. Bacteriological
51 analysis retrieved *E. cloacae* displaying cephalosporinase hyperproduction phenotype from all
52 biological samples (4 out of 4). At the end of July 2015, 4 weeks after the beginning of
53 antibiotherapy, she underwent a surgical revision with medial fibula transport and an Ilizarov
54 external fixator was set because of tibial pseudarthrosis. A total of 3 surgical specimens were
55 sampled (2 soft tissues and one bone). Cultures on Columbia agar plates supplemented with 5
56 % sheep blood (Oxoid, Dardilly, France) incubated both under anaerobic and aerobic
57 atmosphere, and cultures on chocolate agar plates (Oxoid, Dardilly, France) incubated under
58 5% CO₂ remained sterile after 96 hours. Culture was positive on Rosenow (Biorad, Marnes-
59 la-Coquette, France) enriched liquid medium after 2 weeks of incubation. Gram staining
60 showed large spore-forming Gram-variable bacilli. Subculture performed on blood agar plates
61 under anaerobic conditions grew with medium size mucoid colonies surrounded by a single
62 zone of beta-hemolysis (two out of three samples). MALDI-TOF mass spectrometry (MS)
63 performed directly on the colonies identified *Clostridium indolis* (Log score value of 2.19
64 matching *Clostridium indolis* reference strain DSM755T; MALDI Biotyper v2.3). This
65 identification was confirmed by the National Reference Center of Anaerobic Bacteria and
66 Botulism (Institut Pasteur, Paris, France) by 16S rDNA gene sequencing using forward
67 AAGGAGGTGATCCAGCCGCA and reverse primers AGAGTTTGATCATGGCTCAG,
68 (99,6% of identity with the sequence of *Clostridium indolis* type strain DSM755T, GenBank
69 accession number Y18184) (Strain 570.15, see *figure* for phylogenetic analysis) [1–5].
70 Antimicrobial susceptibility testing was performed on the isolated strain using E-test strips
71 (bioMérieux, Marcy-l'Etoile, France), with a 1 McFarland suspension, on Brucella agar plate
72 supplemented with vitamin K (1 mg/L) and 5% sheep blood that were incubated under

anaerobic atmosphere as recommended by the CASFM 2013. The Minimal Inhibitory Concentrations (MICs) were read following the CASFM 2013 interpretative standard for anaerobes (see table1). The antibiotic treatment was changed to oral metronidazole (500 mg t.i.d) for a total duration of four weeks. Biological markers of inflammation returned normal at 4 weeks (CRP 4.1 mg/L and normal neutrophils count 2.6 G/L). In February 2016, the patient reported no painful phenomenon and clinical examination showed clean scars and body temperature was normal. CRP was negative. Radiological control did not show signs of osteitis.

Clostridium species are spore forming Gram-positive or Gram-variable anaerobic bacilli that are ubiquitous in the environment. Some species are part of human digestive microbiota. *Clostridium* species like *Clostridium difficile*, *Clostridium perfringens* and *Clostridium tetani* are well known for their involvement in human infections mostly related to the production of specific toxins. *Clostridium* species can also be associated with post-traumatic infections [6] and only few species among the genus *Clostridium* are involved in osteo-articular infections [7–11]. Interestingly, *C. indolis*, which belongs to the *C. saccharolyticum* group along with *Clostridium sphenoides*, *Clostridium celerecrescens*, *Clostridium methoxybenzovorans* and *Desulfotomaculum guttoideum*, has never been reported in any human infection so far. *C.indolis* was isolated only once from a blood culture of a patient presenting without sepsis syndrome [12]. Furthermore, the clinical significance of *C. saccharolyticum* species group in human is not well established. Only 4 cases of infections due to *C. sphenoides* were reported (2 gastro-intestinal infections, one bacteremia and one osteomyelitis) [13–16]. Three cases of *C. celerecrescens* infections were also described including an osteomyelitis, an abscess secondary to an open fracture and a post traumatic wound infection [7,8,17] (see table 2 for details). Here we report the first case of osteitis related to *C. indolis* identified by MALDI-TOF MS using Biotyper database. The recent spread of new identification tools in routine

98 such as MALDI-TOF MS allows an accurate identification of bacterial species that were
99 previously underestimated. Nevertheless, there is only one reference spectrum of *C. indolis*,
100 *C. sphenoides* and *C. celerecrescens* included in the Bruker database (Biotyper v2.3) and
101 there is still no reference for *C. methoxybenzovorans* and *D. guttoideum*. These limits may
102 lead to an underestimation of these species. Hopefully, new reference spectra will be soon
103 included in the upcoming databases. In this study, we further attempted to identify the patient
104 strain (Strain 570.15) using Rapid ID 32A strips (BioMérieux, Marcy-l'Etoile, France) that is
105 still widely used in routine microbiological diagnosis. The test misidentified the strain as *C.*
106 *clostridioforme* with an acceptable significance (biochemical API profile: 45362000, 99,4%
107 T=0.77) except for a positive reaction for indole production. Interestingly, Bouvet *et al.*
108 reported the misidentification of *C. celerecrescens* as *C. clostridioforme* using RAPID ID
109 32A strips [17]. This type of mistakes pinpoints the need to integrate new strains to refine
110 biochemical databases. In order to help identifying these phylogenetically related anaerobic
111 species in routine diagnosis, and waiting for enlarged databases, we provide a summary of the
112 main biochemical characteristics of *C. saccharolyticum* species group in the table 3.
113 Concerning the clinical presentation, the patient was successfully treated by metronidazole,
114 which is active on *C. indolis*, and follow-up consultation at six months after the last surgery
115 reported no physical, biological or radiological signs of infection. Of note, bacteriological
116 samples obtained after the first orthopedic surgery were positive to *E. cloacae* in
117 monobacterial culture, and only four weeks after an appropriate antibiotic treatment with
118 cefepime and ciprofloxacin targeting *E. cloacae*, *C. indolis* was isolated in culture. The strains
119 showed high resistance level to cefepime and ciprofloxacin with MICs values of 256 mg/L
120 and 32 mg/L, respectively. Interestingly, the literature review reported high rates of
121 *Clostridium sp.* susceptible to moxifloxacin, from 69% to 79% [18,19]. This data suggests
122 that, in our case, the resistance of the strain to quinolones was probably acquired under

antibiotic pressure. This could be explained by the pre-existence of *C. indolis* in the wound and selection of resistant mutants after the first course of antibiotics. Indeed, even if we did not detect *C. indolis* in the first samples as co-pathogen, it seems very likely that it has been overgrown by *E. cloacae*. In addition, *Clostridium* species are environmental organisms, considered as soil saprophytes, or are part of normal human intestinal flora [20]. Almost all anaerobic osteomyelitis occur by direct extension from an adjacent focus of infection and are rarely due to bacteremia [21]. These data along with the absence of other positive bacteriological samples and/or digestive symptoms, suggest that this post-traumatic chronic osteitis occurred after a telluric contamination. Furthermore, the prolonged contact between the open wound and the ground after the accident can easily explain this mode of infection.

In conclusion, we described here the first case of osteitis due to *Clostridium indolis* in an immunocompetent patient successfully treated by a multidisciplinary medico-surgical approach while this bacterium was previously considered as non-pathogenic. Data concerning *C. saccharolyticum* species group are still lacking in the literature. Hopefully, the spread of new identification tools in bacterial routine identification such as MALDI-TOF MS and *16S* *rDNA* sequencing would help its diagnosis and provide a better understanding of its clinical significance in human infections.

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References

- [1] Biddle AS, Leschine S, Huntemann M, Han J, Chen A, Kyrpides N, et al. The complete genome sequence of *Clostridium indolis* DSM 755T. *Stand Genomic Sci* 2014;9:1089–104. doi:10.4056/sigs.5281010.
- [2] Sneath PH, Sokal RR. Numerical taxonomy. *Nature* 1962;193:855–60.
- [3] Felsenstein J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* 1985;39:783. doi:10.2307/2408678.
- [4] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–20.
- [5] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;30:2725–9. doi:10.1093/molbev/mst197.
- [6] Brook I, Frazier EH. Anaerobic osteomyelitis and arthritis in a military hospital: a 10-year experience. *Am J Med* 1993;94:21–8.
- [7] Glazunova OO, Raoult D, Roux V. First identification of *Clostridium celerecrescens* in liquid drained from an abscess. *J Clin Microbiol* 2005;43:3007–8. doi:10.1128/JCM.43.6.3007-3008.2005.
- [8] Mischnik A, Zimmermann S, Bekeredjian-Ding I, Egermann M. Relapse of posttraumatic osteomyelitis due to *Clostridium celerecrescens*. *Infection* 2011;39:491–4. doi:10.1007/s15010-011-0125-5.
- [9] Lotte R, Popoff MR, Degand N, Lotte L, Bouvet P, Baudin G, et al. Lumbar discitis caused by *Clostridium perfringens*. *J Clin Microbiol* 2014;52:3813–5.

169 doi:10.1128/JCM.00983-14.

170 [10] Ibnoukhatib A, Lacroix J, Moine A, Archambaud M, Bonnet E, Laffosse J-M, et al.
171 Post-traumatic bone and/or joint limb infections due to *Clostridium* spp. *Orthop Traumatol*
172 *Surg Res OTSR* 2012;98:696–705. doi:10.1016/j.otsr.2012.03.019.

173 [11] Levy P-Y, Fournier P-E, Lotte L, Million M, Brouqui P, Raoult D. *Clostridium tetani*
174 osteitis without tetanus. *Emerg Infect Dis* 2014;20:1571–3. doi:10.3201/eid2009.131579.

175 [12] Woo PCY. *Clostridium* bacteraemia characterised by 16S ribosomal RNA gene
176 sequencing. *J Clin Pathol* 2005;58:301–7. doi:10.1136/jcp.2004.022830.

177 [13] Felitti VJ. Primary invasion by *Clostridium sphenoides* in a patient with periodic
178 neutropenia. *Calif Med* 1970;113:76–8.

179 [14] Sullivan SN, Darwish RJ, Schieven BC. Severe diarrhea due to *Clostridium*
180 *sphenoides*: a case report. *Can Med Assoc J* 1980;123:398.

181 [15] Isenberg HD, Lavine LS, Painter BG, Rubins WH, Berkman JL. Primary osteomyelitis
182 due to an anaerobic microorganism. *Am J Clin Pathol* 1975;64:385–8.

183 [16] Kelesidis T, Tsiodras S. *Clostridium sphenoides* Bloodstream Infection in Man. *Emerg*
184 *Infect Dis* 2011;17:156–8. doi:10.3201/eid1701.101029.

185 [17] Bouvet P, K'Ouas G, Le Coustumier A, Popoff MR. *Clostridium celerecrescens*, often
186 misidentified as “*Clostridium clostridioforme* group,” is involved in rare human infection
187 cases. *Diagn Microbiol Infect Dis* 2012;74:299–302. doi:10.1016/j.diagmicrobio.2012.06.024.

188 [18] Wang FD, Liao CH, Lin YT, Sheng WH, Hsueh PR. Trends in the susceptibility of
189 commonly encountered clinically significant anaerobes and susceptibilities of blood isolates
190 of anaerobes to 16 antimicrobial agents, including fidaxomicin and rifaximin, 2008-2012,

191 northern Taiwan. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol
192 2014;33:2041–52. doi:10.1007/s10096-014-2175-y.

193 [19] White BK, Mende K, Weintrob AC, Beckius ML, Zera WC, Lu D, et al. Epidemiology
194 and antimicrobial susceptibilities of wound isolates of obligate anaerobes from combat
195 casualties. Diagn Microbiol Infect Dis 2016;84:144–50.
196 doi:10.1016/j.diagmicrobio.2015.10.010.

197 [20] Allen SD, Emery L, Lyerly DM. Clostridium. Man. Clin. Microbiol., vol. 1. ASM
198 Press, Washington: P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Jorgensen, and R. H.
199 Tenover; 2003, p. 835–56.

200 [21] Mutoh Y, Hirai R, Tanimura A, Matono T, Morino E, Kutsuna S, et al. Osteomyelitis
201 due to Clostridium innocuum in a patient with acute lymphoblastic leukemia: case report and
202 literature review. SpringerPlus 2015;4:385. doi:10.1186/s40064-015-1176-3.

203