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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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ABSTRACT

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 Cauchoix III fracture of left tibia and fibula with major skin damages and soft tissue defects 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 cell count and C-reactive protein (CRP) were all within normal ranges whereas serum creatinine phosphokinase (CPK) level was increased to 697 UI/L secondary to muscular lysis. She underwent emergency surgery involving orthopedic, vascular and plastic surgical procedures. The treatment consisted in trimming and washing followed by centromedular tibial osteosynthesis, anterior tibial artery bypass, deep peroneal nerve graft and finally soleus muscle flap for covering soft tissue defects. In early 2015, she presented a subcutaneous collection of fluid located close to orthopedic screws which had developed during the previous four months. On 15 June 2015, she was readmitted because of an acute purulent discharge that had started four days earlier. Her body temperature was normal and 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 were sampled as recommended [1]. Three samples of the fluid collection and two necrotic bones fragment located next to the proximal and distal part of the screw were sent to our microbiology laboratory for analysis. All samples grew wild-type *Enterobacter cloacae* (on all media seeded: 5 out of 5) displaying natural resistance pattern to beta-lactams. On the basis of these results and according to the recommendations of *Société de Pathologie Infectieuse de Langue Française* (SPILF) [2] for osteoarticular infections on osteosynthesis material, a six-weeks

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antibiotic course of intra-venous (IV) cefepime (2 g t.i.d) and ciprofloxacin IV (400 mg b.i.d) was initiated. One week later, a new intervention was performed to remove the intramedullary nail materiel. Four surgical samples (3 soft tissues and 1 bone) were sent to our laboratory. Bacteriological analysis revealed *E. cloacae* displaying cephalosporinase hyperproduction phenotype from all biological samples (4 out of 4). At the end of July 2015, 4 weeks after the beginning of antibiotic therapy, she underwent a surgical revision with medial fibula transport and an Ilizarov external fixator was set because of tibial pseudarthrosis. A total of 3 surgical specimens were sampled. Two tibial soft tissues interpositions and one tibial necrotic bone fragment were directly placed into a specific transport medium Ultra Turrax tube (Dutscher, Brumath, France) and sent to our laboratory. Cultures on Columbia agar (Oxoid, Dardilly, France) plates supplemented with 5% sheep blood incubated in anaerobic and aerobic atmosphere, and cultures on chocolate agar plates (Oxoid) incubated in 5% CO_2, remained sterile after 96 h. Culture was positive on Rosenow enriched liquid medium (Biorad, Marnes-la-Coquette, France) after 2 weeks of incubation. Gram staining showed large spore-forming Gram-variable bacilli. When subcultured on blood agar plates under anaerobic conditions two out of three samples grew with medium size mucoid colonies surrounded by a single zone of beta-hemolysis. MALDI-TOF mass spectrometry (MS) performed on the colonies by direct transfer onto target identified *Clostridium indolis* Log score value of 2.19 matching *Clostridium indoli*s DSM 755T; MALDI Biotyper v2.3 (Brüker Daltonics, Bremen, Germany). This identification was confirmed by the National Reference Center of Anaerobic Bacteria and Botulism (Institut Pasteur, Paris, France) by *16S rDNA* gene sequencing using forward AAGGAGGTGATCCAGCCGCA and reverse primers AGAGTTTGATCATGGCTCAG, displaying 99.6% of identity with the sequence of *C. indolis* type strain DSM 755T (GenBank accession number Y18184 [3]). Phylogenetic relationship between the isolated strain (Strain 570.15) and the type strain of the species is shown in Fig. 1. The tree was constructed using a neighbor-joining method (Kimura 2 parameter method) and 500 bootstraps with MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) software as previously described [4]. Values above the

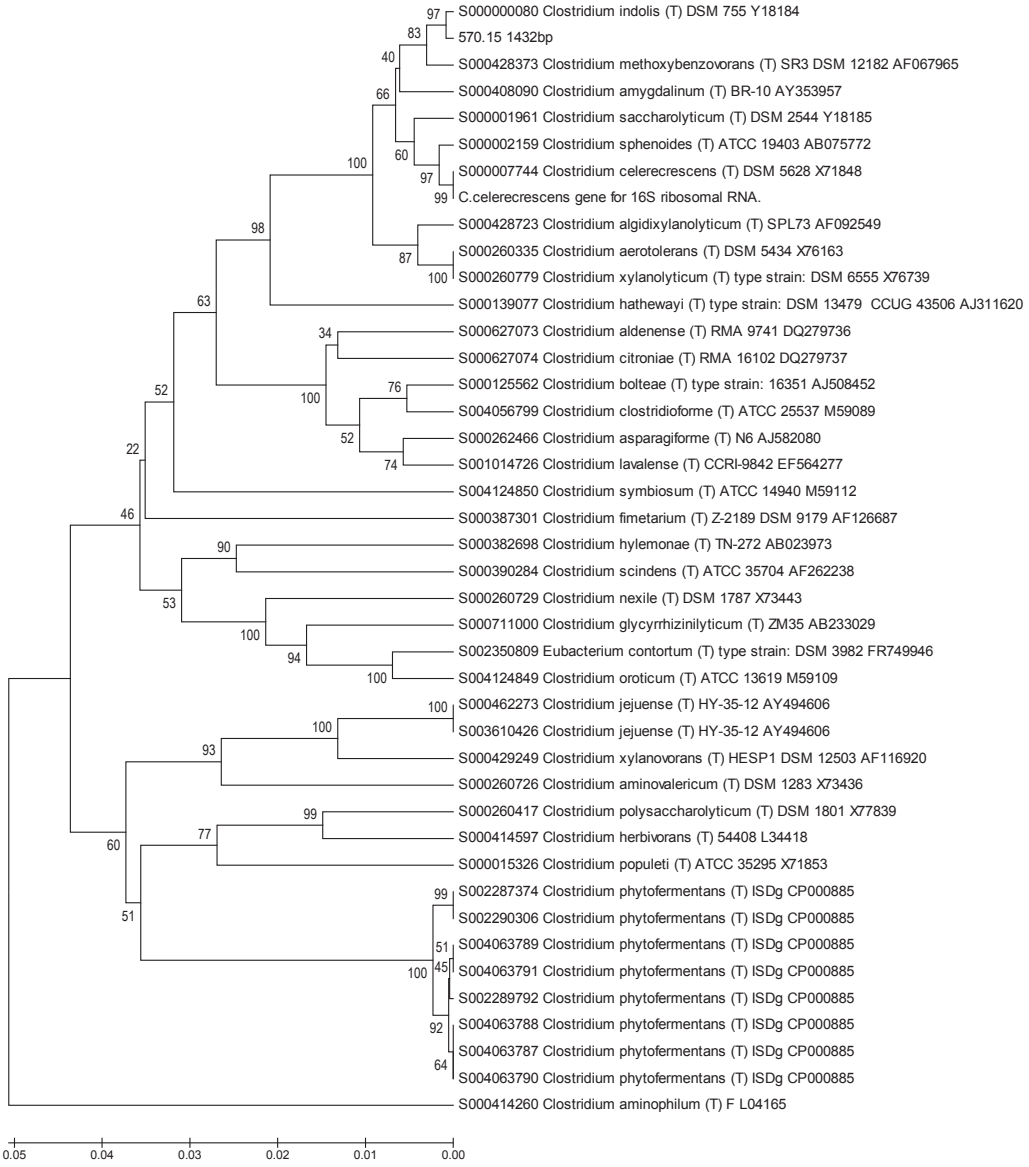

Fig. 1. Evolutionary relationships of strain 570.15. Phylogenetic tree based on 16r DNA sequence analysis as described in the text shows that the strain 570-15 is related to *C. indolis* type strain DSM755T and more distantly related to the clostridia of the *C. saccharolyticum* group.