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1 First description of bacteremia caused by *Oscillibacter valericigenes* in a patient
2 hospitalized for leg amputation.

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12 **Keywords:** Joint and Bone infection; *Oscillibacter*; 16S sequencing; molecular diagnosis

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18 **Highlights :**

- 19 • Culture of *Oscillibacter valericigenes*, a Gram-negative rod, remains very difficult
20 • This Bacterium was, for the first time, isolated during bacteremia
21 • 16S rDNA sequencing is not of interest to predict disease severity

22 Abstract

23 Initially isolated from the alimentary canal of a Japanese corbicula clam, *Oscillibacter*
24 *valericigenes* is a Gram-negative rod, of which culture remains very difficult. Herein we
25 present the first case of bacteremia due to *Oscillibacter valericigenes*, in humans.

26 A 55-year-old man was hospitalized for clinical management of multiple neglected leg
27 wounds (colonized with maggots) that had occurred during a motorcycle accident. Following
28 radiological confirmation of the bone infection, a transfemoral amputation was performed to
29 limit the risk of extended infection.

30 During hospitalization, before the amputation, the patient experienced fever, biological
31 inflammation justifying the sampling of multiple blood cultures. Anaerobic blood culture was
32 positive after 34 hours, without identification by routine procedure (MALDI-TOF), justifying
33 identification by 16S DNA sequencing. In the absence of possible subculture, antibiotic
34 sensitivity testing could not be performed. A pre-emptive treatment by piperacillin-
35 tazobactam was introduced for 14 days. The evolution was good, except for a local disunion.

36 Complete phylogenic analysis of the clinical strain showed that it significantly differed from
37 the reference strain, which is distantly related to the *Clostridia* cluster IV. Due to the culture
38 conditions and specialized identification method by sequencing, prevalence of *O.*
39 *valericigenes* may be underestimated. Optimization of blood culture procedures and
40 utilization of 16S rRNA gene sequencing are tools needed for identification of rare pathogens
41 that could help to optimize clinical management of infected patients.

42 **Introduction**

43 The endospore-forming anaerobic Gram-positive clostridial bacteria are extremely
44 heterogeneous phylogenetically. They form nineteen clusters based on their 16S rDNA gene
45 sequence [1]. Within these clusters, the fourth (Clostridial cluster IV) is also phenotypically
46 heterogeneous. The bacteria belonging to this cluster are present mostly in the alimentary
47 canal and feces of numerous hosts as wood-feeding termites, anaerobic sewage sludge and
48 anaerobic digesters.

49 *Oscillibacter valericigenes* is a Gram-negative rod, motile due to a peritrichous flagella that
50 optimally grows at 30°C (range 15-35°C) [2]. This bacterium remains very difficult to culture.
51 This could explain the underrepresentation of its clinical impact in humans.

52 Herein we present the first case of bacteremia due to *Oscillibacter valericigenes* in humans.

53 **Case Study**

54 A 55-year-old man was hospitalized in the orthopedic department (University Hospital of
55 Poitiers, Poitiers, France) for clinical management of multiple chronic wounds on the left leg
56 that had occurred one year before during a motorcycle accident. Mostly on account of his
57 depression and alcoholism, these wounds were neglected, leading to multiple necrosis and
58 fibroses. Given the overall aspect of the leg and radiological confirmation of the bone
59 infection, it was decided to perform a transfemoral amputation to limit the risk of bacteremia
60 and extension of the infection.

61 During the hospitalization for pre-resection check-up, it was locally observed that the chronic
62 wounds were colonized with maggots. Periosteal tibial and fibular affections (evocative of
63 bone infection) were observed and vascular echography was abnormal, with decreased arterial
64 flow. Before the amputation, the patient experienced fever (39°C) and biological

inflammation (increase in neutrophil counts *ie.* 15220 neutrophils per mm³ for 17700 leukocytes per mm³, elevation of C-Reactive protein *ie* 302 mg/l, associated with inflammatory anemia *ie* 5g/L, necessitating multiple blood transfusions). Given this septic presentation, multiple blood cultures were sampled and anaerobic blood vials were positive after 34 hours of culture. Bacteria could not be identified by routine procedure using MALDI-TOF (bioMérieux, Marcy-l'Étoile, France), justifying the need for 16S DNA sequencing. Given subculture failure, Antimicrobial Susceptibility Testing could not be performed. A probabilistic treatment by Piperacillin-Tazobactam (4 grams three times a day) was introduced for a 14-day post-operative period. Evolution was good, except for local disunion of the scar due to the patient's agitation.

Sanger sequencing of the 16S rDNA V1-V3 regions was performed on 3500 Dx Genetic Analyzer (ThermoFisher Waltham, MA, USA). Obtained sequences were then compared to other available ones, using the LeBibi website to identify *Oscillibacter valericigenes* [3].

A complete phylogenetic analysis of the strain based on 16S analysis showed that this clinical strain differs from the reference strain (Sjm18-20^T) with a pairwise alignment score of 7862963, with dissimilarities on 9 nucleotides (2.34%) out of 384 nucleotide coordinates (24 nucleotides for *Oscillispira guillermondi* on the same coordinates). Phylogenetic analysis is summarized on Figure 1. Unlike some other pathogens, no differentiation could be observed between pathogenic or symbiotic strains based on exclusively phylogenetic analyses of the 16S rDNA gene [4]. Sequences were archives on Sequence Read archives under the accession number n° SRR9717275.

Discussion

We described herein the first case of severe human infection caused by a very rare bacterium, *Oscillibacter valericigenes*.

89 Described for the first time in 2007, in Japan (Shimane Prefecture), the Clostridial bacterium
90 is a Gram-negative, non-sporulating strictly anaerobic rod that belongs to the *Firmicutes*
91 phylum and the *Oscillospiraceae* family [2,5]. A member of the Clostridial cluster IV, one of
92 the clusters based on 16SrRNA gene sequences, this bacterium is distantly related to other
93 members (similarity values less than 91%, on the whole-sequenced genome of more than 4.41
94 Megabases), and most similar to those of uncultured bacteria in animal digestive tracts and
95 feces [1]. It is very interesting to note that phylogenetic analysis using protein-coding genes
96 (such as *ileS*, *valS*, *gyrB* and *rplKLM*) allowed classification of *O. valericigenes* reference
97 strain Sjm18-20^T within cluster IV. As the most closely neighboring anaerobic species
98 described up until now (in our phylogenic analysis of the clinical strain), *Oscillispira*
99 *guillermundii* differ from *Oscillibacter* sp. strain due to several properties, particularly the
100 capacity to generate multicellular structure and endospore, which is impossible for
101 *Oscillibacter valericigenes* [6].

102 This bacterium was initially isolated from the alimentary canal of a Japanese corbicula clam
103 (*Corbicula japonica*) sampled on a seacoast. Similarly, the habitat of anaerobic bacteria
104 belonging to Clostridial cluster IV consists in the alimentary canal and feces of numerous
105 organisms including wood-feeding termites, anaerobic sewage sludge and anaerobic digesters.
106 *O. valericigenes* has also been detected physiologically in human intestinal microbiota. Its
107 association with Crohn's disease suggests its use in clinical diagnosis and/or therapies [7,8].

108 In the present case, the wounds of the patient were colonized with maggots, which may have
109 been the inoculation source of the infection. Bacteraemia involving rare bacteria (like
110 *Ignatzschineria indica* or *Wohlfahrtiimonas chitiniclastica*) have previously been described in
111 patients with chronic wounds colonized by maggots [9,10]. Poor personal hygiene,
112 alcoholism, vascular disease, chronic wound or maggot colonisation have been identified as
113 risk factors for infections due to these bacteria. As described in our case study, identification

114 and culture of *O. valericigenes* is difficult, as 16S rDNA was mandatory to identify the
115 bacteria, and its high susceptibility to oxygen led to non-recovery of a live bacterial strain.
116 Databases of the MALDI-TOF instruments commonly used for rapid diagnosis should be
117 improved in order to allow laboratories not using costly methods (16s rRNA gene
118 sequencing...) to make the diagnosis.

119 In conclusion, due to the difficult culture conditions and specialized identification method by
120 sequencing, the prevalence of *O. valericigenes* bacteremia may be underestimated. Medical
121 biologists and clinicians should pay more attention to rare bacteria such as these in order to
122 clearly determine their clinical significance. Finally, optimization of blood culture procedures
123 (allowing study in an anaerobic atmosphere using an automatic device) and utilization of 16S
124 rRNA gene sequencing are powerful tools for rare pathogen identification from blood and
125 other sterile body fluids; as such, they should be widely utilized in view of optimizing clinical
126 management of infected patients.

127 **Conflict of interest:** none

128 **Authors' contributions :**

129 L.B.: analyzed and wrote the manuscript; L.D.: analyzed and wrote the manuscript; A.M.:
130 ensured clinical management of the patient ; G.L.M.: ensured clinical management of the
131 patient ; C.B.: ensured clinical management of the patient ; L-E.G.: ensured clinical
132 management of the patient ; C.P.: collected the data, wrote the manuscript ; M.P.: collected
133 the data, analyses, wrote the manuscript

134 All the authors approved the final version of the article.

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180 **Table and Figure**

181 **Figure 1. Phylogenic analysis highlighting the position of the clinical strain relative to**
182 **other representative strains within the Clostridial cluster IV.** The phylogenic tree was
183 constructed by the ML-Method based on the alignment of 1,614 informative sites of 16S
184 rDNA gene sequences. Corresponding NCBI accession numbers are shown in parentheses.
185 Numbers at nodes indicate support values obtained from 1,000 bootstrap replications.
186 *Clostridium butyricum* does not belong to *Clostridia* cluster IV and was used as an outgroup.
187 The analysis was performed using the V1-V3 consensus sequence obtained by sequencing the
188 cultured strain of the bacteria. In blue, the bacterial strains never associated with a human
189 clinical outcome or pathological status are represented. The bacterial strains associated with a
190 beneficial or detrimental outcome are represented in green and red respectively.

