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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

65 inflammation (increase in neutrophil counts *ie.* 15220 neutrophils per mm<sup>3</sup> for 17700  
66 leukocytes per mm<sup>3</sup>, elevation of C-Reactive protein *ie* 302 mg/l, associated with  
67 inflammatory anemia *ie* 5g/L, necessitating multiple blood transfusions). Given this septic  
68 presentation, multiple blood cultures were sampled and anaerobic blood vials were positive  
69 after 34 hours of culture. Bacteria could not be identified by routine procedure using MALDI-  
70 TOF (bioMérieux, Marcy-l'Étoile, France), justifying the need for 16S DNA sequencing.  
71 Given subculture failure, Antimicrobial Susceptibility Testing could not be performed. A  
72 probabilistic treatment by Piperacillin-Tazobactam (4 grams three times a day) was  
73 introduced for a 14-day post-operative period. Evolution was good, except for local disunion  
74 of the scar due to the patient's agitation.

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

78 A complete phylogenetic analysis of the strain based on 16S analysis showed that this clinical  
79 strain differs from the reference strain (Sjm18-20<sup>T</sup>) with a pairwise alignment score of  
80 7862963, with dissimilarities on 9 nucleotides (2.34%) out of 384 nucleotide coordinates (24  
81 nucleotides for *Oscillispira guillermondi* on the same coordinates). Phylogenetic analysis is  
82 summarized on Figure 1. Unlike some other pathogens, no differentiation could be observed  
83 between pathogenic or symbiotic strains based on exclusively phylogenetic analyses of the 16S  
84 rDNA gene [4]. Sequences were archived on Sequence Read archives under the accession  
85 number n° SRR9717275.

## 86 **Discussion**

87 We described herein the first case of severe human infection caused by a very rare bacterium,  
88 *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<sup>T</sup> 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 *guillermondii* 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.

