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1 **Surgical site infection after hip replacement due to a novel *Peptoniphilus* species,**
2 **provisionally named '*Peptoniphilus nemausus*' sp. nov.**

3

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23

24 **Abstract**

25 We report a case of surgical site infection after total hip prosthesis replacement due to an
26 ofloxacin-resistant *Peptoniphilus* isolate belonging to an unknown species for which the name
27 '*Peptoniphilus nemausus*' sp. nov. is proposed. Follow-up was favourable under clindamycin
28 and rifampin for 3 months in this patient whom had a *Proteus mirabilis* infection treated by
29 fluoroquinolone.

30

31 **Key words:** *Peptoniphilus*, infection, anaerobe, resistance, surgical site infection, prosthetic
32 joint infection.

33

34 **Text**

35 Gram-positive anaerobic cocci (GPAC) are important members of the human
36 microbiota, that can also act as opportunistic pathogens in humans. GPAC were shown to be
37 the more frequently isolated anaerobes in microbiological laboratories (24-31% of the total
38 number of isolated anaerobes) [1,2]. While *Finegoldia magna* and *Parvimonas micra*
39 represent about half of the isolated GPAC [3,4] and are the most studied, several less known
40 genera of GPAC like *Anaerococcus* and *Peptoniphilus*, are involved in various opportunistic
41 human infections, mainly as part of polymicrobial infections [2].

42
43 A 66-year old woman was admitted to the rehabilitation unit of the University
44 Hospital of Nîmes on March 8, 2018, after revision of her total hip prosthesis on March 1.
45 The patient presented with morbid obesity (body mass index 52 defining grade 3 obesity). She
46 had no hormone replacement treatment since menopause that occurred at the age of 55. Her
47 history includes arterial hypertension, breast cancer in remission after surgery and
48 radiotherapy, and under current hormonal therapy by letrozole, an aromatase inhibitor with
49 bone loss side effects. Initial arthroplasty was performed on February 8, for painful hip and
50 functional impotence revealing extensive osteolysis of the femoral head with previously
51 undiagnosed osteoporosis. At the same time, a Vitamin D deficiency of 10 nmol/L (normal
52 range: 30-100 nmol/L) was found requiring supplementation. Early periprosthetic fracture
53 occurred at weightbearing initiation and hip prosthesis replacement, including removal of the
54 failed implant, lavage and implantation of a femoral component that has a long stem, was
55 performed on March 1. Microbiological investigations showed an early prosthetic joint
56 infection (PJI), as the 3 samples taken during hip prosthesis revision were positive for *Proteus*
57 *mirabilis*. PJI was treated by intravenous ofloxacin (600 mg per day) and the introduction of
58 bisphosphonates to correct the osteoporosis and the vitamin D deficiency.

59 In the rehabilitation unit, a surgical site infection was suspected at the beginning of April and
60 confirmed by CT-scan on April 10. Surgical lavage and debridement were performed on
61 April, 12th as part of the management of the infection with Debridement, Antibiotics,
62 Irrigation and Retention (DAIR). Eight surgical samples were obtained (1 periprosthetic fluid,
63 4 periprosthetic tissue and 3 bone samples). Direct examination showed either rare or rather
64 numerous polymorphonuclear depending on the sample and Gram-positive cocci were
65 visualized after Gram stain of a periprosthetic tissue sample leading to the instauration of an
66 intravenous antimicrobial therapy by cefotaxime plus vancomycin. Samples were analyzed
67 according to national recommendations [5]. Anaerobic cultures were positive after 7 days of
68 incubation of the periprosthetic fluid and the 4 tissue specimens and grew a strictly anaerobic
69 Gram-positive coccus. The three bone samples remained negative. Identification by MALDI-
70 TOF mass spectrometry (Vitek® MS, bioMérieux, Marcy-l’Etoile, France) was unsuccessful.
71 Antimicrobial susceptibility testing was performed using Etest strips (bioMérieux) according
72 to the recommendations of the Antibiogram committee of the French Society for
73 Microbiology for anaerobes [6]. The isolate was susceptible to all antibiotics tested (MICs of
74 imipenem and rifampin < 0.02 mg/L, MICs of amoxicillin and coamoxiclav. < 0.016 mg/L,
75 MIC of metronidazole 0.016 mg/L, MIC of linezolid 0.125 mg/L and MIC of clindamycin
76 0.75 mg/L) except ofloxacin (MIC > 32 mg/L). The multidisciplinary team for the
77 management of PJI of our hospital decided an antimicrobial treatment switch to clindamycin
78 (2400 mg per day) and rifampin (1200 mg per day) for 3 months, on April 23th. A favourable
79 outcome was noted after the end of the treatment and a one-year period of clinical follow-up
80 after a novel total hip prosthesis has been implanted in July 2018.
81 For the identification of the GPAC isolated in pure culture from a deep-tissue infection, we
82 tested the isolate with another commercially available MALDI-TOF MS system, (Maldi
83 Biotyper Microflex®, Bruker Daltonics, Bremen, Germany), as differences in identification

84 performances between MALDI-TOF systems have been previously reported for identification
85 of anaerobes [7]; however, no identification was obtained for the clinical isolate using this
86 alternative MALDI-TOF MS system. We also performed 16S rRNA gene sequencing as
87 previously described [8]. Sequence analysis (1388 nt) showed the isolate, belonged to the
88 genus *Peptoniphilus*, but to an as yet unknown species. Indeed, a similarity table constructed
89 using utilities implemented in Biological sequence alignment editor (BioEdit) software
90 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>) revealed that the type strains of
91 *Peptoniphilus coxii* (97.9% of 16S rRNA gene sequence identity) and *Peptoniphilus ivorii*
92 (94.6%), as well as the type strains of the two non validated species '*Peptoniphilus*
93 *urinimassiliensis*' (96.6%) and '*Peptoniphilus pacaensis*' (96.1%) were the most closely
94 related species of the clinical isolate [9-12]. However, the highest 16S rRNA gene sequence
95 identity observed between the clinical isolate (strain 1804121828, GenBank accession
96 number: MK945758) and the type strain of *Peptoniphilus coxii* was below the threshold for
97 species identification, *i.e.*, less than 98.7% of 16S rRNA gene identity [13], suggesting the
98 clinical isolate to belong to a novel species in the genus *Peptoniphilus* [14]. The 16S rRNA
99 gene sequence of the clinical isolate was also compared with those of the type strains of
100 species of the genus *Peptoniphilus* through phylogenetic analysis. Evolutionary distances
101 were analysed using the neighbour-joining (NJ) method (Kimura two-parameter substitution
102 model) using phylogenetic analyses available at <http://www.phylogeny.fr> [14]. Phylogenetic
103 analysis supported the inclusion of the isolate in a new species based on a clearly
104 individualized branching within the genus *Peptoniphilus* and the cluster *P. coxii* / '*P.*
105 *pacaensis*' / '*P.urinimassiliensis*' / *P. ivorii* (Figure 1). A formal characterization of the novel
106 species is ongoing and the name '*Peptoniphilus nemausus*' sp. nov. is proposed for this novel
107 species pertaining to the Nîmes town in the south of France, where the strain supporting the
108 description of the species was isolated.
109

110 The genus *Peptoniphilus* was individualized in 2001 to accommodate strictly
111 anaerobic Gram-positive cocci previously classified in the genus *Peptostreptococcus* that
112 were butyrate-producers, non-saccharolytic and that used peptone and amino acids as major
113 energy sources [15]. Since then, a growing number of species has been described and
114 currently 17 species are validly published (<http://www.bacterio.net/peptoniphilus.html>) [16]
115 and 9 others have been proposed without current valid publication (May 20, 2019) (Figure 1).
116 Among the genus, species can be distinguished by phenotypic assays (allowing the
117 determination of a metabolic profile) that are not routinely performed in clinical microbiology
118 laboratories, particularly since the development of MALDI-TOF MS; therefore, species
119 identification is currently based on mass spectrometry and, when unsuccessful, on molecular
120 tools [17]. MALDI-TOF MS is a powerful and rapid identification tool; however, databases
121 are currently incomplete and optimization of current databases for anaerobes is ongoing [18-
122 20]. MS was unable to identify our clinical isolate and 16S rRNA gene sequence analysis was
123 required revealing the clinical isolate to belong to an unknown species and showing a still
124 underestimated diversity in this genus. A formal description of this new species based on a
125 polyphasic taxonomy approach has been undertaken.

126 Members of the different human microbiota, *Peptoniphilus* spp. have been reported in a large
127 variety of human endogeneous polymicrobial infections due to the pathogenesis process of
128 such infections, *i.e.*, polymicrobial infections involving members of the contiguous
129 microbiota through contamination of initially sterile anatomical sites. Clinical relevance of
130 *Peptoniphilus* spp. has been mainly demonstrated after isolation from skin and soft tissues,
131 chronic wounds (pressure ulcer, diabetic foot wounds), osteoarticular samples, genitourinary
132 (vaginal infections) and respiratory tract (pleural empyema, chronic rhinosinusitis) [2].
133 Anaerobic infections remain rare in patients with prosthetic joints and mostly involved
134 species originating from the cutaneous microbiota like *Cutibacterium* (formerly

135 *Propionibacterium) acnes* and *Finegoldia magna* [21-24]. Despite *Peptoniphilus* spp. have
136 been previously identified during osteoarticular [21,25,26] and soft tissue infections [27,28],
137 we were unable to find a case similar to that described herein, *i.e.*, surgical site infection
138 following PJI, among the 122 publications retrieved in the PubMed database using the
139 “*Peptoniphilus*” search term (July 8, 2019). In the present case, despite anaerobes were not
140 reported during initial infection and the portal of entry or origin of the *Peptoniphilus* isolate
141 remained unidentified, it is likely that it has been selected by ofloxacin therapy towards initial
142 *P. mirabilis* infection, as the isolate displayed high level resistance to ofloxacin.
143 Fluoroquinolones are one of the therapeutic options in the management of osteoarticular
144 infections in case of susceptibility of the causative microorganism, as they displayed good
145 penetration profiles into bone tissues and synovial fluid [29]. If the antimicrobial
146 susceptibility patterns of the main encountered anaerobic pathogens in bone and joint
147 infections, *C. acnes* and *F. magna*, is documented, antimicrobial resistance patterns of the
148 overall GPAC have received less interest being for long considered as microorganisms
149 susceptible to antibiotics with anti-anaerobic activity. However, studies including or focused
150 on GPAC revealed high rate of resistance towards some antibiotics used in the management
151 of osteoarticular anaerobic infections, 25% of GPAC displayed resistance to clindamycin in
152 most recent studies for example [22,29]; reported some multidrug resistant clinical isolates
153 [30] while revealing heterogeneity in antibiotic susceptibility patterns between species
154 [4,29,31,32]. Regarding ofloxacin, a large study conducted in France, *i.e.*, 170 GPAC isolated
155 from diverse anatomical sites including 16.5% of *Peptoniphilus* spp. all identified by 16S
156 rRNA gene sequencing, showed a global rate of resistance of 63% but revealed that all
157 *Peptoniphilus* - but also all *Anaerococcus* - clinical isolates studied displayed resistance to
158 ofloxacin [3]. In case of deep monomicrobial infection of a normally sterile body site as in the
159 present case, antimicrobial susceptibility testing is recommended to guide the treatment [33];

160 however, in case of polymicrobial infection involving both aerobes and anaerobes or several
161 anaerobes, antimicrobial susceptibility testing is usually less systematically performed on all
162 isolated anaerobes and one should then consider the presence of potentially resistant
163 microorganisms, not only members of the *Bacteroides fragilis* group but also some GPAC,
164 among the cultivable microbiota in the choice of the best therapeutic option.

165

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175

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283

284 **Legend to figure**

285 **Fig. 1.** Neighbor-joining phylogenetic tree showing the relationship between the 16S rRNA
286 gene sequences of *Peptoniphilus* strain 1804121828^T (type strain of the proposed novel
287 species '*Peptoniphilus nemausus*') and of species, either validated or not, in the genus
288 *Peptoniphilus*. Alignment length was 1166 nt. Names for effectively published but non-
289 validated species are indicated between quotes. GenBank accession numbers are indicated in
290 parentheses. Bootstrap support was computed after 1000 reiterations. Bootstrap values are
291 indicated at the corresponding nodes when >70%. *Ezakiella peruensis* was used as the
292 outgroup microorganism.

293 * indicates species with uncertain taxonomic status, as *P. senegalensis* and '*P. rhinitis*' might
294 be synonym species of *P. tyrrelliae* and *P. lacydoensis*, respectively.

