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

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Abstract

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

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

Text

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

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

In the rehabilitation unit, a surgical site infection was suspected at the beginning of April and confirmed by CT-scan on April 10. Surgical lavage and debridement were performed on April, 12th as part of the management of the infection with Debridement, Antibiotics, Irrigation and Retention (DAIR). Eight surgical samples were obtained (1 periprosthetic fluid, 4 periprosthetic tissue and 3 bone samples). Direct examination showed either rare or rather numerous polymorphonuclear depending on the sample and Gram-positive cocci were visualized after Gram stain of a periprosthetic tissue sample leading to the instauration of an intravenous antimicrobial therapy by cefotaxime plus vancomycin. Samples were analyzed according to national recommendations [5]. Anaerobic cultures were positive after 7 days of incubation of the periprosthetic fluid and the 4 tissue specimens and grew a strictly anaerobic Gram-positive coccus. The three bone samples remained negative. Identification by MALDI-TOF mass spectrometry (Vitek® MS, bioMérieux, Marcy-l'Etoile, France) was unsuccessful. Antimicrobial susceptibility testing was performed using Etest strips (bioMérieux) according to the recommendations of the Antibiogram committee of the French Society for Microbiology for anaerobes [6]. The isolate was susceptible to all antibiotics tested (MICs of imipenem and rifampin < 0.02 mg/L, MICs of amoxicillin and coamoxiclav. < 0.016 mg/L, MIC of metronidazole 0.016 mg/L, MIC of linezolid 0.125 mg/L and MIC of clindamycin 0.75 mg/L) except ofloxacin (MIC > 32 mg/L). The multidisciplinary team for the management of PJI of our hospital decided an antimicrobial treatment switch to clindamycin (2400 mg per day) and rifampin (1200 mg per day) for 3 months, on April 23th. A favourable outcome was noted after the end of the treatment and a one-year period of clinical follow-up after a novel total hip prosthesis has been implanted in July 2018.

For the identification of the GPAC isolated in pure culture from a deep-tissue infection, we tested the isolate with another commercially available MALDI-TOF MS system, (Maldi Biotyper Microflex®, Bruker Daltonics, Bremen, Germany), as differences in identification

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

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

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

Propionibacterium) *acnes* and *Finegoldia magna* [21-24]. Despite *Peptoniphilus* spp. have been previously identified during osteoarticular [21,25,26] and soft tissue infections [27,28], we were unable to find a case similar to that described herein, *i.e.*, surgical site infection following PJI, among the 122 publications retrieved in the PubMed database using the "*Peptoniphilus*" search term (July 8, 2019). In the present case, despite anaerobes were not reported during initial infection and the portal of entry or origin of the *Peptoniphilus* isolate remained unidentified, it is likely that it has been selected by ofloxacin therapy towards initial *P. mirabilis* infection, as the isolate displayed high level resistance to ofloxacin.

Fluoroquinolones are one of the therapeutic options in the management of osteoarticular infections in case of susceptibility of the causative microorganism, as they displayed good penetration profiles into bone tissues and synovial fluid [29]. If the antimicrobial susceptibility patterns of the main encountered anaerobic pathogens in bone and joint infections, *C. acnes* and *F. magna*, is documented, antimicrobial resistance patterns of the overall GPAC have received less interest being for long considered as microorganisms susceptible to antibiotics with anti-anaerobic activity. However, studies including or focused on GPAC revealed high rate of resistance towards some antibiotics used in the management of osteoarticular anaerobic infections, 25% of GPAC displayed resistance to clindamycin in most recent studies for example [22,29]; reported some multidrug resistant clinical isolates [30] while revealing heterogeneity in antibiotic susceptibility patterns between species [4,29,31,32]. Regarding ofloxacin, a large study conducted in France, *i.e.*, 170 GPAC isolated from diverse anatomical sites including 16.5% of *Peptoniphilus* spp. all identified by 16S rRNA gene sequencing, showed a global rate of resistance of 63% but revealed that all *Peptoniphilus* - but also all *Anaerococcus* - clinical isolates studied displayed resistance to ofloxacin [3]. In case of deep monomicrobial infection of a normally sterile body site as in the present case, antimicrobial susceptibility testing is recommended to guide the treatment [33];

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

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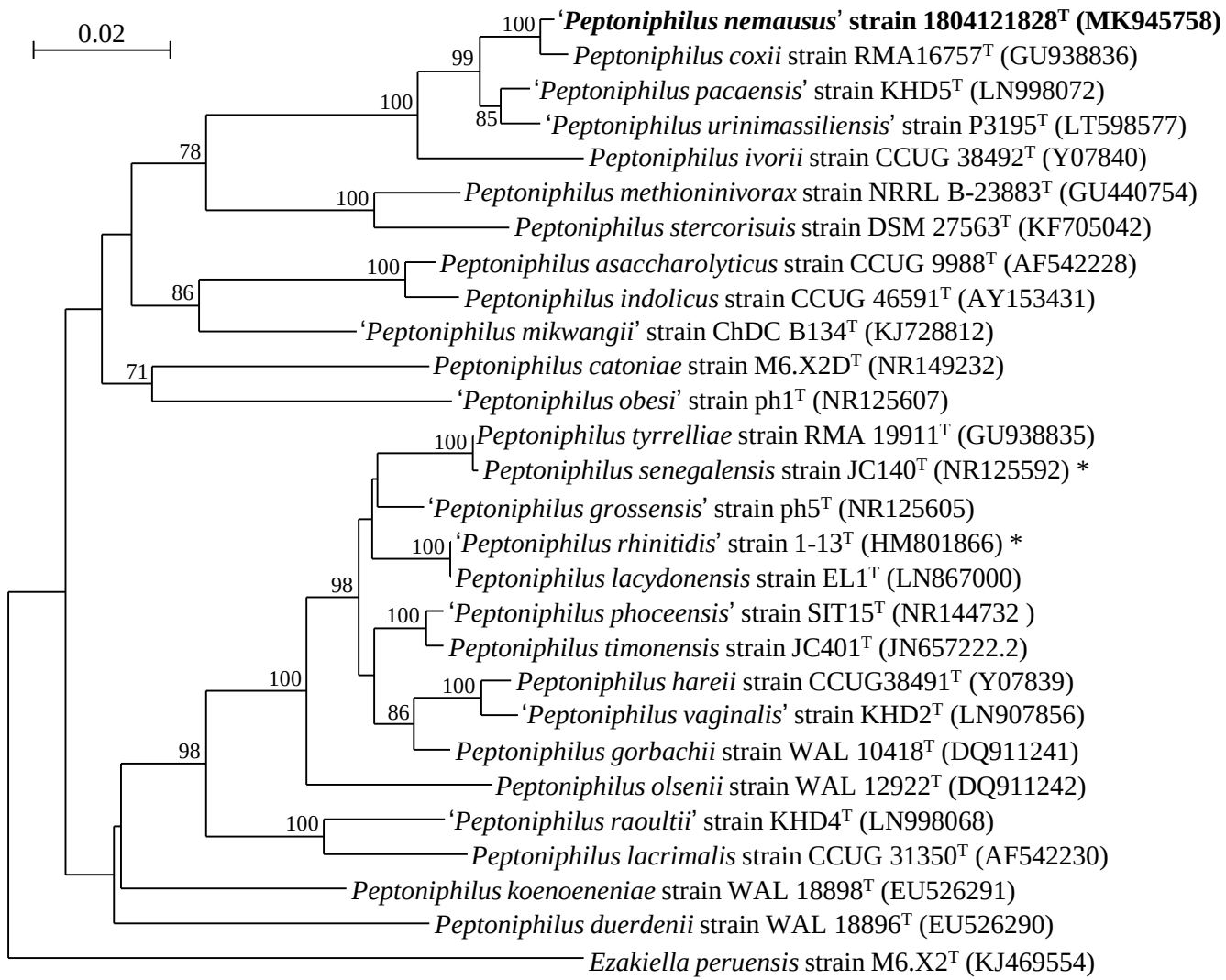
283

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.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‘*Peptoniphilus nemausus*’ strain 1804121828^{T} (MK945758)
Peptoniphilus coxii strain RMA16757^{T} (GU938836)
‘*Peptoniphilus pacaensis*’ strain KHD5^{T} (LN998072)
‘*Peptoniphilus urinimassiliensis*’ strain P3195^{T} (LT598577)
Peptoniphilus ivorii strain CCUG 38492^{T} (Y07840)
Peptoniphilus methioninivorax strain NRRL B-23883^{T} (GU440754)
Peptoniphilus stercorisuis strain DSM 27563^{T} (KF705042)
Peptoniphilus asaccharolyticus strain CCUG 9988^{T} (AF542228)
Peptoniphilus indolicus strain CCUG 46591^{T} (AY153431)
‘*Peptoniphilus mikwangii*’ strain ChDC B134^{T} (KJ728812)
Peptoniphilus catoniae strain M6.X2D^{T} (NR149232)
‘*Peptoniphilus obesi*’ strain ph1^{T} (NR125607)
Peptoniphilus tyrrelliae strain RMA 19911^{T} (GU938835)
Peptoniphilus senegalensis strain JC140^{T} (NR125592) *
‘*Peptoniphilus grossensis*’ strain ph5^{T} (NR125605)
‘*Peptoniphilus rhinitidis*’ strain 1-13^{T} (HM801866) *
Peptoniphilus lacydonensis strain EL1^{T} (LN867000)
‘*Peptoniphilus phoceensis*’ strain SIT15^{T} (NR144732)
Peptoniphilus timonensis strain JC401^{T} (JN657222.2)
Peptoniphilus hareii strain CCUG38491^{T} (Y07839)
‘*Peptoniphilus vaginalis*’ strain KHD2^{T} (LN907856)
Peptoniphilus gorbachii strain WAL 10418^{T} (DQ911241)
Peptoniphilus olsenii strain WAL 12922^{T} (DQ911242)
‘*Peptoniphilus raoultii*’ strain KHD4^{T} (LN998068)
Peptoniphilus lacrimalis strain CCUG 31350^{T} (AF542230)
Peptoniphilus koenoeneniae strain WAL 18898^{T} (EU526291)
Peptoniphilus duerdenii strain WAL 18896^{T} (EU526290)
Ezakiella peruensis strain M6.X2^{T} (KJ469554)