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Letter to the Editor

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Is *Propionimicrobium lymphophilum* a new urinary tract infection cause?

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Sir,

Propionimicrobium lymphophilum is a non-sporulating anaerobic Gram-positive bacillus belonging to the family *Propionibacteriaceae* and is part of the microbiota of the skin and the human urinary tract. Originally described as *Bacillus lymphophilum* in 1916, after several taxonomic changes, which included *Corynebacterium lymphophilum* and *P. lymphophilum*, the current nomenclature of *P. lymphophilum* has been adopted [1]. After reviewing the literature, five cases of infections caused by this microorganism have been published, among which only two of them corresponded to urinary tract infections (UTIs) [2-5].

An 83-year-old woman visited her family physician for dysuria and bladder urgency, without fever or other associated symptoms. Her medical history of interest included osteoporosis treated with subcutaneous denosumab every 6 months and repeated UTIs with an average of four episodes per year. In addition, an urological study was carried out two years ago due to the chronic picture of difficulty in initiating urination and sensation of incomplete emptying. The abdominal ultrasound showed a simple cortical cyst of 8.6 cm in the upper pole of the right kidney and a sinus cyst of 3.7 cm in the upper pole of the left kidney, with both kidneys showing good corticomedullary differentiation and no dilatation of the excretory tract. It was decided to initiate continuous antibiotic prophylaxis, at first with a dose of fosfomycin 500 mg every 24 hours orally and later modified to 3 g of fosfomycin-trometamol orally weekly, although adherence to treatment was irregular.

One month before the current episode, she had presented UTI due to *Escherichia coli*, and was treated with 500 mg of fosfomycin orally (every 8 h for 10 days). Given the persistence of symptomatology, an urine sample was again collected by spontaneous urination and referred to the Microbiology Department.

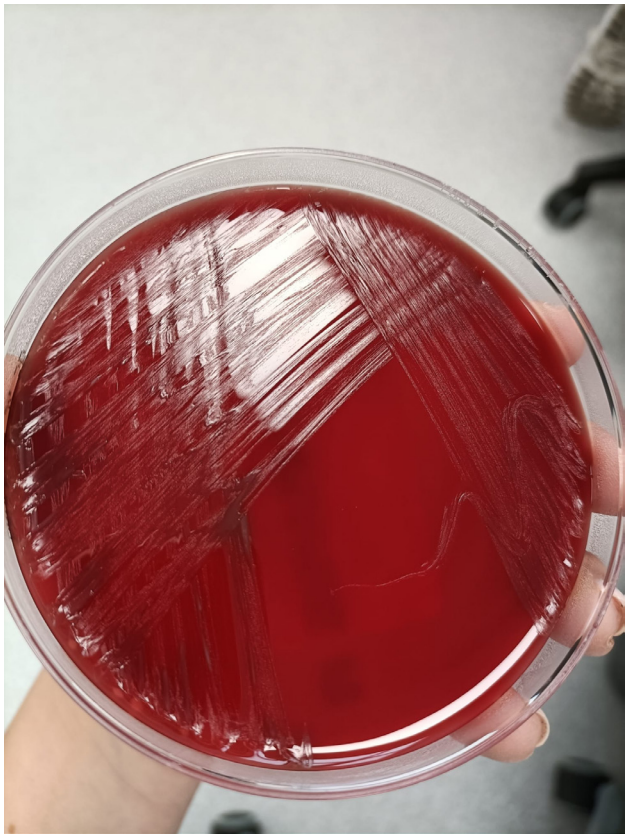

Figure 1 Growth of dark-greyish colonies in Brucella-blood agar with hemine and vitamin K1 after 48 h of incubation in anaerobiosis.

An urinalyses showed positive nitrites and 500/µL leuko-

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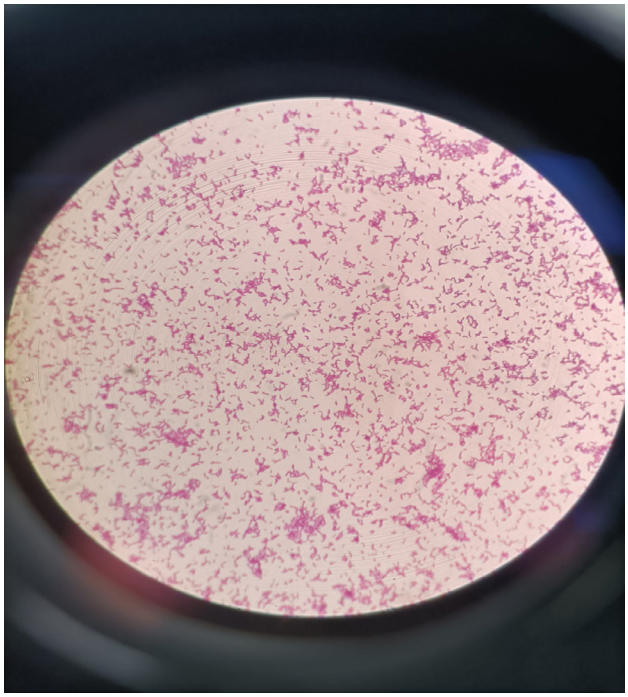

Figure 2 | **Gram-positive bacillus were observed in the gram stain (10x100 scale).**

cytes. Furthermore, according to the work routine established in our laboratory, urine was also analyzed by flow cytometry in the UF-5000 Sysmex© system, which detected bacteriuria and leukocyturia (83,000 bacteria/µL and 208 leukocytes/µL). Then, 1 µL of the urine was seeded in BD© Chromagar Orientation Medium incubated in aerobiosis at 35±2ºC, but no growth was observed at 24 hours. Due to the patient's history and the high bacteriuria detected by cytometry, much higher than 500 BACT/µL -our center's cut-off point for seeding urine from women in Primary Care-, the sample was cultured in BD© Brucella Blood Agar with Hemin and Vitamin K1 and in BD© Chocolate Agar (GC II Agar with IsoVitaleX) at 35-37ºC, in anaerobiosis and with 5% CO_2, respectively. After 48 hours of incubation, small non-hemolytic and grayish colonies (Figure 1) were isolated on Brucella agar, with a count >100,000 CFU/ml. A Gram stain was performed directly from the culture and Gram-positive bacillus were observed (Figure 2). Identification was carried out by mass spectrometry (MALDI-TOF®, Bruker Daltonics), resulting in *P. lymphophilum* with score 2.01.

Because of the isolation of this microorganism is very infrequent, we confirmed the identification by sequencing the 16S rRNA gene (primers 27F and rP2R), following the protocol proposed by Oldham AL et al. [6]. The obtained sequence of 597 bp was introduced in Blast©, whose identification percentage of 97.65% with the *P. lymphophilum* type sequence. In addition, the 16S sequence was registered in GenBank (NCBI) with accession number ON564646.

The susceptibility test was performed using gradient diffusion strips (Liofilchem®) on Brucella agar and anaerobiosis. Interpretation was performed according to the CLSI committee (2022) cut-off points for anaerobic microorganisms, except for ciprofloxacin and trimethoprim-sulfamethoxazole which consulted the CLSI «fastidious» bacteria document (2015) [7,8]. Our strain was determined to be susceptible to penicillin (MIC = 0.125 mg/L), amoxicillin/clavulanate (MIC = 0.125 mg/L), ceftriaxone (MIC = 0.5 mg/L), ciprofloxacin (MIC = 1 mg/L) and moxifloxacin (MIC = 1 mg/L), while it was considered resistant for clindamycin (MIC > 256 mg/L), metronidazole (MIC > 256 mg/L), fosfomycin (MIC >256 mg/dL) and trimethoprim-sulfamethoxazole (MIC > 32 mg/L).

The patient was finally diagnosed with lower UTI and treated with penicillin V 500 mg/8 h for 10 days with good evolution and complete resolution of symptoms. In addition, two urine samples were sent one month after the episode, whose urinalyses yielded negative nitrites and none leukocytes. The other sample was analyzed by flow cytometry in the UF-5000 Sysmex© system, which only detected 191 bacteria/µL and 125 leukocytes/µL.

The pathogenic value of *P. lymphophilum* as a cause of human infections remains to be determined. Only three cases of bacteremia have been described, one of them of urinary focus, and two cases of UTI previously, this being the third case of urinary tract infection described to date [2-5]. Being part of the commensal flora of the skin and urinary tract, a key point should be to differentiate whether the finding of this microorganism has clinical significance. In our case, the patient referred urinary symptomatology, the culture of *P. lymphophilum* was pure and at a significant count of ≥10^5 UFC/ml and the symptoms subsided after the prescribed antibiotic.

In the case of UTIs caused by infrequent microorganisms with demanding growth, the use of flow cytometry can be very useful not only as a screening method prior to urine culture. In the presence of a negative culture on standard media in a symptomatic patient with high bacteriuria detected by cytometry, we suggest seeding the sample on additional culture media, a strategy that allows isolation of demanding pathogens such as *Aerococcus spp* or *Actinotignum schaalii* [9].

The emergence of MALDI-TOF mass spectrometry and the advance of molecular biology with techniques such as 16S rRNA gene sequencing or whole genome sequencing have been a turning point in microbiological identification. The introduction of MALDI-TOF in the routine of microbiology laboratories has replaced phenotypic identification by biochemical reactions, since it is a fast, economical and accurate tool. Its usefulness in the identification of anaerobic microorganisms and detection of new species when the value obtained is ≥ 2.0 has been widely demonstrated [10].

Regarding antibiotic susceptibility, our strain presented low MICs to beta-lactams and moxifloxacin, and resistance to clindamycin, metronidazole, fosfomycin and trimethoprim-sulfamethoxazole, an antibiotic widely used in low UTIs, a profile consistent with published cases [2-5]. Although ciprofloxacin is not active against most anaerobic bacteria, in this

case the MIC was 1 mg/L, which could have been a treatment option if the patient was allergic to beta-lactams.

With the publication of this case we add scientific evidence to consider *P. lymphophilum* as an emerging anaerobe responsible for human infections, although more cases are needed to analyze the risk factors and clinical characteristics of infection by this microorganism. In addition, we would like to highlight the usefulness of combining flow cytometry in urine samples together with MALDI-TOF in daily laboratory practice to identify new emerging demanding pathogens as in our case.

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None to declare

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Stackebrandt E, Schumann P, Schaal KP, Weiss N. *Propionimicrobium* gen. nov., a new genus to accommodate Propionibacterium lymphophilum (Torrey 1916) Johnson and Cummins 1972, 1057AL as *Propionimicrobium lymphophilum* comb. nov. Int J Syst Evol Microbiol. 2002;52(Pt 6):1925-7. doi: 10.1099/00207713-52-6-1925.
2. Cobo F, García-Salcedo JA, Navarro-Marí JM. Bacteremia caused by *Propionibacterium (Propionimicrobium) lymphophilum*. Enferm Infecc Microbiol Clin (Engl Ed). 2021;39:48-49. doi: 10.1016/j.eimc.2020.05.003.
3. Ikeda M, Kobayashi T, Suzuki T, Wakabayashi Y, Ohama Y, Maekawa S, Takahashi S, Homma Y, Tatsuno K, Sato T, Okugawa S, Moriya K, Yotsuyanagi H. *Propionimicrobium lymphophilum* and *Actinotignum schaalii* bacteraemia: a case report. New Microbes New Infect. 2017;18:18-21. doi: 10.1016/j.nmni.2017.03.004.
4. Cobo F, Gómez E, Rodríguez-Granger J, Sampedro A, García-Salcedo JA, Navarro-Marí JM. A rare case of bacteremia caused by *Propionibacterium (Propionimicrobium) lymphophilum*. Anaerobe. 2020;62:102166. doi: 10.1016/j.anaerobe.2020.102166.
5. Williams GD. Two Cases of Urinary Tract Infection Caused *by Propionimicrobium lymphophilum*. J Clin Microbiol. 2015;53:3077-80. doi: 10.1128/JCM.00438-15.
6. Oldham AL, Duncan KE. Similar gene estimates from circular and linear standards in quantitative PCR analyses using the prokaryotic 16S rRNA gene as a model. *PLoS One*. 2012;7:e51931. doi: 10.1371/journal.pone.0051931
7. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.
8. CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
9. Pérez-Ramos IS, I. Angulo López, M. de la Pena-Trigueros et al. Utilidad de la citometría de flujo en el diagnóstico de infecciones del tracto urinario por microorganismos exigentes: a propósito de un caso de cistitis aguda por *Actinotignum schaalii*. Enferm Infecc Microbiol Clin. 2021. Doi: 10.1016/j.eimc.2021.08.007
10. Li Y, Shan M, Zhu Z, et al. Application of MALDI-TOF MS to rapid identification of anaerobic bacteria. BMC Infect Dis. 2019;19(1):941. Published 2019 Nov 7. doi:10.1186/s12879-019-4584-0