



First report of *Myroides phaeus* bacteraemia identified by Polymerase chain reaction and genetic sequencing

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ABSTRACT

We report the first case of *Myroides phaeus* isolated from blood, causing bacteremia in an immunocompromised patient using the automated MicroScan Walk Away 96 system, followed by bacterial identification by amplification-sequencing of the 16S rDNA. The sequences obtained were compared with the reference sequence of the BLAST[®] platform - National Library of Medicine, USA, and the isolation was identified as *Myroides phaeus* strain with 99.67% identity in Blast report. In the literature we did not find previous reported cases of infections by this bacterium, however its pathogenic role is still controversial; therefore, this isolation alerts us to carry out an exhaustive surveillance of other possible acquisition routes.

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Introduction

The genus *Myroides* is a heterogeneous group of gram negative, aerobic, non-fermenting, non-mobile bacilli. In 1996 Vancanneyt et al. reclassified *Flavobacterium odoratum* into 2 different species: *Myroides odoratum* and *Myroides odoratimimus* [1]. Both are considered low-grade opportunistic pathogens that cause community-acquired and in-hospital infections, including urinary tract infections, skin and soft tissue infections, bacteremia, pneumonia, and intra-abdominal infections, most often described in immunocompromised hosts [2,3]. In recent years, 2 species that had not previously been described as human pathogens, *Myroides phaeus* isolated from saliva [4] and *Myroides injenensis* isolated from urine [5] were isolated. We present the case of an immunosuppressed patient, due to multiple myeloma in addition to chronic kidney disease on hemodialysis who developed bacteremia due to *Myroides phaeus*.

Case report

A 57-year-old female patient with a history of high blood pressure, diabetes mellitus for 4 months and chronic kidney disease in renal replacement therapy (RRT). 8 years ago he was diagnosed with multiple myeloma, so she received initial treatment with thalidomide and dexamethasone cycles with partial remission of the disease. Due to this, it was decided to change the therapeutic scheme and start bortezomib (3 cycles), treatment that she maintained until the current hospitalization. For RRT, it required temporary catheter placement in the right femoral vein, which would be replaced by a long-term catheter within twenty days. The results of laboratory examinations at admission indicated: leukocytes 3380/mm³, hemoglobin 7.5 g/dL, platelets 190 000/mm³ and C-reactive protein 5.4 mg/L.

During the first hemodialysis session, fever rose to 38 °C with chills, so that a set of peripheral blood cultures and the femoral catheter were obtained prior to the start of empirical antimicrobial therapy with ceftazidime. At 24 h an increase in the acute phase reactants (C-reactive protein 112.2 mg/L) was found, and the microbiology laboratory reported the growth of slightly rough white-yellowish colonies with gram negative staining; therefore the temporary catheter was removed. The final report of the

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MicroScan Walk Away 96 automated equipment identified *Myroides* sp, with the following susceptibility profile: amikacin MIC > 32 (resistant), aztreonam MIC > 8 (resistant), cefepime MIC > 8 (resistant), cefotaxime MIC > 16 (resistant), ceftazidime MIC > 16 (resistant), ciprofloxacin MIC > 2 (resistant), gentamicin MIC > 8 (resistant), imipenem MIC > 8 (resistant), levofloxacin MIC > 4 (resistant), meropenem MIC > 8 (resistant), piperacillin-tazobactam MIC < 16 (sensitive), tobramycin MIC > 8 (resistant), trimethoprim-sulfamethoxazole MIC > 2/38 (resistant); therefore, it was decided to optimize the antimicrobial coverage with piperacillin-tazobactam for 7 days at 96 h, with favorable evolution and disappearance of the fever 48 h after the start of the targeted antimicrobial treatment. The peripheral blood cultures at 48 h of targeted therapy were negative. The patient was discharged with a new catheter located in the left femoral vein.

The microbiological isolation and initial identification of the strain of *Myroides* spp. was carried out by the MicroScan Walk Away 96 automated system in the microbiology laboratory of the Guillermo Almenara Irigoyen National Hospital. Subsequently, this strain was sent to the Molecular Biology Laboratory of the Institute of Nutritional Research, Lima-Peru. The 16S rDNA gene was amplified by conventional PCR, from the DNA extracted from a pure culture of the bacterium, the fragments were visualized on a 3 % agarose gel (Fig. 1) and the amplified products were purified for direct sequencing with the Sanger method (Macrogen® Korea). The sequences obtained were compared with the reference sequence of the BLAST® platform - National Library of Medicine, USA. Isolation was identified as *Myroides phaeus* strain (99.67 % identity in Blast report).

Discussion

Myroides phaeus, is a bacterial species that was isolated from human saliva from a Chinese student who was asymptomatic, the phylogenetic analysis was close to *Myroides marinus*, *Myroides odoratimimus* and *Myroides profundus* with similarity in genetic sequences of 96.5 %, 96.3 % and 96.1 %, respectively [4]. So far, this species has not been reported as a pathogen, and in general due to the limited reports related to infections caused by *Myroides*, it is not yet possible to determine the true role of this species as a

human pathogen. *Myroides* spp. is commonly found in environmental sources, particularly in soil and water, but also in wastewater from hospitals [6,7].

The case of our patient is a catheter-associated bacteremia in an immunocompromised host, with diabetes and chronic kidney disease in hemodialysis. *Myroides* spp. infections have been described receiving these risk groups, but the contamination scenario is also possible during venipuncture, in the preparation of the culture media or during the processing of the culture, which could be considered as pseudobacteremia [8]. Although this possibility is present, the application of standardized protocols for sampling and processing, as well as biosecurity, make the contamination introduced at any stage of the blood culture, from extraction to processing in the laboratory, to a minimum.

In the report by Douce et al. isolates of *Myroides odoratus* were described in peripheral blood and central venous catheter samples, related to contaminated injection water in a hospital in Ecuador, however, they were not considered as pathogenic agents [9]. Another report documented urinary tract infection with *Myroides odoratimimus* in an elderly patient [10]. However, in the Americas there have been eight reports of clinical isolates, one in Jamaica associated with endocarditis and bacteremia in a hemodialysis patient, also in Argentina and six cases in the United States [2,11,12]. In the scientific literature, so far there are no reports of isolates of the *Myroides* genus in Peru, and *Myroides phaeus* has not been reported as a cause of bacteremia or any other infectious process.

The identification of *Myroides* by automated methods such as MicroScan Panel or VITEK 2 have limitations in the identification of species, in contrast both MALDI-TOF MS and 16S rDNA sequencing allow to differentiate *Myroides odoratimimus*, *Myroides odoratus* and other species of the genus *Myroides* [2,13]. In addition, the relevance of this species is that most strains are resistant to multiple drugs, and have various virulence factors such as their ability to form biofilms [14]. The strain isolated in this case only showed susceptibility to piperacillin-tazobactam. The sensitivity to this antimicrobial drug is variable in the strains of *Myroides odoratimimus* and *Myroides odoratum* [15].

In conclusion, we report the first case of *Myroides phaeus* isolated in blood culture, causing bacteremia in an immunocompromised patient using MicroScan Panel in addition to PCR - genetic sequencing. More studies are needed to determine the relevance of *M. phaeus* as a pathogen.

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Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Contributions

Pérez-Lazo G: Study design, Writing, Data collection.
 Morales-Moreno A: Data collection.
 Soto-Febres F: Data collection.
 Jove-Quimper H: Sample preservation and processing.
 Morales-Castillo L: Sample preservation and processing.
 Palomares-Reyes C: Sample preservation and processing.
 Del Valle-Mendoza J: Writing.

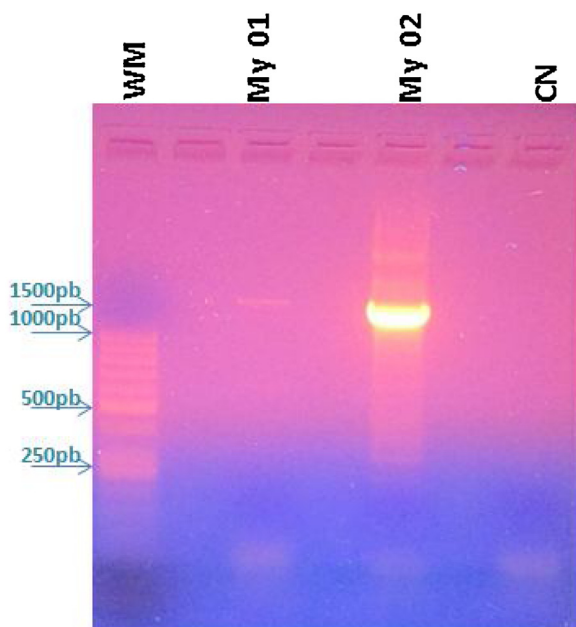


Fig. 1. The band My 01 and My 02 show the fragments amplified from the culture.

Aguilar-Luis M: Sample preservation and processing.
Silva-Caso W: Study design, Writing.

Ethical approval

This case report has not required approval by the ethics committee of the hospital where it was performed. This is because the case was described as part of the hospital epidemiological surveillance program for multiresistant microorganisms. The patient signed an informed consent for this work. This research was funded with the support of the Technological University of Peru.

Declaration of Competing Interest

The authors declare no conflicts of interest for this article.

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