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CASE REPORT

Identification of *Ochrobactrum oryzae* in Primary Bloodstream Infection in a Dialysis Patient: Can it be an Emerging Pathogen?

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ABSTRACT

Ochrobactrum spp is a gram-negative bacillus currently considered an emerging and opportunistic infection, rare in humans, and generally associated with indwelling foreign bodies. We report a case of primary bloodstream infection related to a dialysis catheter, caused by *Ochrobactrum oryzae* misidentified as *Ochrobactrum anthropi*. *J Microbiol Infect Dis 2016; 6(3): 128-131*

Key words: *Ochrobactrum oryzae*, *Ochrobactrum anthropi*, Dialysis, Bloodstream infection, MALDI-TOF MS, Sanger sequencing.

INTRODUCTION

Ochrobactrum spp is a strictly aerobic, oxidase-positive, urease-positive, non-fermentative Gram-negative bacillus (NFGNB). In 1988, the Centers of Diseases Control and Prevention (CDC) classified as "*Achromobacter* Group Vd", belonging to the family of *Brucellaceae*. Currently, the genus *Ochrobactrum* consists of the fifteen species, but only five of them were described from human clinical specimens: *O. anthropi, O. intermedium, O. pseudointermedium, O. haematophilum, O. pseudogrignonense*; where the first three species have been described as more pathogenic [1-4].

Widely found in the environment, the *Ochrobactrum spp*. has the same microbiological niche of *Pseudomonas* spp. It is distributed in soils, vegetation, industrial environments, and primarily in water sources (pools, fluids used in dialysis and antiseptics). Nowadays it is considered an emerging opportunistic pathogen, related to infections in patients with indwelling devices and the immunocompromised. I Its first report as an intra-abdominal infection in humans was in 1980 [5]. In this report, we describe a case of bloodstream infection caused by *Ochrobactrum oryzae* misidentified as *Ochrobactrum anthropi* in a dialytic patient.

CASE REPORT

The patient was an 86-years-old Asian man, with a history of hypertension, Type II diabetes mellitus, dyslipidemia, and end stage renal disease. He was undergoing hemodialysis by percutaneous catheter for 10 months, had a total atrial ventricular blockage (TAVB), and had a transvenous pacemaker. He presented to the emergency room due to his declining medical conditions in March 2014. He reported having mild dyspnea, sweating, fever (39.8°C), and chills during the hemodialysis session. Upon admission, the patient was stable, afebrile, with normal vital signs, and without inflammatory signs at the site of insertion of the percutaneous catheter. Pertinent laboratory results displayed leukocytes at 10.020 (83.4% neutrophils) and C-Reactive Protein at 4.2 mg/dl (RV<1 mg/l).

The patient was diagnosed with primary catheter related bloodstream infection. Paired blood cultures were collected (from peripheral and percutaneous catheters), piperacilin/tazobactan *plus* vancomycin empirical therapy was initiated, and percutaneous catheter withdrawal was requested. After two days, both blood cultures were reported positive by the BACTEC™ 9240 automated system (BD) with the growth of Gram-negative bacillus (GNB).

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The GNB was identified as *O. anthropi* by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) technology, using Vitek MS system and Myla software (bioMerieux, Marcy-l'Etoile, France).

Based on the literature and susceptibility profile for *O. anthropi*, the antimicrobial therapy was modified to imipenem [2,5,6]. Blood cultures collected before the adjustments of antibiotics, after the seven-day treatment with piperacillin/tazobactam *plus* vancomycin, were negative. After 14 days of imipenem, the patient became stable, afebrile, and a transthoracic echocardiogram did not show any vegetation. The patient was discharged and remained clinically stable at the thirty-day follow-up.

The identification on the Vitek MS system was performed by the direct colony method and the results was based on a mass spectral fingerprint generated by two software database systems of interpretation. The GNB sample was first identified by Myla software database as *Ochrobactrum anthropi* with a high confidence level (99.9%). However, as described, the Myla software database version 3.2 contains only the *Ochrobactrum anthropi* species as an option. Therefore, the identification of the strain was performed by Saramis™ software database that contain 14 different species of *Ochrobactrum*. Unfortunately, the strain was identified as *Ochrobactrum* spp. with a high confidence level (96.5%). The discrepancy in identification at the species level were investigated with PCR and Sanger sequencing using universal primers of the 16S rRNA gene at positions 8-27 (16S-8F, 5´-AGAGTTTGATCCTGGCTCAG–3´) and 1473-1493 (16S-1493R, 5´-ACGGCTACCTTGTTACGACTT-3'). Amplicons were sequenced using BigDye v3.1 with cycle sequencing kit and compared to sequences available in the GenBank data bases. The sequence from 1326 bp (GenBank accession no. KT945235) displayed the best match (100% similarity) with the *Ochrobactrum oryzae* strain MTCC 4195 (NCBI Reference sequence NR_042417.1) deposited in the databases of the National Center for Biotechnology Information.

Antimicrobial susceptibility profile was performed by Etest (bioMerieux) and the interpretative criteria was according to the Clinical Laboratory Standards Institute guidelines (CLSI, M100-S25). The minimal inhibitory concentration (MIC) values were read in terms of µg/mL, where the edge of inhibition ellipse intersects the side of the strip. Quality control was performed by testing *Escherichia coli* ATCC 25922. The *O. oryzae* strain showed susceptibility to imipenem (MIC 1.0 µg/mL), meropenem (MIC 0.38 µg/mL), amikacin (MIC 12 µg/mL), and ciprofloxacin (MIC 0.38 µg/mL), and resistance to polymyxin B (MIC >1024 µg/mL) (Figure 1).

DISCUSSION

Differentiation between *Ochrobactrum* species by physiological tests is extremely difficult due to its high phenotypic similarity index [7]. The performance of MALDI-TOF Mass Spectrometry technology is significantly better for NFGNB identification than conventional biochemical and phenotypic tests [8]. However, some limitations still can be observed at the species identification level of *Ochrobactrum* spp.It is known that, the most of commercially available kits used for clinical sample identification have only the *O. anthropi* species in their databases [7]. In consequence, other species from *Ochrobactrum*, such as *O. oryzae*, might be misidentified as *O. anthropi.* In these cases, it would be beneficial to use a molecular tool such as 16S rRNA gene sequencing as it is able to offer reliable resultsand considered as the gold standard identification method.

There are no reported cases of *O. oryzae* as human pathogenic infections. The *O. oryzae* strain has been described by Tripathi *et al*, being identified on the surface of sterile seeds and plant tissue in deep-water rice plantations in India [9] (MTCC 4195^T). In the literature review, only case reports based on *O. anthropi* infections were found. Thoma B. and collegues [2] reported 73 cases from 35 publications of infections with *O. anthropi* of which 31.5% were associated with neoplasms, 28.8% with surgical procedures, 5.5% were patients on dialysis, and 4.1% had immunodeficiency syndromes. There were no diagnoses of associated comorbidities in 23.3% of the cases. It was also identified that 33% of infections were in children less than 10 years with neoplasms [1,2].

Descriptions of endocarditis cases, pyogenic infections, peritonitis post-dialysis, septic arthritis, infections in pre-term newborns, endophthalmitis, pneumonia, meningitis, and osteomyelitis were found [6,10-14]. However, most of these infections were associated with invasive devices, mainly in patients on hemodialysis,with a peritoneal catheter, or drainage sites due to the potential of its adhesion to several kinds of synthetic materials. Although considered a low-virulence bacteria, severe and fatal cases have been reported [1,2,15].

In Brazil, Menezes et al published a case of bacteremia *O. anthropi* in a newborn, possibly as-

sociated with the presence of central device. The identification of the microorganism was initially by automated method (BATEC system), with subsequent use of MALDI-TOF, PCR, and genetic sequencing of 16S ribosomal RNA [16].

Regarding the antimicrobial susceptibility, there was reported production of AmpC, conferring resistance to third and fourth generation cephalosporins and chloramphenicol [1,13,17]. They are considered sensitive microorganisms to fluoroquinolones, imipenem, sulfamethoxazole/trimethroprim, and aminoglycosides [1,18]. There is low level of susceptibility for colistin. In our case, the treatment was initiated with piperacilin/tazobactam *plus* vancomycin according to the institution protocol, and the percutaneous catheter was removed. After the primary bacterial identification of *O. anthropi*, blood cultures were repeated and antimicrobial therapy was changed to imipenem. These control blood cultures before the antimicrobial change were negative, suggesting that the bacteremia has been resolved by device removal. Similar resolution due to device removal has been reported in a few cases reported including the soft tissue infection with resolution of symptoms after surgical debridement [8,18].

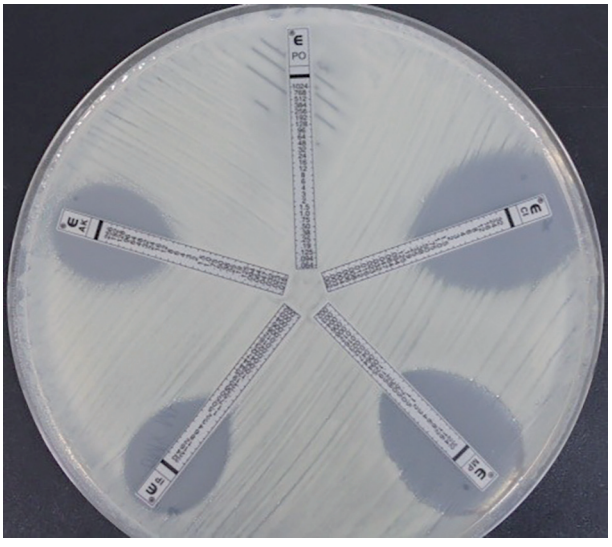

Figure 1. Intersection of the inhibition ellipse with the strip showing the MIC value of the *Ochrobactrum oryzae* strain from Polymyxin (PO), Ciprofloxacin (CI), Meropenem (MP), Imipenem (IP) and Amikacin (AK).

We concluded that this is the first *Ochrobactrum oryzae* bacteraemia described as a possible emerging microorganism in patients under dialysis. There is no report in the literature of pathogenic infections with this species. The species identification of this agent is extremely difficult, requiring molecular biology techniques. Infectious disease specialists must be on the lookout for prompt detection of these microorganisms.

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Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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