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Case Report

KPC-2 producing *Pseudomonas putida* as an unexpected pathogen of catheter-associated bloodstream infection

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Abstract

Infections due to multidrug resistant Gram-negative pathogens are of great concern worldwide, as they are frequently associated with high mortality and morbidity rates. The occurrence of *Pseudomonas* spp. producing *Klebsiella pneumoniae* carbapenemases (KPCs) imposes a great challenge through treatment course of bloodstream infections (BSIs). *Pseudomonas putida* has been recognized as an emerging pathogen of healthcare associated infections (HAIs). Therefore, we aimed to report a case of a non-fatal case of peripheral line associated BSI (PLA-BSI) in an immunocompromised host due to *P. putida* harboring bla$_{KPC-2}$ gene in Brazil. A *P. putida* isolate was recovered from a blood culture of a 72-year-old man admitted at a University Hospital, identified by BD Phoenix™ 100 (Becton, Dickinson and Company), causing PLA-BSI. The species identification was confirmed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and resistance to carbapenems were confirmed by Epsilometer test (E-test®). Additionally, the presence of important carbapenemases genes (*bla$_{KPC}$*, *bla$_{NDM}$*, *bla$_{OXA-48-like}$*, *bla$_{SPM}$*, *bla$_{IMP}$*, *bla$_{VIM}$*) was investigated by Polymerase Chain Reaction. The bacterial isolate was confirmed as meropenem resistant *P. putida* harboring bla$_{KPC-2}$ gene.Thereofre, these fidings suggest that *P. putida* can work as a reservoir for resistance genes as this bacterium has the ability to disseminate through water-fluids inside hospital and community settings. Moreover, this paper highlights that a frequent and worldwide disseminated mechanism of resistance (*bla$_{KPC-2}$*) is currently occurring among uncommon agents of BSI.

Key words: *Pseudomonas putida*; bloodstream infection; *Klebsiella pneumoniae* carbapenemase, KPC.

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Introduction

Multidrug resistant (MDR) Gram-negative bacterial (GNB) infections are a global health problem associated with substantial burden on healthcare systems [1,2] due to recurrent bloodstream infections (BSIs), longer length of hospital stays, and increased inpatient costs [3]. MDR organisms are frequent agents of healthcare associated infections (HAIs), including central line associated BSI (CLA-BSIs) [4]. These infections have been described as an important cause of health loss and are frequently related to high mortality rates [4,5]. Recently, the proportion of MDR Gram-negative causing CLA-BSIs has increased, and early removal of the suspected vascular catheter is an essential step during the management of these infections [4,6].

Pseudomonas species are aerobic Gram-negative non-fermentative bacilli, being able to colonize soil, plants (including fruits and vegetables), animal tissues and water [7]. *Pseudomonas aeruginosa* is the most common species of the genus related to HAI worldwide [8]. *Pseudomonas putida* is an opportunistic pathogen which was previously thought to have low pathogenicity [7]. Currently, *P. putida* has been recognized as an emerging pathogen of HAI, especially, in immunocompromised individuals, such as those with malignance and neutropenia [7,9]. In a case-series of 28 adult patients with infections caused by *P. putida*, it was

observed that most of these individuals (85.7%) had an immunosuppression condition and many of these infections were CLA-BSIs [9]. Despite considered as a rare agent of infection in humans, *P. putida* can cause a wide range of infections, such as CLA-BSI, cholangitis in patients with biliary devices, pneumonia, urinary tract infections and war wounds infections [7,9].

Emergence of MDR organisms, including carbapenem-resistant *P. putida*, is concerning [10-12]. Carbapenemases production is one of the most common mechanisms of resistance to β-lactams antibiotics in Gram-negative bacteria [8]. *Klebsiella pneumoniae* carbapenemases (KPCs) are the carbapenemase mostly described in Enterobacteriaceae, mainly in *K. pneumoniae* [13]. However, KPCs have been already found in other Enterobacteriaceae and non-fermentative Gram-negative organisms [8]. KPCs are able to hydrolyze penicillin, cephalosporin, monobactam and carbapenem; and KPC-producing strains often have acquired resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, becoming MDR organisms [12]. Genes encoding KPC enzymes (bla_{KPC}) are frequently located on transferable plasmids and KPC-producing clinical isolates have been associated with hospital outbreaks in the United States of America, Central and South America, Europe and Asia [8]. In fact, KPC-producing Enterobacteriaceae are spread worldwide [8,9].

Infections caused by KPC-2-producing *P. putida* are rare, and was only described twice around the world [11,14]. In 2008, the first case of infection due to KPC-2-producing *P. putida* was reported in Texas, USA [11]. In 2012, four years later, KPC-2-producing *P. putida* was described causing CLA-BSI in a child with lymphoma in Brazil [14]. However, some authors have suggested that *P. putida* act as an exchange platform for resistance genes to pathogenic organisms within a hospital [15]. Therefore, we aimed to describe a case of peripheral line associated BSI (PLA-BSI) due to KPC-2-producing *P. putida* isolated from a patient admitted to a Brazilian university hospital (UH), highlighting the occurrence of a frequent and worldwide disseminated mechanism of resistance (bla_{KPC-2}) among an uncommon agent of BSI.

Case Report

On 14th of July of 2018 (Day 1; D1), a 72-year-old man with relapsing chronic lymphocytic leukemia was admitted to a daycare clinic at UH to initiate a new cycle of chemotherapy with cyclophosphamide and vincristine. During the physical exam, besides the enlargement of the spleen the patient presented fever (axillary temperature: 38°C) and cough, being hospitalized at the UH in order to investigate a possible respiratory infection. Empirical intravenous antimicrobial therapy with amoxicillin/clavulanate plus azithromycin was administered for a suspected pneumonia during a period of seven days. The pneumonia was confirmed by right lobar consolidation on chest X-ray and positive blood culture for *Streptococcus pneumoniae*. On D14 the patient had a fever relapse (Axillary temperature: 38°C), associated with the presence of a peripheral venous line, without an identified site of infection. Additionally, the patient presented elevated serum levels for leucocytes (12.000 cell/mm^3) and C-reactive protein (3.85 mg/dL; Reference value: 0.3 mg/dL). At this moment, the diagnosis of PLA-BSI was suspected, and two blood samples were obtained from two different venipuncture sites (20 mL each) for diagnosis investigation, according to protocols of the Infection Control Section of HU. Besides, the peripheral venous line was immediately removed. On D17 the patient was discharged with any signs of infection. However, on D18, the blood cultures showed growth of carbapenem-resistant *P. putida* with susceptibility to polymyxin and aminoglycoside only, identified by BD Phoenix™ 100 (Becton, Dickinson and Co., New Jersey, USA) automated identification and susceptibility testing system. Thus, the diagnosis of PLA-BSI was established, considering clinical and microbiological criteria. Once carbapenem-resistant *P. putida* is an unusual agent of infection, the species identification was further confirmed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) and resistance to meropenem [Minimum Inhibitory Concentration (MIC) > 32 mg/mL] and imipenem (MIC > 32 mg/mL) tested by Epsilometer test (E-test®) (AB bioMérieux, Solna, Sweden), according to Clinical and Laboratory Standards Institute (CLSI) guidelines interpretation. Additionally, the presence of important carbapenemases genes (bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{SPM}, bla_{IMP}, bla_{VIM}) was investigated in *P. putida* isolate by Polimerase Chain Reaction (PCR) [16], being the bla_{KPC-2} gene the only one detected. This study was approved by the Ethics Committee of the University Hospital Antônio Pedro (reference number 32570).

Discussion

Here we report a case of peripheral venous line associated bloodstream infection caused by KPC-2-producing *P. putida* is reported. To the best of our

knowledge, this is the second episode [14] of KPC-2-producing *P. putida* infection described in our country and the third one in the world [11].

The dissemination of KPC-2 gene in emerging Gram-negative pathogens is of growing concern for clinicians because infections caused by carbapenemase producing bacteria usually have limited therapeutic options and elevated mortality rate [8]. Therefore, this report emphasizes the potential ability of *P. putida* to acquire resistance genes and became an MDR pathogen of HAIs. In the present case, the rapid removal of the peripheral catheter, which was the focus of infection, probably contributed to the benign outcome, as previously described by other authors [4,6]. Once *P. putida* is usually widespread in environmental and humid surfaces, the transmission of this organism to the peripheral catheter inserted in the patient may had occurred by contaminated hands or solutions. Some authors had described outbreaks caused by *P. putida* in intensive care units (ICU) and non-ICU, generally related to the transmission of contaminated fluids [17,18], highlighting the ability of this pathogen to persist in fluids and in water-associated hospital environments. It has been already suggested that environmental bacteria, such as *P. putida,* can behave as reservoirs and vectors of mobile genetic elements, being able to share resistance genes to clinical strains among hospital water systems [15].

In this context, precise identification and detection of the etiological agent and its antimicrobial resistance mechanisms are essential to surveil its dissemination. In the present case, the MALDI-TOF MS was a useful microbiological tool in order to confirm the species identification. MALDI-TOF MS is considered a quick, reliable, accurate and relatively inexpensive technique, being a cost-effective alternative for characterizing rare and microorganisms that are difficult to identify [19]. Additionally, the early detection of resistance genes in new pathogens, here done by PCR, is essential to recognize and prevent dissemination of MDR organisms [20].

Conclusion

Our findings suggest that *P. putida* can work as a reservoir for resistance genes as this bacterium has the ability to disseminate through water-fluids inside hospital and community settings. Moreover, this paper highlights that a frequent and worldwide disseminated mechanism of resistance (bla_{KPC-2}) is currently occurring among uncommon agents of BSI. These infections have few therapeutic options and can be associated with elevated mortality. Thus, the surveillance of this pathogen using accurate microbiological and molecular methods must be stressed out. In this context, microbiological approaches like MALD-TOF MS and PCR are essential to recognize bla_{KPC-2} producing *P. putida* and guide therapeutic and preventive measures.

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