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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Carbapenem-resistant IMP-1-producing *Pseudocitrobacter vendiensis* emerging in a hemodialysis unit

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Abstract

Hemodialysis patients are at high risk for bloodstream infections associated with highest morbidity and mortality rates. Bacterial species not commonly related to such infections has been hardly identified by traditional methods. *Pseudocitrobacter* is a novel genus of the order *Enterobacterales* that is associated with carbapenemase genes and nosocomial infection. In this context, we have investigated nine cases of bloodstream infections by carbapenem-resistant Gram-negative bacilli in patients assisted at a hemodialysis unit in Brazil. The infections were caused by a metallo-β-lactamase (IMP-1)-producing clone (> 90% *XbaI-PFGE* similarity) of *Pseudocitrobacter vendiensis*, displaying a multidrug-resistant profile to broad-spectrum cephalosporins, carbapenems, chloramphenicol, and trimethoprim-sulfamethoxazole. *S1-PFGE* and Southern blot hybridization revealed that $bla_{\text{IMP-1}}$ was carried by a 200-kb IncC/ST3 plasmid. Patients were successfully treated with amikacin, and strict disinfection procedures and hand washing protocols were reinforced. We report the emergence of *P. vendiensis*, a recently described species of the genus, in bloodstream infections of patients undergoing hemodialysis. Considering the epidemic potential of carbapenemase-producing *Enterobacterales* in hospital settings, surveillance of this emerging pathogen is of utmost importance.

Keywords Nosocomial infection · Metallo-β-lactamase · Carbapenemase · *Enterobacterales* · Outbreak

Patients undergoing hemodialysis are at high risk for bloodstream infections (BSI), with both morbidity and mortality being highest in this population [1]. Because of the dialysis process and conditions, they are vulnerable to acquiring such infections by water-borne bacteria not commonly related to them, and the consequent identification of isolates by standard methods is not trustworthy [2, 3]. *Pseudocitrobacter* is a novel genus of the order *Enterobacterales* with recent taxonomic revaluation, which has differentiated two species, *Pseudocitrobacter faecalis* (with *Pseudocitrobacter anthropi* being a posterior heterotypic synonym) isolated from feces of patients attending military hospitals in Pakistan, and *Pseudocitrobacter vendiensis*, recently isolated from a hospitalized patient transferred from Spain to Denmark [4, 5]. Worryingly, *P. faecalis* and *P. vendiensis* have been associated with production of NDM-1 and KPC-2 carbapenemases, respectively [4, 5]. We hereby report the emergence of IMP-1 metallo-β-lactamase (MβL)-producing *P. vendiensis* causing bloodstream infection in hemodialysis patients in Brazil.

In January 2018, a 56-year-old male patient undergoing hemodialysis in a tertiary-care hospital presented symptoms of bacteremia (i.e., fever, chills and shivering). In this regard, a Gram-negative bacilli (GNB) labeled as

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HMD01 was isolated from blood culture. Over the following 9 months, eight novel cases of bloodstream infections by GNB (strains HMD02 to HMD09) were confirmed in patients assisted at the same hemodialysis unit. Patients were successfully treated with amikacin 500 mg IV every 48 h for 10 days, whereas strict disinfection procedures and hand washing protocols were reinforced. Initially strains were identified and tested for antimicrobial susceptibility using the VITEK 2 Compact system (bioMérieux). The automated system identified five isolates (HMD03, HMD04, HMD05, HMD06, and HMD09) as *Pantoea* sp., and four (HMD01, HMD02, HMD07, and HMD08) as *Enterobacter cloacae* complex. All nine isolates presented identical resistance profile to ampicillin, cefepime, ceftazidime, ceftriaxone, ertapenem (MIC > 32 µg/mL), imipenem (MIC > 32 µg/mL), meropenem (MIC > 32 µg/mL), chloramphenicol, and trimethoprim-sulfamethoxazole according to the CLSI guidelines [6]. Molecular typing was performed by *XbaI-PFGE*, and carbapenemase genes were screened by PCR and DNA sequencing [7]. Localization of carbapenemase encoding gene was determined by *S1-PFGE* and Southern blot hybridization [8]. Molecular typing by *XbaI-PFGE* revealed clonal relatedness (> 93%) among carbapenem-resistant isolates, which carried the $bla_{\text{IMP-1}}$ MβL gene onto a 200-kb IncC/ST3 plasmid. Total DNA of a representative clone (strain HMD06) was extracted and sequenced using the MiSeq platform (Illumina Inc., San Diego, CA), with paired-end reads (250 bp). Reads were submitted to de novo assembly using Unicycler v.0.4.0, and then subjected to automatic annotation using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.3.2 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Prediction of bacterial species, resistome, and plasmidome were analyzed using online tools (http://www.genomicepidemiology.org/). Separation of contigs into different networks concerning plasmids and the chromosome was obtained using the PLACNETw tool (https://castillo.dicom.unican.es/upload/). Since 16S ribosomal DNA sequence analysis (SpeciesFinder-2.0) and fast K-mer algorithm (KmerFinder-3.1) failed to resolve the species identification, the GTDB Toolkit (GTDB-Tk) [9] was used to achieve a taxonomic assignment for the HMD006 genome. This tool allocated the strain within the genus *Pseudocitrobacter*, but it did not match perfectly with the only validly accepted species of this genus until that moment, *P. faecalis*. Therefore, a phylogenetic analysis of the 16S rRNA gene was performed with type strains of *P. faecalis* and the recently validly published *P. vendiensis*, as well as with other type strains of related *Enterobacterales* species [4, 5]. 16S rRNA gene sequences were obtained from public databases. Multiple alignments were constructed based upon the CLUSTAL W (http://www.clustal.org/clustal2/). The phylogenetic tree was constructed using the neighbor-joining method by using the MEGA X software (https://www.megasoftware.net/). Distance matrices were calculated by the Kimura 2-parameter method and bootstrap analysis was performed based on 1000 re-samplings. Type strains of the genus *Pseudocitrobacter* formed a monophyletic cluster (Fig. 1), and the strain HMD006 did not cluster with any of the *Pseudocitrobacter* spp., although it shared 16S rRNA gene similarities of 99.0% and 99.2% with the strain *P. faecalis* CIT 52 ^{T} and *P. vendiensis* CP020170097^{T}, respectively. Therefore, average nucleotide identity (ANI) and Tetra analyses were performed by using online tools (http://jspecies.ribohost.com/jspeciesws/; https://www.ezbiocloud.net/), in order to confirm the taxonomic position of this strain. The ANI between HMD006 and *P. faecalis* CIT 52 ^{T} strains ranged from 90.8 to 92.2%, and between HMD006 and *P. vendiensis* CP020170097^{T} ranged from 97.5 to 98.2%;

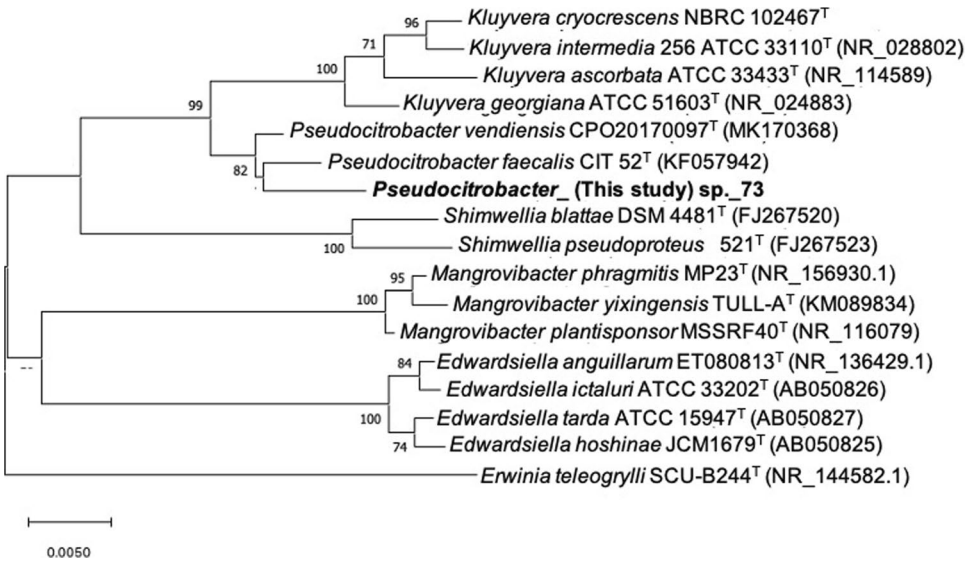

Fig. 1 Neighbor-joining phylogenetic tree of the 16S rRNA gene (1357 bp) of *Enterobacterales* species showing the position of *Pseudocitrobacter* sp. strain 73 (HMD006) within the genus *Pseudocitrobacter* and other close genera. Bootstrap values (> 70%) based on 1000 replications are shown at the nodes of the tree. Bar, 5 substitutions per 1000 nt

therefore, considering a threshold ≥ 95% for new species definition [10, 11], the strain HMD006 was confirmed as belonging to the species *P. vendiensis*.

Resistome analysis of *P. vendiensis* HMD06 revealed the presence of genes conferring resistance to β-lactams [$bla_{\text{IMP-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-15}}$], aminoglycosides [*aac(6')-31*, *aac(6')-Ib-cr*, *ant(3")-Ia*, *aph(3'')-Ib*, and *aph(6)-Id*], fluoroquinolones [*aac(6')-Ib-cr*, *qnrB1*], tetracycline [*tet(A)*], phenicols [*catB3* and *floR*], sulphonamides [*sul1*, *sul2*], trimethoprim [*dfrA14*], and quaternary ammonium compounds [*qacE*]. On the other hand, plasmidome analysis confirmed only the presence of the IncC/ST3 plasmid. In order to better characterize the plasmid involved in the dissemination of the $bla_{\text{IMP-1}}$ gene, the distinct FASTA files containing the separated contigs were additionally submitted to the ResFinder 4.1 tool, and we obtained the localization of each antimicrobial resistance gene. The plasmid IncC/ST3 was determined as presenting about 206.3 kb and containing $bla_{\text{IMP-1}}$, $bla_{\text{OXA-1}}$, *tet(A), catB3, floR, qacE*, *sul1*, *sul2*, *aac(6')-31*, *aph(3")-Ib*, *aph(6)-Id*, *ant(3'')-Ia*, *aac(6')-Ib-cr*, and *qnrB1*. A second plasmid of about 77.5 kb, also identified on *S1-PFGE*, able for mobilization and transferring since it possesses MOB genes, was not identified because it did not present any known replicon or antimicrobial resistance genes (according to the analysis performed on PLACNETw). The $bla_{\text{CTX-M-15}}$ (preceded by an IS*Ecp1* and succeeded by a Tn*3* truncated by an IS*26*) and *dfr*A14 genes were identified inserted into the chromosome of *P. vendiensis* HMD06.

Genomic approaches in microbiology are elucidating genetic backgrounds and taxonomic changes of clinically relevant pathogens [12]. Clinical isolates of *Pseudocitrobacter* spp. and other species of the order *Enterobacterales* are commonly incorrectly identified due to the use of traditional methods in clinical laboratories. Phenotypic tests are only partially reliable to distinguish species of bacteria not recognized as usually pathogens to humans, resulting in misidentification, such as the observed in the present study and in others [13, 14]. Genome sequencing is an important taxonomic tool to resolve these issues, and correct identification of potentially pathogenic bacteria is important to establish the appropriated treatment. In this study, we report the emergence of IMP-1-producing *P. vendiensis* causing an outbreak of invasive infections in hemodialysis patients, in Brazil. Considering the epidemic potential of carbapenemase-producing *Enterobacterales* in hospital settings, and unfavorable prognosis of critical patients with carbapenem-resistant infections [15], surveillance of this emerging pathogen is of utmost importance.

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Author contribution Letícia Kellen de Andrade, Arturo Levican, Louise Cerdeira, Tiago Casella, Quézia Moura, Bruna Fuga, and Nilton Lincopan performed the investigation process. Andressa Batista Zequini de Morais, Melissa Maia Braz, and Evelin Rodrigues Martins performed the investigation and data curation about the laboratory characterization of *P. vendiensis*. Nilton Lincopan and Mara Corrêa Lelles Nogueira conducted the project administration and supervision. All authors contributed to the analysis and the writing of the final manuscript.

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Data availability The draft genome sequence of strain HMD006 was deposited at DDBJ/ENA/GenBank, as *Pseudocitrobacter* sp. 73, under accession number VTZO00000000.1 and BioProject PRJNA563339.

Code availability Not applicable.

Declarations

Ethics approval The study was approved by the Research Ethics Committee of the Faculdade de Medicina de São José do Rio Preto under the approval #E: 3.300.442. This study does not require a consent form from the participants.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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