Genomic analysis of Acinetobacter bereziniae co-harboring blaNDM-1 and blaOXA-58 isolated from fatal pneumonia and stomatitis in a Burmese python (Python bivittatus)

GABRIEL SIQUEIRA DOS SANTOS[1], JULIA DE MENEZES MACEDO PAUSA[2], CAROLINE DA COSTA ABRAHÃO[2], ALICE SOARES DE OLIVEIRA[2], JOÃO PESSOA ARAÚJO JUNIOR[3], AMANDA HAISI[3], LUCIANA SARTORI[4,5], JOSÉ SOARES FERREIRA NETO[1], MARCOS BRYAN HEINEMANN[1]

[1.] FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA, UNIVERSIDADE DE SÃO PAULO, DEPARTAMENTO DE MEDICINA VETERINÁRIA PREVENTIVA E SAÚDE ANIMAL, SÃO PAULO/SÃO PAULO, BRASIL
[2.] DIVISÃO DA FAUNA SILVESTRE, PREFEITURA MUNICIPAL DE SÃO PAULO, CENTRO DE MANEJO E CONSERVAÇÃO DE ANIMAIS SILVESTRES, SÃO PAULO/SÃO PAULO, BRASIL
[3.] UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", INSTITUTO DE BIOTECNOLOGIA, SÃO PAULO/SÃO PAULO, BRASIL
[4.] ESCOLA PAULISTA DE MEDICINA, UNIVERSIDADE FEDERAL DE SÃO PAULO, DEPARTAMENTO DE INFECTOLOGIA, SÃO PAULO, SÃO PAULO
[5.] INSTITUTO PAULISTA DE RESISTÊNCIA AOS ANTIMICROBIANOS, SÃO PAULO/SÃO PAULO, BRASIL

Introduction: Carbapenemase-producing *Acinetobacter* (CPA) are multi-drug-resistant (MDR) pathogens frequently associated with healthcare-associated infections (HAIs). However, considering the transboundary aspect of the MDR bacteria threat, wild animals, especially those kept under human care, are frequently exposed to human sources and, therefore, often harbor MDR bacteria or even suffer an infectious process caused by MDR bacteria. **Objectives**: Analyze an *Acinetobacter bereziniae* strain harboring plasmid-borne bla_{NDM-1} and bla_{OXA-58} causing pneumonia and stomatitis in a non-native Burmese python (*Python bivittatus*) rescued in a public park. **Materials and Methods:** A Burmese python was rescued from a public park in Brazil, and the veterinary staff reported pneumonia and stomatitis resulting in the animal death. A caseum sample was streaked on blood agar and cultured at 36°C overnight. Gray and non-hemolytic colonies were obtained, and the isolate was identified as *Acinetobacter bereziniae* by MALDI-TOF. Antimicrobials minimum inhibitory concentration (MIC) was assessed by the broth microdilution method, testing for cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), meropenem (MPM), gentamicin (GEN), amikacin (AMI), ciprofloxacin (CIP), colistin (COL), tetracycline (TET), and trimethoprim/sulfamethoxazole (SUT), performed and interpreted according to CLSI M100 ED35 guidelines. Whole genome sequencing of the strain PY02 was performed using the MiSeq i100 platform and Oxford Nanopore Technology. The quality of the reads was assessed with FastQC v.0.12.1, the reads were trimmed with Trimmomatic v.0.39 and the hybrid genome assembling was performed with Unicycler v.0.5.1. PY02 was confirmed as *A. bereziniae* with Kraken2 and genomic data was deposited in GenBank Nucleotide database. Resistome and mobilome were predicted with the ABRicate v1.0.1 platform with the ResFinder v4.6 and CARD v4.0.1 databases and MGEfinder v1.0.3. Plasmid contigs were predicted with mlplasmids v2.1.0. **Results**: The PY02 isolate showed resistance to cefotaxime (MIC > 64), ceftazidime (MIC > 32), cefepime (MIC > 8), meropenem (MIC > 16), gentamicin (MIC > 16), amikacin (MIC > 32), ciprofloxacin (MIC > 4), and tetracycline (MIC > 32) and was sensitive to colistin (MIC ≤ 2) and

trimethoprim/sulfamethoxazole (MIC ≤ 1/19). The antimicrobial resistance profile was a result of the presence of a 114448 bp plasmid-located resistance encoding genes for beta-lactams (*bla*$_{NDM-1}$ and *bla* $_{OXA-58}$), aminoglycosides (*aph(3')-VI*, *aph(3')-VIb* and *aac(3)-IId*) and macrolides (*mph(E)* and *msr(E)*), chromosome-located resistance genes for cephalosporins/penams (*bla*$_{OXA-355}$) and tetracycline (*tet(39)*), besides GyrA Ser81Leu substitution frequently related to quinolone resistance in *Acinetobacter baumannii* strains. **Discussion**: *Python bivittatus* is not a native species from Brazil and the insertion of this animal in a human context can explain the acquisition and further infection caused by a CPA harboring two acquired carbapenemase-encoding genes. **Conclusion**: The report of an *Acinetobacter bereziniae* co-harboring *bla*$_{NDM-1}$ and *bla*$_{OXA-58}$ isolated from fatal pneumonia and stomatitis in a Burmese python (*Python bivittatus*) reveals how antimicrobial-resistant bacteria represent a threat to wildlife and reinforce a One Health approach to fight AMR.

Agradecimentos: GSS is grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (24/17243-8) for his scholarship. MBH and JSFN are grateful to the Conselho Nacional de Desenvolvimento Cinetífico e Tecnológico for their fellowships (grant: 310462/2021; 302899/2022, respectively).