TITLE: A NOVEL INTEGRON CARRYING *bla*$_{IMP-1}$ IN MULTIDRUG-RESISTANT *PROTEUS MIRABILIS* CLINICAL ISOLATES

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Proteus mirabilis is a member of the Enterobacteriaceae family and exhibits intrinsic resistance to polymyxins and tigecycline. The acquisition of mechanisms conferring resistance to cephalosporins, and more recently to carbapenems is very worrisome because drastically limits the therapeutic options. Metallo-β-lactamases (MβLs) production has been described in a variety of Enterobacteriaceae species. However, the acquisition of MβL genes, like *bla*$_{IMP}$ by *P. mirabilis* still represents a rare event. The aim of this study was to characterize microbiologically the first report of a *bla*$_{IMP-1-}$ producing *P. mirabilis* outbreak in Brazil. A total of 10 carbapenem-resistant *P. mirabilis* isolates were collected from distinct units at a tertiary hospital in the city of Diadema, São Paulo. The confirmatory bacterial identification and carbapenemase activity were performed by MALDI-TOF MS. The susceptibility profile was determined by broth microdilution and showed resistance to all β-lactams but susceptibility to aminoglycosides (except Pm-7 strain), and ciprofloxacin. PCR and sequencing demonstrated that all isolates carried *bla*$_{IMP-1}$, *bla*$_{TEM-1}$ and *bla*$_{CTX-M-2}$. However, additional resistant genes were detected in Pm-3 (*bla*$_{KPC-2}$) and Pm-7 (*bla*$_{CTX-M-14}$ and *rmtB-1*) isolates. The *bla*$_{IMP-1}$ was inserted at the first position of a new class 1 integron, named *In*1359, followed by two aminoglycoside modifying enzymes (AME) encoding genes, the novel *aacA4* variant, named *aacA4-26*, and the *aadA1e*. The plasmid profile showed that *bla*$_{IMP-1}$ was located on a 150 Kb conjugative plasmid in all isolates, and successfully transferred to the recipient *E. coli* J53 strain. The MICs for the transformant strain (T-3.1) showed an 8- to 32-fold increase to carbapenem and 64- to 528- fold increase to cephalosporins compared to *E. coli* J53. In addition, the hybridization assay showed that *bla*$_{KPC-2}$ was inserted on a ~95.4 Kb plasmid in the Pm-3 isolate. The analysis of the genetic similarity by PFGE showed that all isolates were clonally related (similarity of >84.7%; distributed in three subtypes). This report describes for the first time an outbreak of IMP-1-producing *P. mirabilis* isolates carrying a variety of resistance genes, including *bla*$_{KPC-2}$, and *rmtB*. In addition, the confirmation of a new transferable genetic element, *In*1359, might enable the dissemination of this resistant determinant to many other bacterial species, representing a serious challenge to clinicians and infection control teams.

Keywords: *bla*$_{IMP-1}$, carbapenem-resistant *Proteus mirabilis, aacA4-26, In*1359.