

TITLE: OprD AND RESISTANCE TO CARBAPENEMS IN NON-CARBAPENEMASE-PRODUCING *Pseudomonas aeruginosa*

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ABSTRACT:

The emergence of *Pseudomonas aeruginosa* MDR isolates leads to reduction of therapeutic options, increasing morbidity and mortality rates. Carbapenems are the first line of choice for the treatment of infections by *P. aeruginosa* MDR. OprD protein regulates the entry of these antibiotics into the bacterial cell, and the loss of its functions is the main determinant of carbapenem resistance in non-carbapenemases-producing strains. This study aimed to determine the MIC for imipenem and meropenem in seven *P. aeruginosa* strains collected from patients and environment in a Burn Center. Six of them carbapenems resistant and non-carbapenemases-producing and one carbapenem susceptible. Furthermore, mutations in *oprD* gene were investigated in all isolates, which belonged to two different Sequence Type (ST2236 and ST2237). MIC for imipenem and meropenem was achieved by using M.I.C.Evaluator™ strips, according to manufacturer's instructions. *Pseudomonas aeruginosa* ATCC27853 was used as control. *oprD* mutations were detected by whole amplification of this gene through PCR, followed by sequencing. Sequences analysis were performed by Lasergene Software and compared with PAO1 reference strain. Protein alignments were carried out using ESPript 3.0. MIC values ranged from 16 to ≥ 32 $\mu\text{g}/\text{mL}$ to meropenem and imipenem in 5/6 carbapenem resistant strains. These results demonstrated high resistance to carbapenems, since the breakpoint for both antibiotics is ≥ 8 $\mu\text{g}/\text{mL}$. The carbapenem-susceptible strain (PA26) presented MIC=1 and 0.25 $\mu\text{g}/\text{mL}$ for imipenem and meropenem respectively. Mutations found in *oprD* were similar among strains of the same ST. ST2236 strains (PA3, PA24 and PA26) showed several mutations by bases replacement, present in all strains, comprising the susceptible one. However, only the carbapenem resistant strains PA3 and PA24 exhibited a point mutation at position 1270, which generate a stop codon, resulting in a smaller protein. On the other hand, ST2237 carbapenem resistant strains PA2, PA4, PA5 and PA31 presented frameshift mutations, like insertions and deletions. Deletion of two nucleotides (TC) at positions 122-123 respectively was common to all strains of this ST and suggests loss of OprD protein. These results suggest that carbapenems resistance in the studied strains can be attributed to the OprD protein, being distinct among the STs, since mutations found in ST2236 suggest a non-functional protein and those of ST2237 suggest protein loss.

Keywords: *P. aeruginosa*, carbapenem resistance, OprD

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