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# Loss of the OprD Homologue Protein in *Acinetobacter baumannii*: Impact on Carbapenem Susceptibility

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In an article recently published in *Antimicrobial Agents and Chemotherapy*, Catel-Ferreira et al. (1) demonstrated that the lack of OprD in *Acinetobacter baumannii* did not affect imipenem and meropenem susceptibilities compared with those of the wild-type parent and suggest that an *A. baumannii* OprD homologue is likely not involved in the carbapenem resistance mechanism. We report herein the impact of another *A. baumannii* Δ*oprD* homologue mutant strain on carbapenem susceptibilities. The gene disruption method was used to inactivate the *A. baumannii* OprD homologue. The internal fragment of the *oprD*-like gene (559 bp) from *A. baumannii* strain ATCC 17978 was amplified by PCR using the primers OprDintFW (5′-CGATGGTTCAGCTTACGATCATTG) and OprDintRV (5′-GCTGTTCTGTTGGTACGCTAACATC) and cloned into the pGEM-T Easy vector by A/T cloning, following the manufacturing instructions (Promega, Spain) (2). The resulting construct incorporated into *Escherichia coli* strain DH5α was purified and electroporated into *A. baumannii* strain ATCC 17978 in order to knock out its *oprD*-like gene by allelic replacement. Transformants were selected on Luria-Bertani agar plates containing 80 μg/ml of ticarcillin. *oprD*-like-gene disruption within the resulting strain, designated JPAB03, was verified by PCR using a combination of primers matching the upstream region of the *oprD*-like gene (OprDextFW, 5′-ATGCTAAAAGCACAAAAACTTAC) and the pGEM-T Easy vector (M13R, 5′-CAGGAAACAGCTATGAC) and by outer membrane protein (OMP) profiling using SDS-PAGE, PCR, and OMP profile analysis, which confirmed the disruption of the *oprD*-like gene and the absence of OprD expression. A microdilution carbapenem (imipenem and meropenem) susceptibility assay was used. Compared to the wild-type parent strain, ATCC 17978, strain JPAB03 showed similar MICs of imipenem and meropenem, 0.5 and 0.5 μg/ml, respectively. These data confirm, as Catel-Ferreira et al. have described (1), that the *A. baumannii* OprD homologue would not be related to permeability to carbapenems. The confirmation of these previous results is important to rule out the contribution of OprD downregulation to carbapenem resistance in *A. baumannii*. Contradictory previous studies showed that the reduction of OprD expression affects carbapenem susceptibility by increasing the imipenem and meropenem MICs (3–5). It is noteworthy that CarO, another porin involved in permeability to carbapenems, is downregulated in these carbapenem-resistant *A. baumannii* strains (3–5). Thus, we suggest that only the loss of OprD in *A. baumannii* is not sufficient to increase the carbapenem MICs. Therefore, more investigations are needed to confirm this hypothesis.

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***Ed. Note:*** *The authors of the published paper (Catel-Ferreira et al.) did not feel that a response was necessary.*



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