TITLE: INSERTIONAL INACTIVATION OF THE OMPK36 GENE BY AN IS903 IN KPC-producing *Klebsiella pneumoniae*

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ABSTRACT:

Production of KPC and reduction of membrane permeability are of great relevance for carbapenem resistance. In this study, we analyzed the contribution of the outer membrane proteins (Omps) for the different ertapenem susceptibility profiles of two isogenic isolates of KPC-producing *Klebsiella pneumoniae* (KPN133-resistant and KPN132-susceptible). The genes encoding the Omps *ompK*35 and *ompK*36 were analyzed by PCR and sequencing, followed by homology analysis with sequences deposited in the *ISFinder* database. The expression of these genes and bla_{KPC-2} was evaluated by RT-qPCR. The gene *rpoB* was used as endogenous control and the susceptible strain of *K. pneumoniae* KPN077 as reference sample. The presence of the Omps was investigated by SDS-PAGE. Analysis of the *ompK*36 genetic environment showed the presence of the *IS903* insertion sequence disrupting this gene in KPN 133. The expression of the *ompK*35 and *ompK*36 genes was repressed in both KPN132 and KPN133. Moreover, in KPN133, the expression of *ompK*36 was undetectable. SDS-PAGE results also showed a significant decrease of OmpK36 in the outer membrane. For bla_{KPC-2} gene, its expression in KPN 133 was lower than in KPN132. This could be a consequence of the decreased amount of OmpK36 in the outer membrane, since a lower amount of antimicrobial entering the cell would dispense the production of so much KPC. Whereas ertapenem has already been described as the preferential substrate of KPC-type carbapenemases, this data suggests a strict control over the regulation of carbapenemase gene expression, especially in strains presenting alterations in Omps. According to our findings, it was estimated that membrane permeability in KPN132 was less affected than in KPN133. Therefore, a higher antimicrobial concentration would be reached into the cell. In addition, the higher expression of $bla_{KPC-2,}$ when compared do KPN133, could indicate drug hydrolysis by KPC, but not enough to achieve resistance levels. Thus, we suggest that the increased MIC for ertapenem in KPN133 was due to inactivation of *ompK*36 gene by an *IS903* in this isolate, which resulted in a decrease of this protein in the outer membrane. Different mechanisms may play a role in the resistance to carbapenems, but our findings showed that the decrease in membrane permeability, due to loss of *ompK36* gene integrity and its decreased expression, associated to the decreased OmpK36 production, were determinant for occurrence of the resistance phenotype observed.

Keywords: Resistance, ertapenem, Omps, KPC.

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