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## **CHARACTERIZATION OF NOVEL *Acinetobacter baumannii* PATHOGENICITY FACTORS DURING *Galleria mellonella* INFECTION**

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*Acinetobacter baumannii* (Ab) is a human pathogen of major concern due to its multi-drug resistance (MDR). Ab belongs to the small group of bacteria that escape from the lethal action of antibiotics and cause most nosocomial infections, the ESKAPE pathogens. The continued increase in resistance to carbapenems (carbR),  $\beta$ -lactams considered one of the last resources for the treatment of infections caused by MDR Gram-negative pathogens (BGN), is of great concern in the clinical settings. The World Health Organization (WHO) has included Ab carbR in a list of critical priority pathogens for the development of R&D strategies focused on the control of infections by these MDR microorganisms. Ab pathogenesis is associated with the MDR condition and with the presence of virulence factors (VF), including outer membrane proteins (OMP) and lipoproteins, among others, that can be associated with secreted outer membrane vesicles (OMV), which are scarcely characterized at the moment. We previously reported the characterization of seven AB5075 mutants in genes predicted as putative VF. Four of these genes (#1, #2, #4 y #11) revealed roles in the pathophysiology of Ab for the gene products, as reduced A549 cell adherence and invasion. These mutants (?#1, ?#2, ?#4 and ?#11) also showed higher levels of biofilm formation on abiotic surfaces, lower motility in semisolid media and different colony phenotypes in Congo red assay, as compared to WT. Thus, indicating an altered cell envelope for the mutants. Also, ?#1 and ?#2 showed enhanced sensitivity towards oxidative stress ( $H_2O_2$ ), a condition that mimics intra macrophages surviving. Thus, suggesting roles for the corresponding proteins during oxidative stress or contributing to Ab survival during infection. Here, we further analyze the role of these four gene products in Ab virulence using *Galleria mellonella* larvae as infection model. These larvae are an alternative model of infection, with a myriad of advantages: maintenance and handling are quite easy, it is considered to be more ethically acceptable than other models and their immune system has a lot of similarities to those of mammals. We first optimized the use of *G. mellonella* larvae for AB5075 infection by using different bacterial doses, from  $10^3$  to  $10^7$  UFC, and observing larvae survival along 96 hs post-infection.  $10^5$  UFC was the selected condition. Infection assays were performed with 15 larvae inoculated with ?#1, ?#2, ?#4, ?#11 and

WT, including the corresponding controls. We observed a lower killing of *G. mellonella* when they were injected with the mutants as compared to the parental strain. In particular, ?#4 was the least lethal mutant followed by ?#11, ?#2 and ?#1. Although more work is needed, these results indicate a relevant role for #4 gene product during Ab infection, and contribute to the understanding of Ab virulence mechanisms, revealing novel possible targets for therapeutic development.

Palabras clave: *Acinetobacter baumannii* - *Galleria mellonella* - Virulence - Secreted proteins