

Virulence Characteristics of Biofilm-Forming *Acinetobacter baumannii* in Clinical Isolates Using a *Galleria mellonella* Model

Abstract:

Acinetobacter baumannii is a Gram-negative coccobacillus responsible for severe hospital-acquired infections, particularly in intensive care units (ICUs). The current study was designed to characterize the virulence traits of biofilm-forming carbapenem-resistant *A. baumannii* causing pneumonia in ICU patients using a *Galleria mellonella* model. Two hundred and thirty patients with hospital-acquired or ventilator-associated pneumonia were included in our study. Among the total isolates, *A. baumannii* was the most frequently isolated etiological agent in ICU patients with pneumonia (54/165, 32.7%). All *A. baumannii* isolates were subjected to antimicrobial susceptibility testing by the Kirby–Bauer disk diffusion method, while the minimum inhibitory concentrations of imipenem and colistin were estimated using the broth microdilution technique. The biofilm formation activity of the isolates was tested using the microtiter plate technique. Biofilm quantification showed that 61.1% (33/54) of the isolates were strong biofilm producers, while 27.7% (15/54) and 11.1% (6/54) showed moderate or weak biofilm production. By studying the prevalence of carbapenemase-encoding genes among isolates, blaOXA-23-like was positive in 88.9% of the isolates (48/54). The blaNDM gene was found in 27.7% of the isolates (15/54 isolates). blaOXA-23-like and blaNDM genes coexisted in 25.9% (14/54 isolates). Bap and blaPER-1 genes, the biofilm-associated genes, coexisted in 5.6% (3/54) of the isolates. For in vivo assessment of *A. baumannii* pathogenicity, a *Galleria mellonella* survival assay was used. *G. mellonella* survival was statistically different between moderate and poor biofilm producers ($p < 0.0001$). The killing effect of the strong biofilm-producing group was significantly higher than that of the moderate and poor biofilm producers ($p < 0.0001$ for each comparison). These findings highlight the role of biofilm formation as a powerful virulence factor for carbapenem-resistant *A. baumannii* that causes pneumonia in the ICU.