IMPACT OF SURFACE-ASSOCIATED VIRULENCE FACTORS ON THE CYTOTOXICITY OF ACINETOBACTER BAUMANNII OUTER MEMBRANE VESICLES

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Acinetobacter baumannii is an opportunistic pathogen notable for its high levels of antibiotic resistance and its role in hospital-acquired infections [1]. Carbapenem-resistant *A. baumannii* was designated a top priority for antibiotic development by the WHO in 2017 [2]. Despite the identification of some virulence factors, the mechanisms underlying its pathogenicity remain poorly characterized compared to other Gram-negative bacteria [3]. It is known that *A. baumannii* releases outer membrane vesicles (OMVs) – nanoscale, outer-membrane-derived particles. OMVs are lipid bilayer vesicles released into the extracellular environment, containing outer membrane lipids and proteins, periplasmic enzymes, genetic material [4]. OMVs play an important role in pathogenesis by delivering virulence factors, inducing inflammation, promoting immune evasion and antibiotic resistance. By enabling host damage without direct bacterial contact, OMVs significantly enhance *A. baumannii* virulence [5]. This study aims to evaluate the influence of *A. baumannii* surface virulence factors on the cytotoxicity of OMVs.

In this study, five different *A. baumannii* strains were used: wild-type clinical isolates (52 wt and 169 wt), previously constructed polysaccharide capsule-deficient mutant strain (52 ΔgalU), outer membrane protein OmpA deficient mutant strain (52 ΔompA), spontaneous mutant strain with lipooligosaccharide (LOS) structural changes (169 ΔLOS). The *ompA* gene was knocked out of *A. baumannii* clinical isolate by generating a markerless gene-deletion mutant. The spontaneous mutant strain was obtained by colistin-dependent loss/modification of LOS, evaluated by SDS-PAGE and Alcian Blue dye. The OMVs were purified from each *A. baumannii* strain by ultracentrifugation of overnight cultures. The concentrations of OMVs were determined by overall protein content detected by Bradford assay. Cytotoxicity of OMVs was evaluated using A549 lung carcinoma epithelial cell line and assessing their viability via the MTT method.

The gene *ompA* was successfully knocked out from *A. baumannii* clinical isolate and confirmed by sequencing. Complete loss of LOS in the spontaneous mutant was not observed, but structural modifications were detected. OMVs from all strains exhibited cytotoxicity towards A549 cells compared to the PBS negative control. However, when comparing OMVs from the 52 wt strain and the capsular polysaccharide-nonproducing 52 ΔgalU strain, the latter showed a reduced cytotoxic effect. Similarly, the OMVs from the 169 wt caused lower viability of A549 cells when compared with OMVs from the 169 ΔLOS strain. Therefore, variation in OMV composition causes different outcomes towards cytotoxicity, highlighting the critical contribution of specific surface components to OMV-mediated host cell damage and represent promising targets for future therapeutic interventions.

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