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Outer Membrane Vesicle Proteomics to Discover the Pathogenicity of *Acinetobacter baumannii*

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Acinetobacter baumannii causes pneumonia, urinary tract infection, bacteremia, meningitis, blood stream infection and wounds of combat casualty [1,2]. The ability of *Acinetobacter* to adhere at abiotic surface and form biofilm helps in its survival in the harsh environmental conditions such as desiccation, nutrient deficiency and antibiotic treatment. There is rising concern about antimicrobial drug resistance among *Acinetobacter baumannii* since the past decade [3-12]. Gram negative bacteria constitutively secrete Outer Membrane Vesicles (OMVs) into the extracellular milieu that play crucial role in the delivery of virulence factors to host cells [13]. OMVs also act as intercellular communicasomes in polyspecies communities by enhancing bacterial survival, nutrient acquisition, biofilm formation, and pathogenesis [2,13,14]. OMVs allow enzymes/proteins to reach distant targets in a concentrated, protected, and targeted form. The gram-negative envelope also contains proteins with several important functions, such as nutrient acquisition, secretion, signaling, adherence, and protection from the environment [15]. Therefore, it is important to explore the OMVs for the better understanding of pathogenesis and virulence of *Acinetobacter baumannii*.

Proteomics emerged as a tool to study the proteome under diverse conditions [4,5]. With the advance of proteomics, a significant advancement has been made in the recent years in the field of OMV proteomics. Methods such as unlabeled (comparative coomassie stained 2D electrophoresis), labeled (DIGE, iTRAQ, ICAT, ICPL) and recently label-free quantification methods (SRM) have emerged which can be engaged in the OMV proteomics. These advancements in the proteomic methods help in the better understanding of outer membrane vesicles proteome.

Kwon et al. performed proteomics of OMVs to identify the secretion of OMVs as well as analyze the comprehensive proteome of *A. baumannii*-derived OMVs [16]. They reported a number of proteins such as β-lactamase, OmpA, chaperonin GroEL and virulence-associated proteins in the OMVs of *Acinetobacter* [16]. Using OMVs proteomics, EF-Tu has been identified in the cell surface and OMVs of *A. baumannii* [17]. Mendez et al., performed proteomics of extracellular proteome such as OMVs and FSEPs (freely soluble extracellular proteins) of *A. baumannii* [18]. The results identified 179 proteins in the OMVs fraction and 148 proteins in the FSEPs fraction. They identified 39 OMVs proteins which are associated with pathogenesis and virulence, attachment to host cells (e.g., CsuE, CsuB, CsuA/B) and specialized secretion systems for delivery of virulence factors (e.g., P. pilus assembly and Fil F) [18]. They also explain the role of proteins of OMVs in the oxidative stress and as defense mechanism against the macrophagic attack [19].

Cabral et al., performed proteomics of *Acinetobacter* cultured in three different conditions (exponential, late stationary phase and biofilms stage) and they also checked the effects of biofilm inhibitory compound (salicylate) on the biofilm formation. This multiple-approach strategy showed a unique lifestyle of *A. baumannii* involved in biofilms formation [20]. Similarly, high-end isoelectric point proteome analysis of *Acinetobacter radioresistens* reveals that 'envelope stress responses' can be induced by aromatic compounds [21-23]. Using 2D-DIGE and mass analysis, Minami et al. demonstrated that alteration in proteomic profiles of detergent-resistant membrane fractions during cold acclimation [24]. Proteomic analyses also showed that histidine metabolism have role in the biofilm formation of *Acinetobacter baumannii* [20]. Yun et al., performed differential quantitative proteomic analysis of outer membrane from multidrug-resistant *Acinetobacter baumannii* and reported that carbapenem induces the expression of resistance-nodulation-cell division transporters, protein kinases and suppress outer membrane proteins expression [19]. Carbapenem resistance of *Acinetobacter baumannii* has been studied using outer membrane proteomics between wild type and carbapenem resistance strain of *Acinetobacter baumannii* [12,16,21,22].

Reports have shown that OMV proteomics also help to develop vaccine and probiotics. Aguilera et al. identified 18 different proteins in the OMVs of *Escherichia coli* strain Nissle-1917. These OMV interact with the host and induce the beneficial effects on the host hence can be used as probiotics [25]. Using OMV proteomics of detergent-extracted OMVs and detergent-free extracted OMVs, Bas et al proved that the immunogenic proteins of OMV vaccines depends partially on the purification procedure and they suggested that detergent-free OMVs are preferred composition for the vaccine development against gram negative bacteria [26].

Therefore, OMVs proteomics helps to explain the role of various factors/proteins of OMVs in the pathogenesis, virulence and survival of *Acinetobacter* in the host. Hence, outer membrane vesicles proteomics has now emerged as a method of choice for the study of pathogenesis of disease and virulence of *Acinetobacter baumannii*.

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