ISOLATION AND CHARACTERIZATION OF OUTER-MEMBRANE VESICLES FROM OPPORTUNISTIC PATHOGEN *ACINETOBACTER BAUMANNII*

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Gram-negative bacterium *Acinetobacter baumannii* is recognized among most dangerous microorganisms in health care settings worldwide. This opportunistic pathogen causes variety of nosocomial infections to immunocompromised patients. Due to its ability to acquire multidrug resistance and persist in clinical environment, infections caused by *A. baumannii* are difficult to cure. An inefficiency of antibiotics against this pathogen encourages the development of alternative treatments [1].

A. baumannii secretes nano-spherical structures called outer-membrane vesicles (OMV) which contain variety of bacterial molecules. Most abundant proteins found in OMVs are outer-membrane porin OmpA and β-lactamase AmpC [2]. OmpA acts as multivirulent factor and possibly influence OMVs biogenesis [3], while AmpC contributes to antimicrobial resistance of *A. baumannii*. Therefore outer-membrane vesicles are considered to play an important role in *A. baumannii* pathogenesis. Due to high prevalence of bacterial antigens on the surface, OMVs are one of the most promising vaccine candidates against *A. baumannii* [4].

The aim of this work was to isolate and characterize outer-membrane vesicles from clinical *A. baumannii* strain. For this purpose we isolated OMVs from *A.baumannii* clinical strain and *ompA* gene knockout mutant. OMVs were visualized using transmission electron microscopy (TEM). Quantity and protein content of OMVs were measured using Bradford assay and SDS-PAGE. Detection of OmpA was performed using Western blot. AmpC β-lactamase activity using nitrocefin assay was performed as well.

According to our results, *A. baumannii* Δ*ompA* mutant produced ~3 times more OMVs comparing with wild-type strain and showed differences in OMVs protein profiles. Also both OMVs from wild-type and *ompA* deletion mutant contained active β-lactamase AmpC. However absence of OmpA resulted in slower hydrolysis of nitrocefin.

In conclusion, we confirmed that OmpA plays an important role in biogenesis of outer-membrane vesicles secreted by clinical *A. baumannii* strain and possibly contributes to the bacterial resistance to β-lactam antibiotics.

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