ISOLATION OF A HIGH MOLECULAR MASS PLASMID FROM OPPORTUNISTIC PATHOGEN *Acinetobacter baumannii*

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Acinetobacter baumannii is a gram-negative aerobic bacterium regarded as opportunistic pathogen due to ability to cause severe illness in persons with deficient immune system, particularly in hospital patients, although community-acquired cases are known as well [1]. Strains belonging to international clonal lineages I (IC I) and II (IC II) are associated with hospital outbreaks and high virulence. Clinical isolates frequently express multi-drug resistance (MDR) including resistance to colistin, a drug of last resort for gram-negative MDR species which highly contributes to worldwide concern about *A. baumannii* as an emerging threat to human health [2]. *A. baumannii* isolates exhibit diversity in variously sized plasmids they harbor [3]. Small to medium sized plasmids containing antibiotic resistance genes are studied extensively unlike large conjugative plasmids which remain mainly uncharacterized and their influence on *A. baumannii* virulence characteristics yet to be elucidated.

During previous laboratory investigations, characteristics of multiple clinical IC I and IC II isolates were described [4]. We speculate that phenotype differences related to pathogenicity among IC II isolates could be attributed to p2AB52 plasmid. *A. baumannii* isolate, harboring this 67 kb conjugative plasmid, was demonstrated to possess more hydrophobic cell surface and reduced lethality in animal infection model compared to another *A. baumannii* isolate, which lacks p2AB52. To confirm this hypothesis pulsed-field gel electrophoresis (PFGE) was performed as described by Barton *et al.* in order to isolate p2AB52 [5]. Single plasmid was isolated by cutting out a piece of agarose in the place of approximately 70 kb size band from the gel, digesting the piece with agarase and precipitating p2AB52 from the solution using isopropanol.

Further assays include transformation of isolated p2AB52 into *A. baumannii* isolate and determination of the phenotype of transformants such as cell surface hydrophobicity and lethality in animal infection model.

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[3] Salto PI, Torres GT, Wibberg D *et al.* Comparative genomic analysis of *Acinetobacter* spp. plasmids originating from clinical settings and environmental habitats. *Sci Rep*. 2018;8(1):7783.

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