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EFFECT OF PABN ON ACINETOBACTER BAUMANNII ANTIBIOTIC RESISTANCE

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Abstract: Acinetobacter baumannii is a multidrug-resistant and an invasive pathogen and is one of the major causes of nosocomial infections in the current healthcare system. It has been recognized as an agent of pneumonia, septicemia, meningitis, urinary tract, and wound infections, and is associated with high mortality. Pathogenesis in A. baumannii infections is an outcome of multiple virulence factors that help the organism to resist stressful environmental conditions and enable development of severe infections. The efflux pump may play a role in antibiotic resistance in A. baumannii isolates. The ability of A. baumannii isolates to acquire drug resistance by the efflux pump mechanism is a concern. Thus, new strategies are required to eliminate the efflux transport activity from resistant A. baumannii isolates causing nosocomial infections. The effects of efflux pump inhibitors such as the phenylalanine-arginine B-naphthylamide (PABN) on the antimicrobial susceptibility have been examined by many studies and concluded that the addition of the PABN at a final concentration of 100 µg/ml greatly reduced the MIC of various antibiotics. In addition, Biofilm plays an important role in persistent infections caused by A. baumannii. Furthermore, the adhesion and biofilm phenotypes of some clinical isolates seem to be related to the presence of broad-spectrum antibiotic resistance. the effects and possible mechanisms of PAβN on A. baumannii biofilm formation and dispersion showed that PAβN inhibited A. baumannii biofilm formation and enhanced its dispersion. The effects of PAβN on A. baumannii biofilm formation and dispersion were independent of the efflux pumps.

INTRODUCTION

Acinetobacter baumannii is gram-negative, aerobic coccobacilli and non-motile (Figure 1) [1, that belongs to “ESKAPE” six pathogens with multidrug resistance and virulence. This group is a responsible majority of nosocomi infections and can avoid biocidal effect of antimicrobial agents. Acinetobacter baumannii can be identified by using 16s ribosomal-RNA as well as conserved region of seven housekeeping genes: gltA, gyrB, gdhB, recA, cpn60, gpi, and rpoD by using multilocus sequence typing (MLST) [2–4]. Infections of A. baumannii have been considered a major concern because it shows extensive resistance to antibiotics and high molarity death associated with its infections [5– 7].

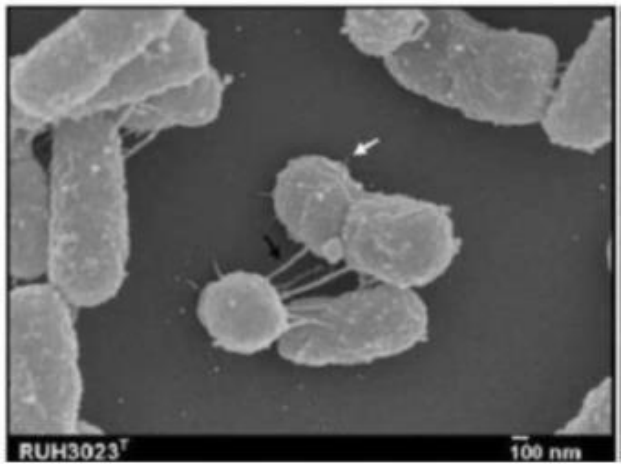

Figure 1: - Scanning electron micrographs of A. baumannii. Black arrows indicatelong cell extensions: white arrows indicate short pili-like structures.

Hospitalized and vulnerable patients are at higher risk of A. baumannii infections because it penetrates through skin and airway defects. Furthermore, most infections caused by this bacterium affect patients staying in the intensive care unit (ICU) [8– 11]. Acinetobacter baumannii causes many infections including skin and soft tissues, wound infections, bacteremia, endocarditis, urinary tract infections (UTIs), meningitis, and pneumonia [9–11]. The most common nosocomial infection associated with A. baumannii is pneumonia mainly in patients admitted to ICU and breathing through the ventilator. The mortality rate from A. baumannii caused ventilator-associated pneumonia (VAP) varies from 40 to 70% [12, 13]. Some risk factors related to acute myocardial infarction involve bloodstream infections, immunosuppression, artificial ventilation, preceding antibiotic treatment, and invasive virus colonization [14]. The rate of mortality Infections of the A. baumannii bloodstream range from 28 to 43% [15]. Acinetobacter baumannii interferes with the development of burn infections, where the existence of Multi-Drug Resistance (MDR) strains and low penetration of many antibiotics are the main problems for chemotherapy [16– 19]. The rate of burn infections associated with A. baumannii is about 22% and mainly spread among military personnel, with an MDR rate of about 53% [20]. Additionally, A. baumannii can cause infections related to the central nervous system (CNS) that can be treated by colistin antibiotics [21]. Multidrug-resistant A. baumannii is recognized to be among the most difficult antimicrobial resistant gram-negative bacilli to control and treat. Increasing antimicrobial resistance among Acinetobacter isolates has been documented, although definitions of MDR vary in the literature [22,23]. The increase in usages of β-Lactam antibiotics has contributed to the emergenceof drug resistant and rapid development of A. baumannii resistant strains. Infections caused by these resistant strains are treated with carbapenems. However, the emergence and spread of resistant A. baumannii (CR-Ab) tocarbapenems has limited the effectiveness of this drug. Furthermore, the emergence of colistin resistant A. baumannii (Col-R-Ab) strains have beenrecorded and this resistance is occurred due to changes in the structure of the lipopolysaccharide (LPS). Colistin resistance is showed to occur due to mutations in lpxA/D/C and pmrA/B genes resulting in regulation down wards and modification of lipid A biosynthesis [24,25,26]. Antimicrobial resistance greatly limits the therapeutic options for patients who are infected with this organism, especially if isolates are resistant to the carbapenem class of antimicrobial agents. Overall, this leads to rapid emerging pathogen in the health care setting, where it causes infections that includebacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism has the ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfacesmake it a frequent cause of outbreaks of infection and an endemic, health care associated pathogen. Because therapeutic options are limited for multidrug-resistant Acinetobacter infection, the development or discovery of new therapies, well controlled clinical trials of existing antimicrobial

regimens and combinations, and greater emphasis on the prevention of health care associated transmission of MDR Acinetobacter infection are essential [22,23].

Diagnosis:

More advanced molecular diagnostic methods have been developed for identification of Acinetobacter to the species level, these include:

- Amplified 16S rRNA gene restriction analysis (ARDRA) [27].
- High-resolution fingerprint analysis by amplified fragment lengthpolymorphism (AFLP).
- Ribotyping [29].
- tRNA spacer fingerprinting [30].
- Restriction analysis of the 16S–23S rRNA intergenic spacer sequences[31].
- Sequence analysis of the 16S–23S rRNA gene spacer region [32].
- Sequencing of the rpoB (RNA polymerase β-subunit) gene and itsflanking spacers [33].

Virulence Potential

Despite extensive research into the virulence potential of this emerging pathogen, little is still known about its true pathogenic potential or virulence repertoire. While it is believed that several factors may contribute to the virulence potential of A. baumannii, one factor in particular, OmpA, a member of the Outer membrane proteins (OMPs), has been determined to contribute significantly to the disease-causing potential of the pathogen [34]. A. baumannii OmpA bind to the host epithelia and mitochondria, once bound tothe mitochondria, OmpA induces mitochondrial dysfunction and causes the mitochondria to swell. This is followed by the release of cytochrome c, a heme protein, which leads to the formation of apoptosome. These reactions all contribute to apoptosis of the cell [34]. OmpA, being the most abundant surface protein on the pathogen, is also involved in resistance to complement and the formation of biofilms [35,36]. two key stress survival strategies and potentially important virulence associated factors that help to promote bacterial survival both inside and outside the host. The ability of A. baumannii to form biofilms allows it to grow persistently in unfavourable conditions and environments. Other key proteins that have been shown to contribute to A. baumannii virulence include phospholipase D and C. While phospholipase D is important for resistance to human serum, epithelial cell evasion and pathogenesis [37]. phospholipase C enhances toxicity to epithelial cells [38]. Along with OmpA, fimbria, also expressed on the surface of the bacterial cell, contribute to the adhesion of the pathogen to host epithelia.

Pathogenicity of A. baumannii:

Acinetobacter baumannii is not considered a community pathogen, but in immunocompromised individuals and in children, it populates tracheostomy sites and can cause community-acquired bronchiolitis and tracheobronchitis. It has also been implicated in community-acquired pneumonia with underlying conditions such as smoking, alcoholism, diabetes mellitus. Acinetobacter baumannii can be transmitted through the vicinity of affected patients or colonizers such as linens fomites, curtains, bed rails, tables, sinks, doors, feeding tubes, and even medical equipment. Contamination of respiratory support equipment, suction devices, and devices used for intravascular access is the key source of infection [39]. Major predisposing factors important in the acquisition of A. baumannii infection include prolonged hospital stay, mechanical ventilation, intravascular device, advanced age, immunosuppression, previous broad-spectrum antimicrobial therapy, previous sepsis, ICU stay, and enteral feedings [40].

Acinetobacter baumannii Biofilm formation:

A biofilm is a community of multiple bacterial cells associated with a surface (either biotic or abiotic), arranged in a tertiary structure in intimate contact with each other and encased in an extracellular matrix that can be comprised of carbohydrates, nucleic acids, proteins, and other macromolecules [41]. Furthermore, this structure can confer resistance to antimicrobial therapies on the order of one thousand times greater than that of their planktonic counterparts [42]. Bacterial biofilm initiation and development is not simply a serendipitous adherence of bacterial cells to a surface. On the contrary, it is a highly regulated series of molecular events, which cells keep under tight regulation. The most common factors that can influence biofilm formation are nutrient availability, bacterial appendages (pili and flagella), bacterial surface components (outer membrane proteins, adhesins), quorum sensing and macromolecular secretions (polysaccharides, nucleic acids and so on) [43]. In addition, complex regulatory networks including two-component regulatory systems and transcriptional regulators are known to be responsible for the expression of a variety of biofilm associated gene products in response to awide range of environmental signals [44]. Acinetobacter baumannii has been shown to form biofilms on abiotic surfaces, which can include glass and equipment used in intensive care units, and/or on biotic surfaces such as epithelial cells [35]. Pili assembly and production of biofilm-associated protein (BAP) both contribute to the initiation of biofilm production and maturation after A. baumannii attach to particular surfaces [35]. When pili attach to abiotic surfaces, they initiate the formation of micro colonies, followed by the full development of biofilm structures. BAP are present on the surface of bacterial cells, and they contribute to biofilm development and maturation by stabilizing the mature biofilm on abiotic or biotic surfaces [35]. The ability of A. baumannii to form biofilms is multifactorial and diverse, dependent upon the surface with which the cells are interacting. The expression of bacterial-associated factors in biofilm development is dependent upon nutrients and sensing of the environment by either the BfmS sensor kinase, crosstalk with other kinases or substrate-level phosphorylation of the cognate response regulators such as BfmR. In addition to these factors, surface proteins such as a Bap homolog could be involved in stabilizing the mature biofilm on abiotic or biotic surfaces. The presence of metal cations and the expression of resistance to broad- spectrum antibiotics can also increase the ability of A. baumannii to adhere to, and form biofilms on, a surface. However, many of the molecular mechanisms by which these bacteria adhere to diverse, medically relevant surfaces and human host cells remain obscure. Elucidating these mechanisms using modern and global approaches could provide missing basic information on these processes, which could be novel targets for future antimicrobial strategies as the age of antibiotics begins to wane. These are realistic and achievable goals since A. baumannii has entered the genomic and postgenomic era after several genomes were fully sequenced and annotated or are close to completion. Comparative genomics has already shed light on the common and unique genetic features of different clinical isolates, such as the presence of a unique resistance island in a multidrug-resistant nosocomial isolate [45]. These advances, together with the possibility of conducting global gene-expression analyses and testing virulence with appropriate experimental models, should provide a quantum leap in our understanding of not only biofilm-formation functions but also how these functions correlate with other cellular factors that contribute to the virulence of A. baumannii and its ability to cause severe infections in humans.

Effect of Phenylalanine-arginine β-naphthylamide (PAβN)

Phenylalanine-arginine β-naphthylamide (PAβN) is a broad-spectrum effluxpump inhibitor that has shown to potentiate the activity of antibiotics in Gram negative bacteria as shown in figure 2 [46 & 47].

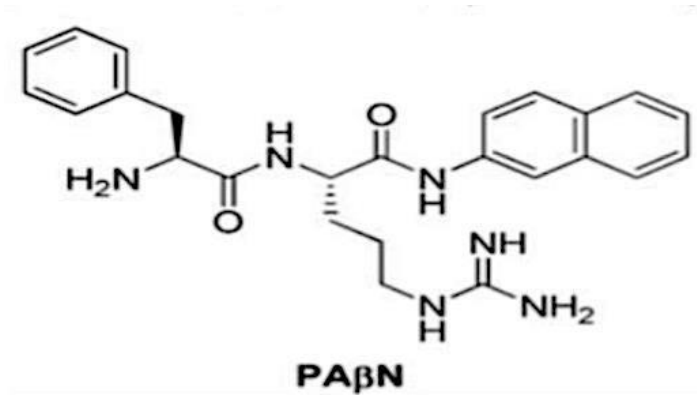

Figure 2: - Composition of Phenylalanine-arginine β-naphthylamide

Efflux pump inhibitors (EPI)

EPIs are the molecules that inhibit efflux pumps by one or more mechanisms, leading to inactive drug transport. Since this could eventually lead to successful build-up of an antibiotic inside the cell, these EPIs can be used as adjuncts in combination with antibiotics to enhance their activity against bacteria expressing efflux pumps [48]. Efflux pump inhibitors (EPIs); their mechanism of action is through competitive inhibition with antibiotics on the efflux pump resulting in increased intracellularconcentration of antibiotic, hence, restoring its antibacterial activity (Figure3) [49].

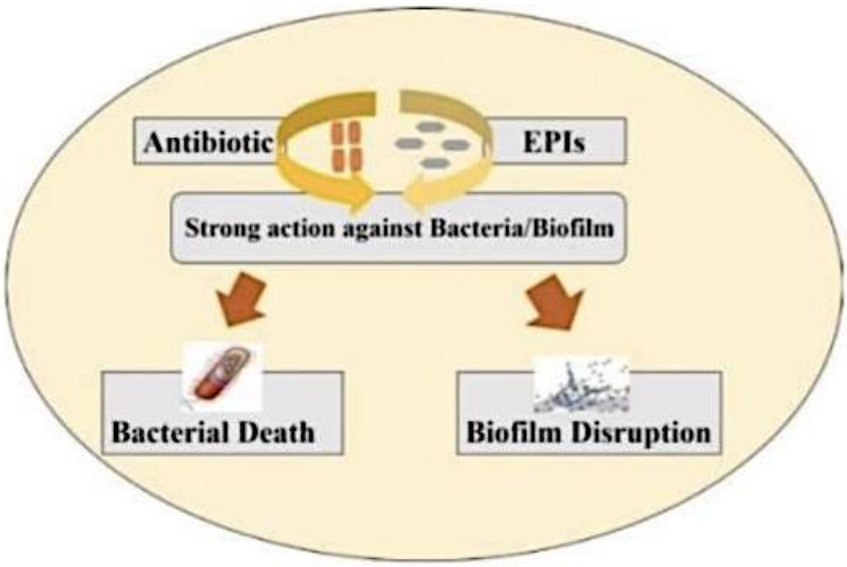

Figure 3: - Efflux pump inhibitors (EPIs) action

Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment (Figure 4). These proteins are found in both Gram-positive and Gram-negative bacteria as well as in eukaryotic organisms [51].

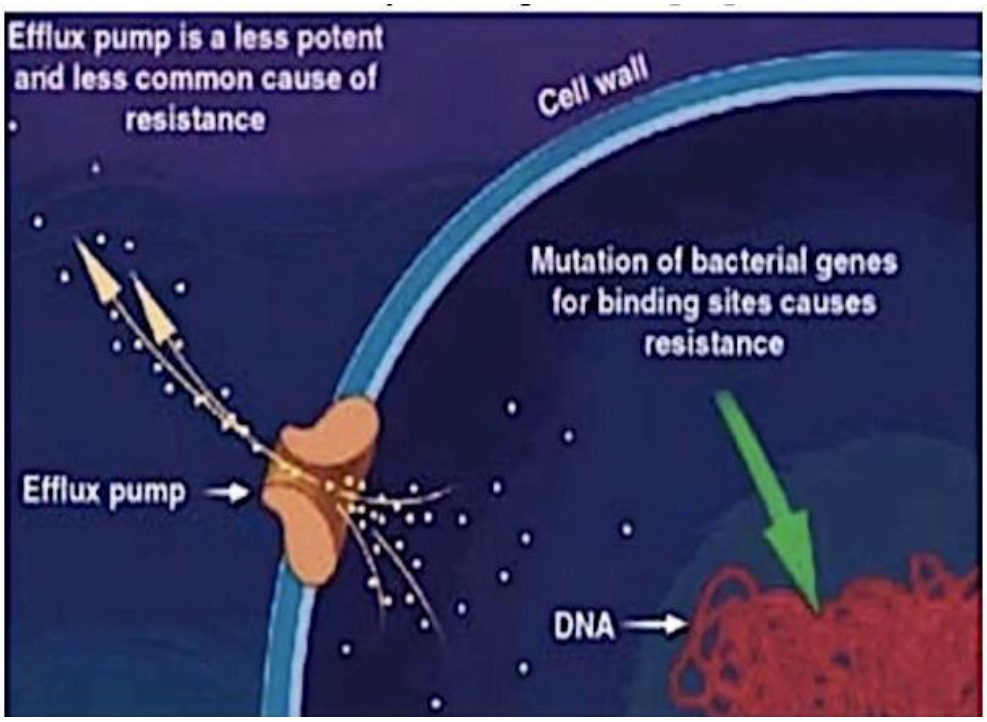

Figure 4: - Efflux pump action.

1) Effects of PAβN as Efflux Pump Inhibitor (EPI)

Data analysis indicated that in the presence of the pump inhibitor, the quantityof the MICs of imipenem in most of the isolates decreased in the presence of the efflux pump inhibitor. Mohajeri et al., 2013 reported that all the isolates in their study were resistant to imipenem and meropenem as well as to other antimicrobial agents [53]. Shahcheraghi et al., 2011 determined that 12% of the A. baumannii isolates in their investigation were resistant to colistin [54]. Fallah et al.,2014 reported that the resistance rate of the A. baumannii strains to colistin in their research was 2 (1.8%) [55]. There-fore, colistin can be helpful in treating A. baumannii related infections in burn patients. Efflux pump systems are an extremely important cause of multi- drug resistance [56]. Valentine et al., 2008 found that the addition of the PABN at a final concentration of 100 µg/ml greatly reduced the MIC of ciprofloxacin from 2- to 8-fold [57]. Pan Hou et al., 2012 noted that after exposure to the efflux pump inhibitor, the PABN, a 4- to 32-fold reduction in the MICs of imipenem was observed in 33 (66%) isolates of imipenem-resistant A. baumannii [58]. Szabo et al., 2006 reported that the addition of the PABN at different concentrations reduced the MICs of various antibiotics [59]. Researchers observed that the imipenem susceptibility of most of the isolates was increased in the presence of the PABN by 4-to 64-fold. The results suggest that multidrug efflux pumps play a role in the mechanism of the resistance in A. baumannii strains. Induction of expression of the adeFGH pump resulted in a 16- and 64-fold increase in resistance to chloramphenicol and trimethoprim, respectively, whilst the minimum inhibitory concentration (MIC) for clindamycin increased by 32- fold. Addition of PABN resulted in the potentiation of chloramphenicol, trimethoprim, and clindamycin by 16-, 128- and 256-fold, respectively. Potentiation of the activity of clindamycin by PABN shows that the outer membrane permeability of A. baumannii does not serve as a barrier for the EPI [60].

2) Effects of PAβN on A. baumannii biofilm formation anddispersion

PAβN significantly inhibited the biofilm formation of the studied isolates in a dose-dependent manner. PAβN at 100 µg/ml inhibited biofilm formation by 57.71%. PAβN also showed weak eradication effect of the formed biofilm; 100 µg/ml PAβN eradicated 19% of the formed biofilm. Because biofilm formation and

the efflux pump system are both related to A. baumannii resistance and survival in the hospital environment, we examined therelationship between biofilm and the efflux pump system. Studies of biofilm and the efflux pump system are limited and have shown controversial results. The effect of PAβN a universal efflux inhibitor, on biofilm formation and dispersion was investigated. As the ability of A. baumannii isolates to acquire drug resistance by the efflux pump mechanism is a concern, most studies of

PAβN have focused on anti-microbic susceptibility changes. PAβN was found to inhibit the ability of the AdeFGH pump to efflux trimethoprim, chloramphenicol, and clindamycin in A. baumannii strains [61]. The minimum biofilm eradication concentration in Burkholderia pseudomallei biofilms with ceftazidime and doxycycline was decreased by twofold to 16-fold in the presence of PAβN [62]. Study found that although efflux pump genes did not differ in the different biofilm formation ability groups, PAβN still effectively inhibited biofilm formation and enhanced biofilm dispersion. This observation agrees with data from another study, in which PAβN was paired with the iron chelators 2,2′-dipyridyl, acetohydroxamic acid, and EDTA, which all inhibited Pseudomonas aeruginosa growth and biofilm formation [63].

Acinetobacter baumannii showed a strong biofilm formation ability. Biofilm formation by A. baumannii was not associated with antibiotic resistance andwas inhibited by PAβN. The mechanisms of the effects of PAβN on A. baumannii biofilm formation and dispersion may be independent of the efflux pumps [64].

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