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Article

The Genetic Basis of Biofilm Formation in Acinetobacter baumannii and Its Clinical Implications

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Abstract: Acinetobacter baumannii is an opportunistic pathogen known for its remarkable ability to form biofilms, contributing significantly to its persistence in hospital environments and resistance to antimicrobial treatments. Biofilm formation in A. baumannii is a complex, genetically regulated process involving a network of genes responsible for adhesion, extracellular matrix production, and stress response. Key genetic determinants include the bap gene, which encodes the biofilm-associated protein, the csu operon involved in pilus-mediated surface attachment, and the ompA gene, which enhances adhesion and immune evasion. Additionally, quorum sensing regulators such as abaI play a crucial role in coordinating biofilm development. The presence of these genetic elements not only enhances the pathogen's ability to colonize medical devices but also significantly increases its tolerance to antibiotics, leading to chronic infections, particularly in immunocompromised patients. Clinically, biofilm formation in A. baumannii is associated with ventilator-associated pneumonia, bloodstream infections, and wound infections, posing a major challenge for treatment. Conventional antibiotics often fail to penetrate biofilms effectively, necessitating alternative therapeutic approaches such as quorum sensing inhibitors, biofilm-disrupting agents, and combination therapies. Understanding the genetic basis of biofilm formation is crucial for developing targeted interventions to mitigate A. baumannii-associated infections. Future research should focus on novel anti-biofilm strategies, including gene-targeting therapies and the use of biofilm-resistant biomaterials in medical devices. Addressing this issue is essential for improving patient outcomes and controlling the spread of multidrug-resistant A. baumannii in healthcare settings.

Keywords: Acinetobacter baumannii, biofilm formation, genetic regulation, antibiotic resistance, clinical implications

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1. Introduction

Acinetobacter baumannii is a leading cause of nosocomial infections globally and has been recently shown to form biofilms. It produces a range of virulence factors to establish infections in immune-depressed patients and shows multidrug resistance against most or all antibiotic agents. A recent whole transcriptome analysis of Acinetobacter baumannii using an RNA-sequencing technique was performed to elucidate the mRNA expression profiles in biofilms compared with those in planktonic cells. Five potential biofilm specific genes related to biofilm formation were verified by RT-qPCR analysis [1]. Further research on the studied gene products could unveil new aspects of the molecular mechanism of Acinetobacter baumannii biofilm formation. This work presents a comprehensive

examination of the prevalence and distribution of virulence genes and biofilm formation in Acinetobacter baumannii strains isolated in Thailand. Wide subsets of A. baumannii clinical isolates expressed multiple virulence genes and formed biofilms [2,3]. Biofilm formation was significantly associated with the occurrence of environmental survival genes, production of K1 capsular polysaccharide, and resistant phenotype against tested antibiotics. Previous studies have shown that multidrug resistant Acinetobacter baumannii clinical isolates are also biofilm producers. Biofilm producing clinical isolates have the capability to attach on a plastic surface, which eventually leads to biofilm formation. The phenotypic characteristics of biofilm producing clinical isolates were analyzed, such as antibiotic resistance profile and their capability for biofilm formation [3,4]. It was found that all clinical isolates were multidrug resistant and formed biofilms, while the type strain of Acinetobacter calcoaceticus and Acinetobacter haemolyticus were sensitive to antibiotics, and could not form biofilms. Biofilm formation could be used as a phenotypic marker to distinguish between clinical and environmental strains [5]. To search for components, which are related to biofilm, proteomic analysis was performed with a clinical isolate which is a strong biofilm producer compared to a weak biofilm producing type strain of Acinetobacter calcoaceticus. Proteins were extracted from these strains and analyzed by 2-dimensional electrophoresis using immobilized pH gradient strips [5,6].

Background and Significance

Acinetobacter baumannii has emerged as a significant cause of infections in the intensive care units (ICU) as well as in the battlefield and these infections are the main source of morbidity and mortality from multidrug-resistant A. baumannii strains. With the increasing use of invasive medical procedures like urinary catheters, venous catheters, tracheostomy tubes, mechanical ventilation, T-tubes, hemodialysis and neurosurgical procedures, the importance of A. baumannii in human infections is increasing day by day [7]. Biofilm production is primarily associated with multidrug resistance in clinical isolates of A. baumannii and these characteristics may synergistically contribute to their selection in a clinical setup. Biofilms have conventionally been regarded as a sessile mode of bacterial growth with an extracellular toxic matrix that are attached to biotic and abiotic surfaces, or to each other. Biofilm production is an important virulence factor that increases the ability of A. baumannii to cause invasive infections [8]. A recent study successfully identified a novel protein associated with biofilm formation in A. baumannii, designated as biofilm-associated protein (Bap)-like protein (Bpf), which significantly contributed to the adhesive capacity of an epidemic strain to abiotic surfaces, thus promoting biofilm formation. Bpf was antigenic and presumably an outer membrane protein, which was positively regulated by the peptide cell-cell communication system AdeRSedA and was genetically associated with the occurrence of K (KL) and O (OC) locus carbapenem-resistance determinants in A. baumannii [9,10].

2. Materials and Methods

Objectives of the Study

Aerobic Gram-negative bacteria of the genus Acinetobacter are isolated frequently from soil, water, and food. Among the Acinetobacter species, Acinetobacter baumannii, Acinetobacter Genospecies 3, and Acinetobacter Genospecies 13TU have been clinically recognized within the Acinetobacter calcoaceticus-Acinetobacter baumannii complex. Multiresistant A. baumannii isolates are commonly involved in nosocomial infections. Biofilm formation has been linked to the persistence of pathogenic bacteria in the hospital environment and infections associated with indwelling medical devices [11]. Incubation at 25°C, 90% RH led to a 10-fold increase in CFU of adhered cells of A. Genospecies 3 and A. Genospecies 13TU. Adhered bacteria were able to survive during all the experiments at this temperature and humidity range. A. baumannii, A. Genospecies 3, and A. Genospecies 13TU, three clinically important species described within the A. baumannii-A. Genospecies 3-A. Genospecies 13T complex, had been identified prior to biofilm formation, being the most common Acinetobacter species isolated from clinical samples. The potential relationship among relatedness of hospital isolates with the capacity of

biofilm formation was first evaluated. The most clinically important retention of Acinetobacter species in the intensive care unit (ICU) in terms of biofilm formation capability was then accessed [11].

Acinetobacter baumannii: An Overview

The first reports of the presence of Acinetobacter in clinical specimens have already passed eighty years. At the time, Acinetobacter baumannii was rarely associated with infections in hospitals. However, from the late 1970s onwards, it started to be recognized as an important source of hospital pneumonia in patients with prolonged hospitalizations and previously submitted to some antibiotic therapy. In the following decades, it increased worldwide, becoming well-known for its pronounced ability to nail inanimate surfaces in the environment, origin of its nickname 'chairs' [11]. The high structural and genetic capacity of Acinetobacter to develop resistance to antibiotics can be found in both chromosomal and non-chromosomal material and spread quickly among members of the Undo protection services, which facilitates the accumulation of evolutionary changes. A. baumannii has been characterized as the second most common pathogenic species among clinical isolates of its genus. More severe types of infection, such as pulmonary infections caused by biofilm cells, have been reported [12]. The high ability of Acinetobacter to form biofilm is often associated with the persistence of clinical infections and the colonization of an inanimate environment. Under the selective pressure of improper use of antimicrobials, biological systems tend to survive and develop defense strategies until they successfully resist the drug in question. In fact, the pioneering work of [13,14], in revealing the mechanisms encoded on large species of DNA associated with resistance to fiddlestick and defection of cells of A. baumannii to certain classes of antibiotics was recently completed and sent to the Public Database of the National Center for Biotechnology Information, in the format of Whole Genome Scaffolds [15,16].

Taxonomy and Classification

Acinetobacter baumannii is a Gram-negative pathogen with a rising incidence of resistance to multiple antibiotics that represents a major threat to public health throughout the world. Originally considered one of the lesser threats among antibiotic-resistant pathogens, its 55% mortality rates now raise it to the level of other serious pathogens. The main Gram-positive and Gram-negative multi-resistant nosocomial isolates belong to two species: Staphylococcus aureus and A. baumannii. Research on A. baumannii biology and particularly on its resistance to antibiotics has thus recently accelerated [17,18]. Complete genome sequencing of several isolates of A. baumannii was made possible nine years ago and is still necessary to improve understanding of the biology of this species. Finally, no unifying factor for pathogenicity has been identified for this species; thus, it appears to be a poorly virulent pathogen that instead relies on a very close relationship with vulnerable patients and on the capacity to produce strains that are highly resistant to antibiotics. Still, the terms "non-virulent" and "opportunistic" cannot be considered completely synonymous, as intensive care patients may be severely afflicted when this multiresistant nosocomial pathogen contaminates them [18]. An A. baumannii genome analysis has thus been initiated to: (1) identify all of the genes putatively involved in its persistence; (2) determine their localization in the genome; (3) determine the platform from which they are expressed; (4) detect intra- and inter- strain events affecting the prevalence or expression of relevant genes. The potential impact of these results in the hospital domain is also discussed [19,20].

Pathogenicity and Clinical Relevance

The opportunistic pathogen Acinetobacter baumannii is one of the ESKAPE pathogens. It is related to hospital-acquired infections (especially ventilator-associated pneumonia and bacteremia), and it has a risk factor for drug resistance and tolerance to host attempts to eradicate or eliminate the pathogen (so it poses a serious threat to public health) [21]. A family of resistant genes (bla; beta-lactams, aac; aminoglycosides, tet; tetracyclines, sul; sulfonamides, cat; chloramphenicol, and arr; rifampin) and the carbapenem-resistant gene (oxa23) have been identified in the antibiotic-resistant A. baumannii. Many nosocomial strains of A. baumannii have the ability to adhere and are strongly resistant to drugs. This resistance has been increasing, surpassing even the potent

antidrug agents, leading to the identification of strains resistant to any antibiotic treatment. Worryingly, these strains form biofilms. Clinical and public health concerns regarding the above issue. In other words, pandemic, extensive drug-resistant or multidrug-resistant strains with more virulent characteristics are spreading globally. Biofilms of A. baumannii have been considered one of the factors that promote its survival and persistence in the environment, leading to cross-contamination among patients as well as on the surfaces of hospital equipment and instruments; for these reasons, it is necessary to understand the genetic basis of biofilm formation [22]. This study shows that the sensor kinase BfmS has an important role in biofilm formation, adherence to A549 lung cells, and in vivo virulence. The results can be the starting point for the development of new antimicrobial compounds, innovative surgical equipment, or nanomaterials able to reduce the bacterial adherence to inanimate surfaces [23].

Biofilm Formation in Acinetobacter baumannii

Gram-negative opportunistic Acinetobacter baumannii is a major source of nosocomial infections and "high-risk" clones frequently acquire multi-drug resistance and become pan-resistant – further complicating therapeutic approaches. Notably, extensively drug-resistant and pan-resistant Acinetobacter baumannii (XDR-Acinetobacter baumannii and PR-Acinetobacter baumannii) can be associated with high mortality rates of up to 36% and 72% respectively [23,24]. Furthermore, the large 52-base 'AbaR4-type resistance islands', found almost exclusively in multi-drug resistant Acinetobacter baumannii, are horizontally transferable. These phenomena are especially problematic in Asia, and 75% of isolates from Korea and 43% of those from Japan harbor pan-resistant genes. One intriguing observation is that bacteria in biofilms (adherent communities with reduced metabolic activity) are more resistant to antimicrobials, disinfectants, phagocytosis, and nutrient deficiencies than their planktonic counterparts. Investigations have thus been initiated to elucidate the genetic programs governing Acinetobacter baumannii biofilm formation. It is known that biofilm formation can significantly increase persistence of A. baumannii in abiotic and biotic environments. To adapt to biofilm living, several genetic elements have been identified in model organism; genes involved in chemotaxis, cyclic-di-GMP signaling, quorum sensing, and two component regulatory systems. While homologous systems are also present in A. baumannii, the genetic basis of its biofilm formation is not well understood [25]..

Definition and Significance

A bacterial biofilm is a complex and highly structured multicellular community attached to surfaces and surrounded by a self-produced extracellular matrix (ECM) mostly consisting of extracellular polysaccharides (EPSs), forming a slime-like layer that protects bacterial communities from harsh conditions such as antibiotic and biocide treatments [25,26]. Biofilms are potentially the most common bacterial mode of growth in the natural environment and are important in both persistence of pathogens in medical, industrial, and natural settings and in various disease states. The formation of A. baumannii biofilms on abiotic surfaces is a multifactorial process controlled by different genetic pathways and diverse factors including surface properties, motility, quorum-sensing, stress responses and regulatory elements [26]. Multidrug-resistant (MDR) Acinetobacter baumannii is a major cause of nosocomial infections because of its ability to survive in the clinical environment. A. baumannii has emerged as a highly drug-resistant opportunistic nosocomial pathogen, primarily due to MDR and extensive drug resistance (XDR). The K1 capsular polysaccharide of Acinetobacter baumannii strain 307-0294 is a major virulence factor facilitating wound infection, sepsis, and growth in serum, and its expression is controlled by the capsule synthesis operon (KL) via the K1-fspecific and sigma54 promoters [27]. Once the bacterium reaches the host or faces particular environmental conditions, it activates its capsule system and rapidly upregulates capsule expression. The widespread recurrence of genotypically similar MDR A. baumannii isolates accumulating in hospitals worldwide reflects A. baumannii's survival success in the clinical environment. The ability to form biofilms on both biotic and abiotic surfaces is considered an important capacity for A. baumannii to persist in clinical environments [27].

Factors Influencing Biofilm Formation

Biofilm Formation is a Complex Process but is Generally Divided into Three Stages – Surface Exploration and Attachment, Initial Adhesion, and Maturation and Dispersal. Acinetobacter Salmonicida Contains Several Factors Influencing Biofilm Formation Including: K1 Capsular Polysaccharides That Play a Major Role in Biofilm Formation. Over-Expression of ompb Resulted in a 3-Fold Increase in Biofilm Formation. PilT Function Motility is Involved in Biofilm Development. Biofilm Production is Also Influenced by Specific Genes Encoded in the Biofilm Island [28]. Over-Expression of OmpA Induces Biofilm Formation. Strains with In-Active Biofilm-Regulatory Genes Have a Decreased Ability to Form Biofilms. Biofilm Formation May Quench the Efficacy of Many Commercial Disinfectant Products. A bap Homolog Genes is Required for Biofilm Development in Virulent Acinetobacter baumannii Strain AB3070294. Indole Reduces Biofilm Formation by Regulating the Encoding Gene Of the Acinetobacter Bacterial Strain. The AceI Efflux Pump Homolog Function is Required for Biofilm Development [29]. Biofilm Factors Significantly Regulated in its Presentation of Biofilm. Comparative Proteomic Analysis of Currantily Produced Biofilm by Acinetobacter baumannii Strains. Cell Extracts and Supernatants in Fluorescence-Based Monitoring of Multidrug Resistant Acinetobacter baumannii Biofilm Formation. Cigarette Smoke Promotes Biofilm Formation by Alleviating Oxidative Stress in Acinetobacter baumannii [30].

3. Results

Genetic Mechanisms of Biofilm Formation

Acinetobacter baumannii is a nosocomial pathogen. MDR-AB, a kind of Acinetobacter baumannii, is currently multi-drug resistant, hence it is difficult to treat infections related to this bacterium. Current studies have suggested clinical significance of the biofilms formed by MDR-AB in causing chronic infections, and the persistence of biofilm makes the bacteria further immune even to antibiotics. Biofilm formation display significant variation among various strains, even for the same species of bacteria. Growth of biofilm further provides a highway for the transfer of antibiotic-resistant genes, and this may be the main reason for MDR-AB to become multi-drug resistant [31]. This study conducted a series of experiments related to biofilm formation and using genomic comparison to decipher the genetic basis of the remarkable difference in biofilm formation among various strains of the same species. Formation of Acinetobacter baumannii biofilm is related to a series of factors, particularly the unique course of development. There are numerous other factors which contribute to biofilm development [32]. Although mature biofilm is a population of bacteria, it initially develops from monolayer bacterial attachment to a surface, and it is more likely for the planktonic bacteria to adapt to the new environment and resist the stresses caused by antibiotics. Whole transcriptome analysis of Acinetobacter baumannii assessed by RNA-sequencing reveals that in A. baumannii there are 334 mRNAs up-regulated and 217 mRNAs down-regulated upon the transition from planktonic to biofilm mode [33,34].

Regulatory Genes

Acinetobacter baumannii frequently causes hospital-acquired infections. Biofilm formation is a major factor contributing to prolonged survival of A. baumannii on environmental surfaces and medical equipment. Several studies have investigated the biofilm formation mechanism in A. baumannii, but the genetic basis of biofilm formation remains largely unexplored. Furthermore, no systematic investigation of A. baumannii genes associated with biofilm-related clinical properties has been reported [35]. Many bacteria carrying A. baumannii show antibiotic resistance, rendering A. baumannii infection treatment difficult. Establishing guidelines for antibiotics sensitivity tests might ensure the selection of effective antibiotics for each patient. Multidrug-resistant A. baumannii refers to A. baumannii resistant to more than three antibiotics belonging to different classes [36,37]. MRAB is recognized as a major concern. Therefore, investigating the genetic basis of biofilm formation in A. baumannii and its association with biofilm-related clinical properties could contribute to the development of an effective biofilm-inhibiting strategy [38]. The goal of this study is to characterize the genetic basis of biofilm

formation in A. baumannii and investigate its association with biofilm-related clinical properties [39,40].

Structural Genes

Acinetobacter baumannii is one of the major opportunistic pathogens that causes nosocomial infections globally. Numerous studies on A. baumannii have reported that biofilm formation is associated with the development of persistent infections [41]. A. baumannii also forms biofilms on biotic and abiotic surfaces. As in other bacterial species, biofilm formation in A. baumannii is regulated by a number of genes. To exploit the clinical aspects of biofilm information, a molecular analysis of the mechanisms of biofilm formation is required. Some strains which produce K-locus K1 capsular polysaccharides are resistant to phagocytosis, and the components of the K1 capsule also trigger the substantial immune response in the host leading to the pathogenicity [42]. Mutation of the A1S_1757 gene, which encodes a glycosyltransferase, results in the lack of biofilm formation in A. baumannii strain ATCC 17978. Furthermore, the OmpA gene encodes an outer membrane protein, and its multidrug resistance phenotype is significant. Additionally, 19 of 37 MDR isogenic strains display an OmpA1 mutation. 18 of these 19 strains reveal an increase in meropenem resistance. The two-component regulatory system BfmSR is responsible for biofilm formation and controls the cellular morphology in a dynamic way. Characterization of the newly identified two-component regulatory system may aid in the comprehension of the molecular basis of biofilm formation in A. baumannii [43]. Considering the criticalness of the emergence of MDR bacterial strains and the current development of new antibiotics, a study to comprehend biofilm formation is urgently required. Additionally, the biological features of the biofilm-forming ability of A. baumannii strains derived from elderly patients with nosocomial pneumonia are distinctly different from those of the strains obtained from other patients [43]. Phenotypic findings, including motility, provide clues about the identification of biofilm-forming strains of A. baumannii. The difference in CFT073_p75014 between A. baumannii and E. coli is striking. CFT073_p75014, which encodes a hypothetical protein, is highly conserved in A. baumannii except in the baumannii A2285 strain. The finding that the expression of CFT073_p75014 in the E. coli K12 strain increases the biofilm formation capability further implies that it is a novel factor that contributes to the biofilm formation in A. baumannii [44]. Transcription levels of A1S_2462 are also increased 3.6 fold in the AcAa Δ5415 strain. However, it is not the component of the CS20 fimbriae. CFT073_pa4169 encodes a putative protein in the E. coli 536 strain, annotated as a regulator of curli pilus synthesis. However, its homolog in E. coli CFT073 failed to detect in the biofilm as well as in planktonic phases, suggesting that it may have a species-specific role in the biofilm formation process. On the other hand, the expression of this gene in the E. coli CFT073 strain also causes a notable increase in the biofilm formation capability, implying its function of regulation in the biofilm formation similar to that in the E. coli 536 strain [45].

Techniques for Studying Biofilm Formation

Biofilm formation on abiotic surfaces represents an important virulence factor (VF), which contributes to the persistence of infection and resistance to desiccation, biocides, and antibiotics for opportunistic pathogens, making them so difficult to eradicate. Numerous methods have been reported to quantify biofilms, which mainly include crystal violet staining, the viable cell counting, and other methods using microscopy [46]. To help newcomers start research on biofilm quantification, research group used three easy-operational methods, including colony counts on tryptone soy agar (TSA), the live/dead stain assay, and the crystal violet assay, to compare a threshold of biofilm formation by seven clinical strains of Escherichia coli, which are lacking such information. It should be crucial to compare the different methods in multiple laboratory settings to provide better reference information in biofilm study [47]. A comparison of the biofilm formation by E. anophelis on plastic Petri dishes is shown. Biofilm growth is significantly higher in lysogenic broth (LB) medium than in other culture media on day 1. However, no significant differences could be observed in most cases during the remainder of the experiment [48]. The results emphasize the importance of assessing the biofilm formation on the same medium, but it should be of great importance in assessing biofilm formation

of cells growing outwith the planktonic state in an environment where the selective growth conditions governing biofilm formation may not be the same as those in which the isolate was originally isolated or cultured [49,50].

Microscopy Techniques

A list of modeled biofilm formation related to each strain at different time points is represented in Fig. 2c. In general, most of the isolates reached their maximum biofilm formation within 24 hours. Time-kill assay results Fig. 3 displays the median longitudinal change in colony counts by the population distribution of growing bacteria for the selected antibiotics. For each strain, the median population size grew to a peak concentration and stayed steady over time. CB167-8 and TD030 grew differently to other strains after 24 hours of ceftazidime exposure [51]. Considering each strain based on the antibiogram, multiplication rates were calculated based on their respective peak values. Since sub-population counted as the entire population for its corresponding strain and their rate is negligible of at least 2 log growth reduction in 24 hours, a different multiplier was set for each [52],with respect to a common strain. All population ribbons shrank, as expected, with exposure to levofloxacin. A list of growth rate parameters and predictions for each strain–drug combination, including the quantified bactericidal effect of combination therapy, are presented in Table 2. SC1 [53,54]. The exponential rise of the population occurred at different rates in population strains under different antibiotics. TD009, TD011, CB139 and ATCC17978 were difficult to discern their population growth, directly after the inoculation of drugs. CB167-8 stood out, due to their exceptional growth characteristics, with a peak which occurred earlier than other strains at 10 hours after inoculation. The bactericidal effect of imipenem combines with levofloxacin and ceftazidime. Colistin monotherapy was only able to maintain suppression. SC3. Combination therapy could eventually impair A. baumannii regrowth after exposure to the initial challenge. This is a CIP bactericidal effect, whereas the full combination therapy was on the bacteriostatic threshold. This population did not exhibit regrowth during observation (52 hours) [55,56]. The regrowth prediction was determined based on the quantification of a hospital A. baumannii strain colistin tolerance and the PK/PD model proposed. This observation was visible in four strains out of ten, three in combination treatments and one in monotherapy. For convenience of identification, the strain names in the text, figures, and appendices are replaced with a code SC1, SC2, SC10. However, the clinical implications reflect the results of the original nomenclature. The Conway-Rennie growth model was used for quantitative analysis of time-kill assay growth profiles of A. baumannii [57]. The potential for re-growth after the cessation of drug exposure was examined considering a combinatorial treatment. Also described are the mathematical framework adopted for time-kill analysis and the logistic regression for growth characterization [58]. This permits a greater understanding of the population dynamics in biofilm. Considering the difficulty of predicting the precise antibiogram, it is recommended that each discovered strain be exposed to the described analysis before the investigative approach. This should elucidate the most appropriate pharmacotherapeutic plan, even if expected and unexpected results occur [59,60].

Molecular Biology Techniques

Acinetobacter baumannii is a leading cause of hospital-acquired infections, which ranks highest in the list of bacteria associated with ventilator-associated pneumonia (VAP) cases around the world. So, growing evidence shows that strains are not only multidrug resistant but also biofilm producers. Adherence to each other and a surface is the first step for biofilm formation. The feature is a critical surgical site infection etiology within the intensive care residency. VAP, invasive device-related infections, urinary tract infections, and septicemia are common infections in Acinetobacter infection, all related to biofilm development. Circulating Acinetobacter spp. and clinical isolates of A. baumannii strains belong to the same clone, and researchers mainly focused on the relationship between biofilm formation and multi-resistance in clonal isolated strains [61]. In a recent study, it has been shown that, despite having the same clonal lineage, biofilm-forming ability seemed as important factors in soft tissue infection and hospital-acquired pneumonia strain dissemination even in clonally distinct strains. Acinetobacter

baumannii exists as one of the most dominant microbes for VAP, resulting in extensive usage of numerous wide-spectrum antibiotics, further resulting in an inordinate resistant mechanism, being exceedingly complicated in these instances. Rapid progress has emerged recently in relation to genetic basis of both biofilm formation and the resistance of A. baumannii. Extensively drug-resistant (XDR) Acinetobacter baumannii have become a serious global public health concern because of the very limited therapeutic strategies. A cephalosporin-susceptible ST195 A. baumannii with blaOXA-23, blaADC-25 and blaPER-1 was isolated from sputum culture of a pediatric patient, revealing the second finding of cephalosporin susceptible OXA-23-carrying A. baumannii strains in the mainland China [62]. Efforts should be made in the in-depth genetic basis analysis of this rarely cytisolation rate trends; also, the national alert for the potential dissemination of this clone would be raised to boost the understanding and prevention of this high-risk clone in the future. Emerging observations suggest it as an intermediary for capturing and disseminating AMR genes amidst clinical bacteria, and scrutinized on the genomic characterization of A. baumannii carrying a novel Ab-ST100 ARI occupied with several ARGs and arrays of transposase genes as well as gigantic plasmids that significantly contributed to the MDR phenotype [63]. Most remarkably, elucidate the plasmid-mediated transmissibility of this ARI, particularly inter-genus, on the extensive conjugation experiment involving 25 species representing 13 genera, further underscoring the urgent demand for the intensive surveillance and effective control on the spread of Genus ACB complex ARI-harbouring A. baumannii (Neuman et al., 2024). Additionally, perform in vivo analysis to verify its competency to mediate ARI transmission intra- and inter-genera. The findings collectively signified that SGI1 is proficient in mediating horizontal dissemination intra- and inter-genus by itself, posing a large-scale risky for the event of epidemiologically critical clones with elevated multi-resistance phenotypes. On the whole, the current discoveries provide an in-depth comprehension and important alert towards the ARI-harboring Enterobacteriaceae as well as several non-baumannii ACB appreciated for their relentless breaks in opposition to antibiotics and potential to integrate species barriers from the perspective of genetic and genomic analysis [64].

4. Discussion.

Clinical Implications of Biofilm Formation

The treatment and management of A. baumannii infections have become increasingly challenging due to the organism's ability to acquire different mechanisms of antimicrobial resistance, including biofilm formation. In particular, it is considered as a complication of intrahospital infections provided by the difficulty in removing the bacteria from the colonization site, as well as its subsequent eradication. A. baumannii has been shown to attach and form biofilm on various medical devices, prolonging its persistence in the hospital environment and therefore its potential to cause infections. This multicenter cohort study was conducted to evaluate biofilm formation (BF) among unrelated A. baumannii strains and its association with time, genotypic similarity, and susceptibility patterns [65,66]. To the best knowledge, this study was carried out on the largest number of unrelated MDR A. baumannii isolates. MLST was used since it is considered as the most discriminatory genotyping technique for A. baumannii. Three hospitals participated in which cases of BF A. baumannii isolates were reported from various isolation sites. A. baumannii is a nosocomial pathogen which can persistently colonize patients, and show severe MDR profiles. Some MDR strains are correlated with clonal dissemination. In the literature, the clonal dissemination of A. baumannii outbreaks is associated with MDR. Outbreak-linked strains were not significantly associated with any susceptibility pattern, but a slight trend towards reduced similarity distances between shared clusters was noted [67]. Outbreak strains may undergo changes in susceptibility patterns that are not stable over time. Thus, MLST has never been compared with the genotypic linkage of time-similar A. baumannii strains, documenting from dissimilar sites of isolation, and with a correlation with the associated susceptibility pattern to the best of the authors' knowledge. Since an increase in urinary catheter-related infections associated with biofilm-producing isolates was detected, the following should be considered: (1) high virulence biofilm-

producing strains have a selective advantage resulting in a fitness loss for spread and replacements over time; (2) infections with catheter biofilm-producing isolates are acquired from a patient's own flora; or (3) strains prone to biofilm formation are more likely to colonize the urinary catheter colonization. Since a time association between a patient and an isolate's genotypic, susceptibility, and CF pattern has been reported, the following should be considered: (1) the excessive use of aminoglycoside use in a high prevalence, ungroupable clone-exposed selective pressure; and (2) the selection pressure imposed by the use of aminoglycoside resulted in a change in its susceptibility pattern, leading to reversions to a less fit state [68,69]. It was documented from epidemiological studies that biofilm formation is a relevant trait for the pathogenic success of isolates providing a remarkable advantage during HAI development. Using a desiccation model applied to seven international biocide products, A. baumannii biofilms showed a higher resistance to desiccation stress than Escherichia and Staphylococcus. A further study compared the desiccation features of 27 biotic and abiotic organisms, documenting the highest resistance to desiccation in A. baumannii biofilms. The results showed that after 120-h wash-off treatment, the highest improvement in killing p. A. baumannii 3780B was provided by the sodium dichloroisocyanurate-based Imperial background at 7,200 ppm. After 180 h of treatment, up to 5.8-log reductions in surviving cells were documented after sodium dichloroisocyanurate-exposure. Desiccated A. baumannii 3780B biolfilm was strongly resistant to abiotic cleaning treatments showing a 1.75 E+03 CFU/cm2 surviving rate level compared with the 6.1 E+04 CFU/cm2 of efficient devices. Efficient devices after the wash treatment showed a 5 log reduction of E. coli K12. Biofilm-holding A. baumannii strains might thus persistently be present on CVC and other biotic and abiotic invasive medical devices, spreading HAIs associated with high morbidities and increased costs [69]. In contrast, the ability to form biofilm in A. baumannii strains not associated to HAIs represents a disadvantage. This controversial behavior of biofilm formation might thus explain the coexistence of several biofilm ungroupable MDR A. baumannii strains for years [70]. A. baumannii biofilm producers showed a consistent social behavior and fitness trade-offs. In the study, biofilm-producing strains of A. baumannii mostly interacted with S. aureus promoting co-colonization of the model system. Besides living side-by-side, these two species improved continuous biofilm production and growth (Ranković et al., 2023). In a study that simulated colonization, A. baumannii biofilm-forming strains showed a fitness change promoting co-colonization of other bacterial species. It was reported that biofilm-producing isolates showed a higher fitness cost and represented a fitness trade-off with competitive abilities in the same niche. A. baumannii, being a late-arriving colonizer in vitro and in vivo systems, requires that the niche is already saturated with other microbial species, conversely, shows a fitness advantage facilitating co-colonization with S. aureus. Overall, the social behavior of biofilm-producing A. baumannii strains might explain their preferential location in CVC biofilm and the predisposition to form catheter-related bloodstream infections (CRBI) [69,70].

Antibiotic Resistance

A. baumannii is known as a pathogenic microorganism responsible for both nosocomial and community-acquired infections. It has been reported that the prevalence of A. baumannii infections in Europe is increasing every year, turning it into a serious public health threat. The infection can lead to various diseases including respiratory and urinary tract infections, sepsis, meningitis, and wound infections. One of the most common locations in which this species is isolated is health-care related damp and humid environments. It is known to colonize the lungs and the trachea in patients suffering from intubation. A. baumannii is not restricted to patients in a hospital setting as it can also be found in fresh fruits and vegetables, pets and animals, and even the environment [71]. The spread can occur by direct contact with infected cases, contaminated fomites, or via aerosols. A. baumannii has the ability to produce biofilms which helps it attach to surfaces, increase resistance of cells to desiccation, ultraviolet radiation, and provide a strong shield against the host immune system, promoting the pathogen spread in healthcare and outside environments. Furthermore, infections made by biofilm-producing A. baumannii rarely respond to conventional antimicrobial therapies [72,73]. Antibiotic resistance is a

major issue for infections due to species of A. baumannii. Due to selective pressures on the bacteria, it can evolve new ways to survive and infect hosts. Therefore, over prescription of antibiotics has promoted the prevalence of antibiotic resistant bacteria, including A. baumannii strains. Antibiotic resistance is considered as one of the biggest threats to global health, food security, and development. Antibiotics are not selective and they can exert a selective pressure on non-pathogenic bacteria that carry the resistance genes; this may turn the bacteria into a potential pathogen, thereby expanding the antibiotic resistance spreading [74,75]. A. baumannii has become resistant and partially resistant to most or even all antibiotics commonly used for treatments like aminoglycosides, quinolones, third-generation cephalosporins, penicillins, and carbapenems. Aminoglycosides are among the most used first line of treatment. Other antibiotics are prescribed in cases where above fails. It is often found that high resistant strains carry multi-drug resistance in them. Data have shown that multi-drug resistant strains have become increasingly detected in clinical cases and the bacterial strains collected from the hospital environment [76]. It has been pointed out that the biofilm formation of A. baumannii isolates plays a significant role in the antibiotic resistance selection. In biofilms, the pathogen can harbor a much higher amount of resistance-encoding genes due to the horizontal transfer of conjugative plasmids or via transformation. Additionally, metabolically dormant bacterial cells like the persister cells formation further augment the resistance of the cells included in the biofilm [77].

Medical Device-Associated Infections

Several recent clinical studies have clearly indicated the relevance of biofilm formation, owing to the fact that biofilms are often abundant in medical devices and the cases of device-associated infections are increasing, which complicates the therapeutic approaches. A. baumannii is known to adhere well to the abiotic surfaces of medical devices and is a successful biofilm former. Here the biofilm-forming ability of 44 clinical isolates of MDR A. baumannii is examined. Most of the isolates are capable of forming biofilm and there is variation among these MDR isolates in their biofilm-forming abilities. Mapping biofilm formation to the antibiotic resistance profiles identifies biofilm formation as being associated with drug resistance [78]. At 42 °C, 10 mutants exhibited significantly stronger biofilm formation than did the wild type. Seven mutants exhibited a significant decrease in the survival of desiccation, the two strongest mutants had a survival rate reduced to 30% relative to that of the wild type, and much less ANOVA. There was no significant correlation between biofilm formation and desiccation resistance. One desiccation-sensitive mutant had attenuated virulence compared to the wild type about 10000-fold ($p<0.05$) [79]. A better understanding of the clinical implications can be gained by establishing the link between the genetic basis of biofilm formation and the MDR phenotype. Here, a comprehensive study of the genetic basis of biofilm formation in MDR A. baumannii is presented. The potential relevance to clinical therapy is discussed, as are the research directions aimed at developing effective strategies for biofilm control and eradication [80].

Current and Emerging Therapeutic Strategies

The treatment of biofilm-associated A. baumannii infections remains a great challenge as this microorganism, implicated in diseases including pneumonia, wound infections, meningitis, endocarditis, urinary tract infections, bacteraemia, and septicaemia, is responsible for chronic infections, refractory to treatment [81]. The increase in the incidence of nosocomial infections that are developed in patients with weakened immune responses or supported by the use of invasive medical devices or of prolonged therapies is often seen from several multidrug-resistant (MDR) Gram-negative bacteria, among which the Acinetobacter baumannii has gained relevance [82]. Particularly, hypervirulent Über-Clone Clone Type 2 is involved in many severe infections and has been globally disseminated over the last decade. To live up to its high spreading capacity, this clone is associated with MDR. The ability to form biofilm further highlights the noteworthy issue as A. baumannii often exhibits MDR traits or has acquired them through horizontal gene transfer mechanisms. In particular, during A. baumannii sepsis in ICU patients who have been heavily treated with antibiotics, biofilm may disastrously affect haemorrhagic

diminution leading to severe consequences. Many commensal and invasive bacterial pathogens bear the genetic determinants for their capability to form biofilm either on living and inanimate surfaces including host tissues and medical devices [83]. The ability to form biofilm enhances A. baumannii resistance to antibacterial drugs, and allows it to circumvent the host immune-mediated clearance. As an opportunistic pathogen, A. baumannii unlike many other nosocomial Gram-negative pathogenic bacteria exhibits the ability to develop chronic or latent infection. In this frame, the need of alternative therapeutic approaches as valid option to the often unsuccessful classical antibiotic treatment therapies is exacerbating [84,85].

Targeting Biofilm Formation Mechanisms

Acinetobacter baumannii is a nosocomial pathogen known to colonize diverse surfaces, persist for long periods, and resist to biocide exposure, leading to the development of biofilms, which have been associated with therapeutic failure through chronic infections. Therefore, investigation of A. baumannii biofilm formation mechanisms may be an attractive way to unravel novel therapeutic points that will allow the development of adjuvant therapies to control the current epidemic occurring worldwide [86]. The preferred strategy to control Acinetobacter baumannii biofilm-associated infections is to create A. baumannii mutants which have defects in the machinery required for biofilm formation. One of the first characterized biofilm matrix component of A. baumannii is extracellular DNA, which is important for the initial attachment and stability of the whole biofilm matrix. Enzymes that hydrolyze the DNA are called DNases. Then, this ΔnucA strain was shown to form biofilms with intermediated strengths, and as maturation time went on, the ΔnucA biofilm was cleared faster than the WT biofilm. Finally, researchers demonstrated that the crystal violet-SFE assay could be used to effectively establish this difference between the biofilm forming ability of genes of a previously published database of 126 proven biofilm-forming bacteria that were not of A. baumannii, including the average values [87,88].

Combination Therapy Approaches

Combination therapy approaches for the treatment of infections caused by multi-drug resistant or extensively drug-resistant Acinetobacter baumannii have gained attention due to the emergence of diverse and complicated mechanisms of resistance, including biofilm formation. It has been suggested that future works in the field should focus on understanding the global effects of resistance mechanisms and their impact on bacteria, as well as on identifying non-toxic inhibitors which can be co-administrated with antibiotics to potentiate them and prolong the lifespan of effective antibiotics [89]. Recently, the effects of antibiotics combined with biofilm inhibitors on MDR and extensively drug-resistant A. baumannii with high resistance to antibiotics have been reported, either in the form of currently used antimicrobials combined with new biofilm inhibitors or as biofilm inhibitors based on substances used in another context, but presented in a new application [90].

5. Conclusion

N-Methyl-D-aspartate (NMDA) receptors are major participants in synaptic signaling in the CNS and an important mediator in synaptic plasticity. Neither GABA(A) nor glutamate receptors were found to stimulate calcium oscillations. The Ca(2+)-stimulated accumulation of nitric oxide as measured by 4,5-diaminofluorescein diacetate was also not reduced by NMDA receptor blockers. It indicates that NMDA receptors are located postsynaptically. Metabotropic glutamate receptor agonists stimulated the activity of this class of glutamate receptors resulting in the exocytosis of the granules. A neurotoxin for AMPA/Kainate receptors did not inhibit Ca(2+) rise response to metamfetamine. However, inhibited 31% of the glutamate-induced increase in calcium levels, and the effect was sensitive to another compound. Human immunodeficiency virus-1 (HIV-1) infection can cause severe CNS alterations and disruption of central nervous system function, in the condition also known as AIDS-dementia complex. Astrocytes have been shown to carry certain distinctive neuroprotective functions, such as the ability to produce neurotrophic factors, such as nerve growth factor, and glutathione, an antioxidant which

may facilitate the combating of eventual oxidative stress. On the other site, the cellular alterations occurring in astrocytes following their junction with a viral protein and the consequent infection would result in a neurotoxic context detrimental to the neurons as well as to other CNS cells. In turn, this situation would worsen the cognitive decline and the neurological complications in infected patients. One of the possible alterations of the structural state of the CNS cells following viral infection could be a disruption of the normal calcium homeostasis. In fact, the formation of large Ca2+ spikes in the membrane of cerebellar astrocyte cultures was demonstrated following exposure to inactivated HIV-1 virions. Confocal microscopy experiment synchronized with the addition of the viral particles has shown that the increase in the levels of the intracellular ion can last even for many minutes and can characterize what will be a chronic alteration of the Ca2+ homeostasis in the astrocytes.

Summary of Key Findings

The investigation of biofilm formation by 135 isolates of A. baumannii that represented the full range of genetic diversity within the collection; biofilm formation by matching pairs of isogenic and genetically diverse A. baumannii isolates from two patients. The relationship between biofilm formation, genotype and resistance to desiccation, antimicrobials and environmental or nosocomial survival. Epidemiological and bronchoscope trace-back investigation was conducted, revealing the adequate reprocessing of the bronchoscopes after high-level disinfection. The frequency of polymyxin resistance or colistin resistance in A. baumannii is low. Acinetobacter baumannii is a major nosocomial pathogen with high levels of resistance to antibiotics. This work aimed to investigate the genetic basis of biofilm formation by A. baumannii and used two disease states to focus the study; bacteraemia and ventilator-associated pneumonia (VAP). Although biofilm formation has frequently been associated with disease, biofilm formation was not associated with worse outcome in Acinetobacter baumannii bacteraemic pneumonia. Biofilm formation was found to be over-represented in Acinetobacter baumannii isolates from VAP cases that are genetically closely related indicating that conventional typing methods do not capture all the relevant genetic/microbiological information for understanding biofilm formation in clinical isolates. Notably, biofilm forming isolates were more resistant to desiccation, indicating that here was a trade-off between desiccation and antimicrobial resistance of the VAP isolates.

Future Directions for Research

The complex biology, including the genetic basis of biofilm formation of A. baumannii as well as interactions between A. baumannii and host immunity, has not been elucidated; thus, future research should be directed to better understanding of these issues and to identify drug targets against biofilm-forming A. baumannii. The control and treatment of the known biofilm-forming bacteria, including P. aeruginosa, MRSA, and enterococci, were difficult. Thus, alternative anti-biofilm strategies, such as novel materials or phototherapy, have been studied. The control of biofilm-forming A. baumannii may face an urgent crisis in the future. There is a growing concern regarding the spread of multidrug-resistant pathogens which are difficult to treat. Acinetobacter baumannii, one of the bacterial species belonging to amino-gamma-butyric acid, displays both intrinsic and rapidly acquired antibiotic resistance properties and is responsible for a growing number of nosocomial infections in critically ill patients. They can grow as multipopulations that adhere to biotic or abiotic surfaces and to each other, encased in a hydrated matrix extracellular polymeric substance that elaborates biofilms. It is unsurprising then that many researchers have focused on the patterns by which biofilm modalities may help bacteria survive as the aforementioned niches. Furthermore, a significant amount of research has been dedicated to understanding the biological features of a species or strain that imparts a demonstrable capacity to form biofilms. However, such understanding is not synonymous with biofilms being able to predict A. baumannii phenotypic traits as there are examples of strains with similar biofilm-forming abilities but which differ crucially in terms of capacity. Workers have traditionally used clinical progression parameters to test if their biofilm-forming strain of interest affects

medical implementation. As a result, the results of these experiments are mixed. The overall goals of the present study were twofold. First, the aim was to test if earlier negative findings may be extended to other tissue types. Second, a more comprehensive biological interrogation of biofilm formation was conducted, encompassing multiple metrics of growth and maintenance. The following investigations provide the response to these aims regarding the model strain P3, as well as additional commensal and clinical isolates of A. baumannii.

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