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ARTIKEL TINJAUAN PUSTAKA

PSEUDOMONAS AERUGINOSA BIOFILM FORMATION AND ITS RESISTANCE TO BETA-LACTAM ANTIBIOTICS

PEMBENTUKAN BIOFILM BAKTERI PSEUDOMONAS AERUGINOSA DAN SIFAT RESISTENSINYA TERHADAP ANTIBIOTIK BETA-LAKTAM

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ABSTRAK
Pendahuluan: *Patogen oportunistik Pseudomonas aeruginosa adalah bakteri gram negatif yang menyebabkan infeksi akut dan kronis pada manusia. Pseudomonas aeruginosa memiliki struktur resistensi yang kuat dengan menempel pada permukaan yangsesuai dan membentuk matriks biofilm. Biofilm bakteri ini dapat menyebabkan resistensi alami yang lebih tinggi terhadap antibiotik karena mekanisme toleransi yang dibuat oleh biofilm. Struktur biofilm terdiri dari matriks eksopolisakarida (PsI, Pel, alginat, dan eDNA) yang sangat sulit ditembus oleh antibiotik. Alginat berperan dalam pembentukan mukus pelindung resistensi antibiotik, dan komponen Psl dan Pel berperan dalam proses pematangan biofilm dan resistensi antibiotik. Pseudomonas aeruginosa dan sistem kekebalan tubuh. Pseudomonas aeruginosa dapat mengembangkan mekanisme resistensi terhadap antibiotik beta-laktam karena adanya hubungan antara pembentukan biofilm dan resistensi antibiotik.*
Tujuan: *Berdasarkan hal tersebut, diperlukan informasi lebih lanjut mengenai hubungan pembentukan biofilm bakteri P. aeruginosa dengan resistensinya terhadap antibiotik beta-laktam.*
Metode: *Artikel ini menggunakan metode tinjauan pustaka dari berbagai literatur mengenai pembentukan biofilm bakteri P. aeruginosa dan sifat resistensinya terhadap antibiotik beta-laktam, seperti artikel penelitian dan studi yang bersangkutan. Tidak ada pembatasan jenis studi referensi artikel ini.*
Diskusi: *Pseudomonas aeruginosa memiliki mekanisme resistensi antibiotik bawaan dan adaptif. Berkurangnya permeabilitas membran, produksi enzim yang kebal antibiotik, perubahan kromosom, dan transfer gen horizontal dari bakteri lain semuanya berkontribusi terhadap resistensi antimikroba. Pseudomonas aeruginosa dapat mengembangkan mekanisme resistensi antibiotik beta-laktam. Sifat resistensi Pseudomonas aeruginosa dapat berubah secara fenotip akibat perkembangan biofilm. Artikel ini diharapkan dapat menambah informasi untuk perkembangan penelitian terhadap penanganan dan terapi obat pada individu yang terinfeksi P. aeruginosa.*
Kata Kunci: *biofilm, beta-laktam, Pseudomonas aeruginosa, resistensi*

ABSTRACT
Introduction: An opportunistic pathogen, Pseudomonas aeruginosa is a Gram-negative bacteria that causes acute and chronic human infections. By adhering to appropriate surfaces and creating a biofilm matrix, Pseudomonas aeruginosa has a high-resistance structure. Due to biofilm resistance mechanisms, this bacterial biofilm may increase natural antibiotic resistance. Antibiotics have difficulty penetrating the exopolysaccharide matrix that makes up the biofilm structure (Psl, Pe, alginate, and eDNA). Alginate is involved in generating mucus, and Psl and Pel components are implicated in biofilm development and antibiotic resistance. Due to the connection between biofilm production and antibiotic resistance, Pseudomonas aeruginosa involves mechanisms of beta-lactam antibiotic resistance.
Objective: On this basis, learning more about the connection between Pseudomonas aeruginosa bacteria's ability to form biofilms and their resistance to beta-lactam antibiotics is essential.
Methods: We use literature methods from various literature on the biofilm formation of P. aeruginosa and its resistance to beta-lactam antibiotics, including research papers and studies with no restrictions types of studies included in this article.

Discussion: Pseudomonas aeruginosa has intrinsic and adaptive antibiotic resistance mechanisms. Decreased membrane permeability, the production of enzymes resistant to antibiotics, chromosomal changes, and horizontal gene transfer from other bacteria contribute to antimicrobial resistance. Beta-lactam antibiotic resistance mechanisms are known to be developed by Pseudomonas aeruginosa. The resistance characteristics of Pseudomonas aeruginosa may change phenotypically due to biofilm development. This article will be helpful in future research on Pseudomonas aeruginosa therapy and medication.
Key Words: biofilm, beta-lactam, Pseudomonas aeruginosa, resistance.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic, motile, non-fermentative, Gram-negative, opportunistic pathogen bacterium found in soil, plants, and hospital reservoirs that causes human infections. These opportunistic bacteria are responsible for infectious diseases such as pneumonia (ventilator-associated pneumonia), urinary tract infections, skin and soft tissue infections, bone and joint infections, especially AIDS patients, cancer, and burns related to immunocompromised patients.[1] Pathogenicity by *P. aeruginosa* results from phenotypic adaptability and a high degree of genomic flexibility. These microorganisms increase the synthesis of virulence and virulence factors. Pseudomonas aeruginosa alters proliferative activity and generates biofilms under stressful conditions.[2]

Antipseudomonas β-lactam antibiotics play an essential role in the clinical management of *Pseudomonas aeruginosa* infections due to their therapeutic efficacy. Mechanisms of antimicrobial resistance found in *P. aeruginosa* include an efflux pump system, porin impermeability modification, target modification, and enzyme-mediated antimicrobial inactivation (e.g., β-lactamase). Multiple resistance mechanisms often coexist, leading to simultaneous resistance to multiple antibiotics. Biofilm formation is one of the causes of multidrug resistance (MDR) in *Pseudomonas aeruginosa*. Biofilms are not degraded by disinfection, heating, or drying and can lead to contamination and transmission of infectious diseases.[3,4]

Biofilms are microorganisms under extreme conditions that adhere to each other on surfaces by producing a self-generated matrix of exopolysaccharides, proteins, metabolites, and extracellular DNA (eDNA) that protects the microbial population in a series of defenses. Microbial cells in biofilms are less sensitive to antimicrobial agents and contain more immune responses than their wild-type cells. Even bacteria that lack intrinsic resistance or protective mutations may become less susceptible to antibiotics under biofilm conditions. *Pseudomonas aeruginosa* undergoes many physiological and phenotypic modifications at the same point of biofilm formation. Most strains can synthesize all three polysaccharides (*Pel*, *Psl*, and alginate) and serve as matrix components in biofilm formation.[5,6]

Infections with *Pseudomonas aeruginosa* are challenging to treat because most isolates exhibit innate resistance to multiple antibiotics. This is attributed to decreased outer membrane permeability, expression of the efflux pump system, and production of enzymes that inactivate antibiotics. Among them are the essential roles of various beta-lactams, such as AmpC, extended-spectrum

beta-lactamases (ESBLs), and carbapenemmases. The coexistence of multiple beta-lactamases in clinical isolates commonly confers resistance to almost all beta-lactam antibiotics.[7]

On this basis, the relationship between the biofilm formation of *Pseudomonas aeruginosa* bacteria and resistance to beta-lactam antibiotics should be further understood.

PSEUDOMONAS AERUGINOSA

Characteristic

Pseudomonas aeruginosa (*P. aeruginosa*) is an aerobic, Gram-negative bacterium with a rod size of 0.5-0.8 µm x 1.5-3 µm, β-hemolytic, simple, non-encapsulated, carbohydrate cannot be fermented, with oxidase activity and a distinctive wine-like odor. The bacterium grows well at 25°C and 35°C and can grow even at 42°C, distinguishing Pseudomonas aeruginosa bacteria from other species. Pseudomonas aeruginosa can also degrade aromatic polycyclic carbons to produce lectins, rhamnolipids, quinolones, hydrocyanic acid, and phenazines when present in the soil. *Pseudomonas aeruginosa* strains produce four pigments: pyocyanin, pyoverdin, pyorubin, and pyomelanin. The tremendous metabolic diversity and flexibility of P. aeruginosa grows in various environments and nutrient sources and can be an opportunistic pathogen. *P. aeruginosa* forms biofilms, is resistant to antibiotics, produces virulence factors, and can rapidly progress to chronic infection.[8,9]

Pseudomonas aeruginosa was first isolated from green pus by Gessard in 1882, and Pseudomonas was first introduced by Migula in 1894. Different colors are produced by different types of pigments produced by *P. aeruginosa*:

a. Pyoverdin/fluorescence is a water-soluble green-yellow fluorescent dye. This pigment is 70-80% derived from the genus Pseudomonas isolates. It also functions as a siderophore at low Fe content.
b. Pyocyanin is a fat-soluble phenazone-derived blue-green pigment involved in iron metabolism. These pigments help maintain the redox balance around bacteria and communication between cells.
c. Pyrubine is a water-soluble reddish-brown pigment. This pigment is derived from 2-3% of Pseudomonas isolates. It has the function of maintaining the redox balance.
d. Pyomelanin is an essential water-soluble, acidic, dark-brown pigment.[10]

Virulence Factors

P. aeruginosa is an opportunistic pathogen that causes several human infections and is resistant to antibiotics. *P. aeruginosa can* adapt to environmental changes, rapidly develop antibiotic resistance, and produce various virulence factors.[11] Due to its capacity to forego innate and adaptive immunity through adhesion, invasion, and biofilm formation, as well as its capacity to create several virulence factors that cause severe tissue damage, *P. aeruginosa* may impact immunocompromised patients. Additionally, this bacterium is responsible for diseases with high death rates in neonates, cancer

patients, cystic fibrosis patients, and people with severe burns.[2,11]

Some virulence may cause disrupted host cell signaling pathways that target the extracellular matrix and/or virulence-promoting adhesion. Several types of virulence caused by *P. aeruginosa* virulence factors are lipopolysaccharides, flagella, fimbriae type IV, excretion system type III, exotoxin A, proteases, alginate, *quorum sensing*, biofilm formation, secretion system type IV. These virulence factors are significant factors that act differently on the immune system.[4] In this article, the production of biofilms, one of the virulence factors, and its relationship to antibiotic resistance will be discussed in greater detail.

Biofilm Formation

A biofilm is a collection of complexes that serve as a barrier between microorganisms and the external or internal environment's microbes. In contrast to the planktonic forms, *P. aeruginosa* commonly develops biofilms in response to exposure to harsh environments, which aids in evading host immune responses and is frequently associated with increased antimicrobial resistance. Microorganisms that generate biofilms also exhibit varying phenotypes based on growth rate and gene transcription.[4] Some bacteria, like *P. aeruginosa* and *P. fluorescens*, can produce biofilms in any environment that supports growth. On the other hand, certain strains of Escherichia coli K-12 and Vibrio cholera only produce biofilms when a minimum medium is supplemented with amino acids.[8,12]

More than 50% of the extracellular matrix of *P. aeruginosa is* composed of three types of extracellular polysaccharides, the capsular alginate polysaccharide and two aggregate polysaccharides (*Psl* and *Pel*), but also contains extracellular DNA (eDNA) and protein. Mature *P. aeruginosa biofilms* are characterized by a “closed” mushroom-like structure and an intricate network of channels for delivering nutrients, oxygen, and waste products.[13] *P. aeruginosa* strains recovered from cystic fibrosis (CF) patients primarily secrete alginate. In contrast, environmental *P. aeruginosa* bacteria primarily secrete *Psl* and *Pel*.[14] It was clearly stated that the polysaccharide synthesis locus (Psl) is a crucial component of the EPS matrix of the *P. aeruginosa* biofilm. Cell-cell interactions and the formation of a matrix are encouraged. *P. aeruginosa* produces *LecA* and *LecB*, which have affinities for the sugars present in the EPS matrix and aid in the attachment of *P. aeruginosa* to particular host cells. Cells and EPS are retained in a persistent biofilm due to *LecB*'s binding to *Psl*. The *Psl* matrix and the biofilm's structure were affected by the chemical separation of *Psl* from its bacterial surface.[8,15,16]

Three different extracellular polysaccharide types were used to gauge the structural integrity of the *P. aeruginosa* biofilm. D-mannuronic acid and L-glucuronic acid combine to form the linear polymer chain known as alginate. The biofilm formations must be safeguarded and stabilized by these two polymers. Alginate

also aids in preserving matrix elements, including moisture and nutrients. *Pel* is a glucose-rich matrix, but its composition is unknown. *Psl*, on the other hand, is a pentasaccharide made up of repeating D-mannose, L-rhamnose, and D-glucose residues. Major structural architectures *Pel* and *Psl* are involved in the early phases of biofilm development. Another important component is eDNA as a food source for bacteria within the biofilm, which serves as a cell-to-cell connection.[11,17,18]

eDNA is an important functional component of the biofilm matrix of *P. aeruginosa*. eDNA is produced from outer membrane vesicles carrying DNA or from DNA released during cell lysis or death. Several lytic agents, including prophages, autolyzing proteins, enzymes, and phenazine, help release eDNA. Some bacterial biofilms' structural integrity is preserved by eDNA, which also acts as a different supply of nutrients like carbon, nitrogen, and phosphate. According to numerous research, eDNA shields biofilms from antibiotic exposure.[19,20]

Biofilms are formed adequately within a few hours. Biofilm formation mainly consists of the following four stages, namely:

a. Planktonic bacteria are initially attracted to surfaces by physical forces, and this initial attachment results in interactions between the bacterium and the surface. Early maturation of a developing biofilm is determined by forming an extracellular matrix that enables mechanical bonding between bacterial cells and supports the transition from “free-living” to “static-living”.
b. Cells irreversibly attach to surfaces and are surrounded by a matrix of extracellular macromolecular substances, causing cell clumping. Transcripted genes involved in alginate production are activated and control the adhesion process' initial matrix creation after bacteriostasis. Cyclic-di-GMP (c-di-GMP), a key messenger found in the cytoplasm of bacterial cells that regulates changes between planktonic organisms and biofilms, is directly responsible for gene regulation.
c. The development of microcolonies that mature three-dimensional biofilm formations. Transport of the nutrients and oxygen required for the growth of sessile cells is made possible by the three-dimensional structure. The biofilm is also regulated from top to bottom regarding acidity and oxygen content.
d. As the infection spreads, the detachment of microcolony cells from the mature biofilm colonizes new attachment sites. Numerous variables, including mechanical disturbances (abrasion), enzymatic breakdown (enzyme secretion determined by quorum sensing), food deficits, or even overpopulation, can cause biofilms to develop. The network is cleared of bacterial components, which are then applied to the environment.[17,21]

Biofilm formation is a complex set of processes involving diffusion, transport, chemicalreactions, and ecological mechanisms

controlled by adhesion, *quorum sensing*, cell death, and thespread of infection. The quorum-sensing system (QS), a cell-to-cell signaling mechanism that controls gene expression in response to variations in cell population density, is primarily responsible for controlling the production of biofilms in *P. aeruginosa*. As a result, biofilms are seen as the dynamically changing structural arrangements of microorganisms that adapt to their surroundings.[17,22]

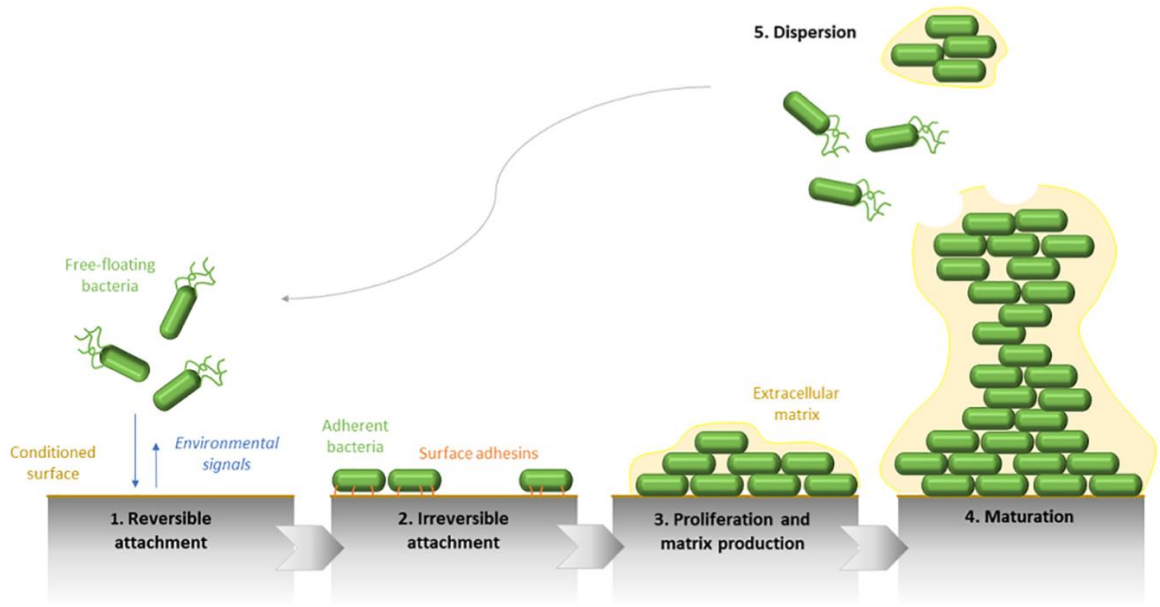

Figure 1. Four Steps of *P. aeruginosa* Biofilm Formation.[21]

Properties of Antibiotic Resistance to Biofilm Formation

Most cells capable of generating biofilms enter the stationary phase over time and survive in large numbers during this stage. One percent of the population developing antibiotic resistance during the stationary phase is the primary cause of decreased susceptibility to them. Biofilms created by antibiotic-resistant biofilm cell states on medical implants like joint implants, urinary and cardiac catheters, and replacement heart valves contribute to human infection. This situation seriously threatens humans because of its pathogenicity and is responsible for most pathogenic infections.[17]

Many endogenous and acquired antibiotic resistance mechanisms, including resistant cell growth and biofilm-mediated MDR, are the leading causes of *P. aeruginosa*-acquired drug resistance. *P. aeruginosa* is susceptible to rapidly developing antibiotic resistance, including aminoglycosides, quinolones, and β-lactams. *P. aeruginosa* gains antibiotic resistance through several processes, including the outer membrane's permeability, efflux-pump systems, and the enzyme that renders drugs ineffective.[18] *P. aeruginosa* exhibits three different types of antibiotic resistance: acquired antibiotic resistance (caused by mutation and the acquisition of resistance genes), intrinsic

antibiotic resistance (caused by the permeability of the outer membrane, the efflux system, and enzymatic antibiotic inactivetion), and adaptive antibiotic resistance (biofilm-mediated resistance). Through horizontal gene transfer, antibiotic-resistance genes carried on plasmids might originatefrom the same or distinct bacterial species. Biofilm production is prompted by quorum-sensing signaling molecules, which are physical barriers stopping antibiotics frompenetrating cells.[17,23]

Due to the extremely low permeability of the outer membrane and the presence of efflux, porin, and beta-lactamase pumps, *P. aeruginosa* is resistant to antibiotics. However, the emergency of pathophysiological and micro-indel mutations in their coding genes due to intensive pharmacotherapy therapy leads to overexpression of efflux pumps, changes in targetantibiotics, hyper lactamase production, and reduced outer membrane permeability due to loss ofporins. In addition, adaptations such as alginate generation, biofilm formation, lipopolysaccharide modifications (lipid A-aminoarabinosylation and O-polysaccharide loss), and *quorum-sensing mutations* contribute to drug resistance. Due to a protective advantage provided by the biofilm phenotype, the resistance mechanism is specific to bacteria encapsulated in biofilms.[13,14,17]

Morphological changes in *P. aeruginosa* emerged when the bacteria dissected meropenemand biapenem to form a “bulge”-like structure in the center. Numerous investigations have revealed a connection between the amount of endotoxin secreted by bacteria and the development of new types. Antibiotics block PBP-2 and PBP-3, which cause bacteria to change their size and form. Bothcontribute to the creation of bacterial cell walls and in Gram-negative bacteria, both proteins are theprincipal targets of beta-lactam antibiotics. PBP-1 inhibition causes cell lysis, but PBP-2 and PBP-3inhibition has been linked to filamentous bacteria.[21]

Antibiotic susceptibility is rapidly restored once the bacteria disperse from the biofilm. Recovery processes under dispersion conditions of dispersal from biofilms suggest an adaptive resistance mechanism rather than genetic modification. The natural protection provided by biofilmscan provide spontaneous breeding grounds for mutants. Furthermore, it has been speculated that the spatial affinity of bacterial cells within biofilms accelerates plasmid transfer. The four hypotheticalstages of the biofilm resistance mechanisms are the slow entry of antibiotics into biofilms, substrateconcentration gradients or metabolites leading to zones of slow or non-growing bacteria and expression by some bacterial cells, adaptive stress responses, and finally, small cells differentiate into highly protected persistence cells.[24]

Beyond resistance mechanisms, bacteria may exhibit a tolerance capacity to withstand transient exposure to high concentrations of antibiotics. Tolerant organisms grow more slowly or have a longer lag time in leaving the stationary phase than intolerant organisms. Targeted common antibiotics, e.g., RNA polymerase, a biosynthetic

cell wall enzyme, are less active in non-growing cells, thus avoiding antibiotic-killing processes. The physical mobility of antibiotics within biofilms does notguarantee they will penetrate the biofilm. Diffusion reactions are sufficient to prevent penetrationof penicillin antibiotics into biofilms formed by β-lactamase-positive bacteria.[24,25]

Planktonic morphology and biofilm bacteria vary in their susceptibility to antimicrobial agents. Microorganisms that form biofilms are more resistant to antibiotics than bacteria that grow as singlecells. *P. aeruginosa* biofilms are considered one of the leading causes of unsuccessful antibiotic therapy. *P. aeruginosa* has both inherent and acquired resistance to various antibiotic classes. Tetracyclines, tigecycline, chloramphenicol, and trimethoprim are associated with natural resistance. Beta-lactam antibiotics (benzylpenicillin, isoxazolyl penicillins, aminopenicillins, and in combination with beta-lactamase inhibitors, first and second-generation cephalosporins) are also linked to natural resistance. The creation of enzymes that break down β-lactam antibiotics is connected to acquired resistance. Production of metallo-β-lactamases plays a significant part in *P. aeruginosa*'s antibiotic resistance (MBL). A broad-spectrum antibiotic with all penicillins, cephalosporins, and carbapenems is what MBL is known for. All β-lactamase inhibitors that are clinically accessible have a mild inhibitory effect on these enzymes. When treating infections brought on by multidrug-resistant strains of Gram-negative bacteria, MBL is frequently referred to as an "antibiotic last resort" due to its potent immobilization of carbapenems.[26]

Antibiotic Beta-Lactam

Beta-lactam antibiotics (BLA) are the most widely recommended and used antibacterial molecules to combat various infectious. BLA has a bicyclic or monocyclic structure composed of four β-lactam cores, which is its weakness. The β-lactam amide group can be hydrolyzed enzymaticallyby the -lactamase enzyme, rendering the compound susceptible to antibacterial activity. In general, β-lactam antibiotics have clinically broad-spectrum activity against Gram-positive and Gram-negative bacteria. Antibacterial activity derives from its ability to inhibit a series of cyclic transpeptidase enzymes known as penicillin-binding proteins (PBPs), essential for synthesizing the peptidoglycan layer of the bacterial cell walls. Without peptidoglycan, bacteria cannot grow and eventually die.[27]

Based on the Amber classification, β-lactamases are classified into four classes: class A (ESBL), class B (MBL), class C (*ampC*), and class D (OXA type). Classes A and B have been described as enzymes that rapidly developed resistance to clinical isolates of *P. aeruginosa*. Severalvariants of the broad-spectrum OXA enzyme have been identified in *P. aeruginosa*. Other ESBLs include PER (penicillins and cephalosporins), GES (penicillin and broad-spectrum cephalosporins), and KPC (carbapenems). Metallo-β-lactamases are unique because they contain zinc (Zn) in their active site. The first metallo-β-lactamase in *P. aeruginosa*

was discovered in Japan, and this bacterial factor has developed rapidly to date.[28]

Mechanism of Beta-Lactam Resistance in *Pseudomonas aeruginosa*

P. aeruginosa has evolved an antibacterial mechanism against the β-lactamase class and many different classes found in the bacterium. β-lactamases can inactivate β-lactam antibacterial antibiotics by breaking the bond of the β-lactam ring. This breaks the intermediate bond, and the resulting product lacks antimicrobial activity. The β-lactamase enzyme and the β-lactam ring characterize the hydrolysis of antibiotics by β-lactamase activity. An enzyme that leads to *P. aeruginosa* disrupts the β-lactamase ring in the periplasmic space and deactivates β-lactam by crossing the outer membrane before reaching the PBP. Some genes encoding β-lactamase enzymes are found in bacterial DNA's flexible regions, such as plasmids and type 1 integrin, while others are found in type 2 and 3 integrins.[2]

The main mechanisms for the development of β-lactam resistance by mutations are changes in PBP3 target proteins, decreased drug uptake, increased export, and drug molecule degradation. Furthermore, horizontal gene transfer could take over the β-lactamase enzyme from other bacteria. Metabolic changes and increased biofilm production may also influence resistance.[29]

β-lactam production has become a major mechanism of antimicrobial resistance following the advent of β-lactam therapies, including piperacillin, ceftazidime, cefepime, ceftolozane, and carbapenems. The major β-lactamases of *P. aeruginosa* are cephalosporins, *ampC* overexpression, broad-spectrum β-lactamases, and carbapenemases.[11] Effective antibiotics are the last resort for treatment for serious illness.[30]

Based on research by Sezahdeghani, et al., *P. aeruginosa* isolates were 40.3%, 43.1%, and 44.9% resistant to meropenem, ertapenem, and imipenem, respectively.[31] Carbapenemases are β- lactamases and include serine-β-lactamases (KPC, OXA, and GES genes) and metallo-β- lactamases (MBL). Approximately 15.5% of the isolates produced strong biofilms, 37.6% of the isolates produced moderate biofilms, and 10.2% of the isolates produced weak biofilms. We concluded that the films from the isolate that produced moderate and strong biofilms were resistant to all antibiotic groups tested. Based on the studies, a significant association was found between the formation of *P. aeruginosa* biofilms and the increased prevalence of antibiotic resistance. Within biofilms, bacteria are protected by exopolysaccharides, which prevent the entry of antimicrobial compounds either through the binding of compounds to the biofilm thickness or matrix. In addition, biofilm-protected bacteria with low metabolic activity slow down the uptake of antimicrobial compounds, rendering antibiotics ineffective. Colonies of organisms form biofilms and communicate with each other through quorum sensing. Most biofilm-producing *P. aeruginosa* isolates are classified as multidrug-resistant (MDR).[31]

CONCLUSION

Pseudomonas aeruginosa (*P. Aeruginosa*) is an opportunistic pathogen capable of producing biofilms. The P. aeruginosa biofilm structure surrounds the bacterium and consists of an exopolysaccharide matrix composed of *PsI*, *Pel*, alginate, and eDNA. Formation of biofilms by *Pseudomonas aeruginosa* bacteria generally proceeds through several stages: attachment of planktonic bacteria to suitable surfaces, irreversible solidification of adherent bacterial cells, a maturation process with the formation of microcolonies, and final stages. Biofilms are a reservoir of bacteria that can form new biofilm structures. *Pseudomonas aeruginosa* has mechanisms of antibiotic resistance: intrinsic resistance and adaptive resistance. Antimicrobial resistance involves reduced membrane permeability, synthesis of enzymes that can inactivate antibiotics, chromosomal mutations, and horizontal gene transfer from other bacteria. *Pseudomonasaeruginosa* is known to develop mechanisms of resistance to beta-lactam antibiotics. Furthermore, biofilm formation may also lead to phenotypic changes in the resistance traits of *Pseudomonas aeruginosa*.

REFERENCES

1. Sarkar S, Dutta S, Namhata A, Banerjee C, Sengupta M, Sengupta M. Beta-lactamase profile and biofilm production of Pseudomonas aeruginosa isolated from a tertiary care hospital in Kolkata, India. J Clin Diagn Res 2020;14:22-7.
2. Rocha AJ, Barsottini MRO, Rocha RR, Laurindo MV, Moraes FLL, Rocha SL. *Pseudomonas aeruginosa*: virulence factors and antibiotics resistance genes. Braz Arch Biol Technol. 2019; 62:e19180503.
3. Ahmed MAS, Khan FA, Sultan AA, Söderquist B, Ibrahim EB, Jass J, et al. β-lactamase-mediated resistance in MDR-*Pseudomonas aeruginosa* from Qatar. Antimicrob Resist Infect Control. 2020; 9:170.
4. Thi MTT, Wibowo D, Rehm BHA. *Pseudomonas aeruginosa* biofilms. Int J of Mol Sci. 2020; 21(22):8671.
5. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanism and alternative therapeutic strategies. Biotechnol Adv. 2019; 37(1):177-192.
6. Ciofu O, Tolker-Nielsen T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents – How *P. aeruginosa* can escape antibiotics. Front Microbiol 2019; 10:913.
7. Heydari S, Eftekhar F. Biofilm formation and β-lactamase production in burn isolates of *Pseudomonas aeruginosa*. Jundishapur J Microbiol. 2015; 8(3):e15514.
8. Almanama AA, Al-Sheboul S, Abu-Dan RI. Antimicrobial resistance and biofilm formation of *Pseudomonas aeruginosa*: A short review article. Int Arab J Antimicrob Agents. 2020; 10(2):1-11.
9. Sekhi RJ. *Pseudomonas aeruginosa*: A review article. Europian Scholar Journal. 2022; 3(3):78-84.
10. Behzadi P, Barath Z, Gajdacs M. It's not easy being green: A narrative review on the microbiology, virulence and therapeutics prospects of multidrug-resistant *Pseudomonas aeruginosa*. Antibiotics. 2021; 10(42):n1-29.
11. Tuon FF, Dantas LR, Suss PH, Tasca Ribeiro VS. Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. Pathogens. 2022; 11(300): 1-9. Tuon FF, Dantas LR, Suss PH, Tasca Ribeiro VS. Pathogenesis of the Pseudomonas aeruginosa biofilm: A review. Pathogens. 2022 Feb 27;11(3):300.
12. Iseppi R, Sabia C, Bondi M, Mariani M, Messi P. Virulence factors, drug resistance and biofilm formation in *Pseudomonas* species isolated from healthcare water systems. Curr Microbiol. 2020; 77(8):1737-1745.
13. Jurado-Martin I, Sainz-Mejias M, McClean S. *Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenal of virulence factors. Int J Mol Sci. 2021; 22(3128):1-35.

14. Liao C, Huang X, Wang Q, Yao D. Lu W. Virulence factors of *Pseudomonas aeruginosa* and antivirulence strategies to combat its drug resistance. Front Cell Infect Microbiol 2022; 12:926758.
15. Chen X, Thomsen TR, Winkler H, et al. Influence of biofilm growth age, media, antibiotic concentration and exposure time on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm removal in vitro. BMC Microbiol. 2020; 20:264.
16. Cho HH, Kwon KC, Kim S, Park Y, Koo SH. Association between biofilm formation and antimicrobial resistance in carbapenem-resistant *Pseudomonas aeruginosa*. Ann Clin Lab Sci. 2018; 48(3):363-368.
17. Vetrivel A, Ramasamy M, Vetrivel P, Natchimuthu S, Arunachalam S, Kim G-S, Murugesan R. *Pseudomonas aeruginosa* biofilm formation and its control. Biologics. 2021; 1(3):312-336.
18. Kamali E, Jamali A, Ardebili A, Ezadi F, Mohebbi A. Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of *Pseudomonas aeruginosa*. BMC Res Notes. 2020; 13:27.
19. Rasamiravaka T, Labtani Q, Duez P, El Jaziri M. The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. Biomed Res Int. 2015;2015:759348:1-17.
20. Das T, Manoharan A, Whiteley G, Glasbey T, Manos J. *Pseudomonas aeruginosa* biofilms and infections: roles of extracellular molecules. In: Yadav MK, Singh BP, editors. New and future developments in microbial biotechnology and bioengineering: microbial biofilms. Elsevier; 2020.p.29-46.
21. Olivares E, Badel-Berchoux S, Provot C, Prévost G, Bernardi T, Jehl F. Clinical impact of antibiotic for the treatment of *Pseudomonas aeruginosa* biofilm infections. Front Microbiol. 2020;10:2894.
22. Mahmoud SF, Fayez M, Swelum AA, Alswat AS, Alkafafy M, Alzahrani OM, Alsunaini SJ, Almuslem A, Al Amer AS, Yusuf S. Genetic diversity, biofilm formation, and antibiotic resistance of *Pseudomonas aeruginosa* isolated from cow, camel, and mare with clinical endometritis. Vet Sci. 2022 May 16;9(5):239.
23. Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, Liang H, Song X, Wu M. Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther. 2022 Jun 25;7(1):199.
24. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol. 2002;292: 107-113.
25. Yan J, Bassier BL. Surviving as a community: Antibiotic tolerance and persistence in bacterial biofilms. Cell Host Microbe. 2019;26(1):15-21.
26. Ratajczak M, Kamińska D, Nowak-Malczewska DM, Schneider A, Dlugaszewska J. Relationship between antibiotic resistance, biofilm formation, genes coding virulence factors and source of origin of Pseudomonas aeruginosa clinical strains. Ann Agric Environ Med. 2021 Jun 14;28(2):306-313.
27. Alfei S, Schito AM. β-lactam antibiotics and β-lactamase enzymes inhibitors, Part 2: Our limited resources. Pharmaceuticals (Basel). 2022 Apr 13;15(4):476.
28. Lin H, Feng C, Zhu T, Li A, Liu S, Zhang L, Li Q, Zhang X, Lin L, Lu J, Lin X, Li K, Zhang H, Xu T, Li C, Bao Q. Molecular mechanism of the β-lactamase mediated β-lactam antibiotic resistance of Pseudomonas aeruginosa isolated from a Chinese teaching hospital. Front Microbiol. 2022 Apr 28;13:855961.
29. Glen KA, Lamont IL. β-lactam resistance in *Pseudomonas aeruginosa*: Current status, future prospects. Pathogens. 2021;10:1638.
30. Mousa RS, El-Kady M, Hussein A, Ahmed A, El-Gendy A. Occurrence of carbapenem multidrug-resistant Pseudomonas aeruginosa carrying bla_{VIM} metallo-β-lactamases and their biofilm phenotypes in Al-Azhar University Hospital. J Appl Pharm Sci, 2022; 12(03):073–081.
31. Sezadehghani A, Dehbashi S, Tahmasebi H, Arabestani MR. Detection of blaOXA-145, blaOXA-224, blaOXA-539, and blaOXA-675 genes and carbapenem-hydrolyzing class D β-lactamases (CHDLs) in clinical isolates of Pseudomonas aeruginosa collected from west of Iran, Hamadan. Int J Microbiol. 2022 Aug 5;2022:3841161.