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Pseudomonas aeruginosa: Burn infection, treatment, and antibacterial resistance

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Abstract
Pseudomonas aeruginosa is a leading cause of burn wound infections, posing significant challenges in clinical management due to its virulence factors and increasing antibiotic resistance. This abstract provides a concise overview of *P. aeruginosa* burn infections, focusing on epidemiology, treatment strategies, and antibacterial resistance. *Pseudomonas aeruginosa* burn infections represent a formidable clinical challenge, requiring comprehensive management strategies to mitigate their impact on patient outcomes. Future research efforts should focus on understanding the molecular mechanisms of antibacterial resistance and developing novel therapeutic interventions to address this urgent public health threat. Additionally, infection prevention measures and surveillance of antibiotic resistance patterns are essential for controlling the spread of multidrug-resistant *P. aeruginosa* strains in burn care settings.

Keywords: Virulence, burn, infection, *Pseudomonas aeruginosa*

Introduction
Germs of the gram-negative kind known as *Pseudomonas aeruginosa* are rod-shaped. The dimensions are 1.5 to 3.0 µm in width and 0.5 to 0.8 µm in length. A single polar flagellum is responsible for motility in the majority of strains. This bacterium is common in both water and soil, and it does not cause parasitism. Belonging to the Pseudomonadaceae family, this rod-shaped bacterium is gram-negative, aerobic, and a member of the gamma proteobacteria class. Organisms from the genus Pseudomonas are the only ones in this family. They are classified into eight groups according on shared macromolecules, such 16S ribosomal RNA. *Pseudomonas aeruginosa* is a member of its taxonomic group and is accompanied by 12 other species. Most clinical cases involving *P. aeruginosa* infection are linked to the impairment of the host's defensive mechanisms, particularly in burn victims. Neutropenia from chemotherapy makes patients more vulnerable to several bacterial and fungal infections, however general immunosuppression, such in AIDS patients, may cause many instances of *P. aeruginosa* infection. Research on these individuals does not, however, provide light on the mechanisms by which *P. aeruginosa* causes disease. Here, three illnesses in animals that have been linked to *P. aeruginosa* are: The third risk is acute ulcerative keratitis in those who use long-wearing soft contact lenses; the fourth is bacteremia in those who suffer from severe burns; and the fifth is recurrent lung infection in those who have cystic fibrosis. The many virulence factors of bacteria have been studied and evaluated experimentally provided significant insights into the mechanisms by which *P. aeruginosa* can induce illness in diverse organs, resulting from the disruption of normal physiological processes. These findings provide a comprehensive understand how the body's molecules and cells work that contribute to the significance of *P. aeruginosa* as a pathogen in Animal infections.

Bacteremia caused by *Pseudomonas aeruginosa* in patients with severe burns
The occurrence of Bacterial infection after severe thermal injury could be associated with the substantial breaches in the skin's protective barrier. Because *Pseudomonas aeruginosa* is so common in nature, it is quite probable that a person with severe burns may encounter this bacterium before their burns can fully heal. Hospitals specializing in burn care often include multidrug-resistant *P. aeruginosa* that might potentially harbour infections. Various surfaces

in hospitals, including flooring, bed rails, and sinks, have been shown to harbour *Pseudomonas aeruginosa*. Also, nurses' hands have been shown to have this bacterium. In addition to transmission via inanimate objects and carriers, the patient themselves may introduce bacterial flora into a hospital setting, which can then lead to infection in the same person after an accident. In relation to multidrug resistance, during a multi-year period, Hsueh *et al*. found *Pseudomonas aeruginosa*, a single strain was resistant to several different treatments. After extensive antibiotic therapy for both Pseudomonas and non-Pseudomonas infections, the researchers found that some individuals harboured this strain asymptomatically. The transmission of *P. aeruginosa* between patients might exacerbate this situation, since this strain has the ability to survive in patients after many rounds of antibiotic therapy. Research has shown that a small set of prevalent strains are responsible for cross-contaminating burn victims after their admission to burn centers, particularly when their wounds are cleaned in the restroom.

P. aeruginosa Virulence Factors in Burn Infection

Burn wound infection is caused by *Pseudomonas aeruginosa* and its many virulence factors. Burn wound infections in rats may be caused by aspects contributing to *Pseudomonas aeruginosa*'s pathogenicity, as reported by Rahme *et al*. *P. aeruginosa* pili and flagella have also been shown to play a substantial function. Bacteria defective in either the pilus or the flagellum have reduced virulence, according to comparative studies on Infection resulting from burns caused by *P. aeruginosa* strains. This is obvious in their diminished power to stay at the location of the lesion and their reduced capacity for organism-wide dissemination. The production of proteases by *P. aeruginosa* is an additional factor in the pathogen's ability to propagate inside hosts. Research has shown that elastase may degrade host collagen and noncollagenous proteins while simultaneously weakening the basement membrane's structural integrity. A number of the host's immunological systems, both innate and acquired, may be compromised by proteases. As an example, elastase blocks monocytes' ability to migrate towards a chemical signal, which might have an adverse effect on the host immune system's ability to quickly remove *P. aeruginosa* bacteria from wound sites by engulfing them. The quorum sensing response, which initiates the synthesis of virulence components and the growth of biofilms, is triggered by a critical protein produced by the lasR gene. This evidence suggests that other variables controlled by lasR burn wound infections induced by *Pseudomonas aeruginosa* include these factors. Additional virulence factors of *Pseudomonas aeruginosa* that may lead to burn wound infections include phospholipase C, exoproducts produced by the type III secretion apparatus, lipopolysaccharide (LPS), and ferripyochelin-binding protein.

Despite the fact that a lack of skin barrier function is a major contributor to burn wound infection, it does not fully account for the limited variety of bacterial pathogens that are usually seen in infected burn wounds. Consequently, it is probable that severe burns result in a greater weakening of some host defensive systems that are targeted towards certain infections. There have been reports of a decrease in infection after applying polyclonal Animal antibody directly to burn sites. This suggests that in untreated burn wounds, there is a deficiency of immunoglobulin at levels that are not sufficient for protection. Another research that supports the idea of an antibody-mediated local immunodeficiency in burn wounds found that polymorphonuclear leukocytes' (PMNs') expression of Fc receptors decrease by day five after burn damage. Burn wounds have been shown to exhibit complement depletion, which may be attributed to the localised usage of complement components. One possible explanation for the reported problems with PMN chemotaxis and random migration at burn wound sites is that there are localised anomalies in the complement proteins and Fc receptors of protective antibodies. Taken together, these findings suggest that many hosts immune pathways must malfunction simultaneously for burn wound colonization to occur. In these infections, one key player is *Pseudomonas aeruginosa*, which may exploit the by releasing an overwhelming number of pathogenic elements into the host's already damaged immune system.

Exoenzyme S and exotoxin A are two protein toxins secreted by *Pseudomonas aeruginosa* outside of the cell. Subunit structure of exoenzyme S is similar to that of bacterial toxin A, and it possesses ADPribosylating capabilities. The bacteria that develop in burnt tissue create an enzyme called Exoenzyme S, even in the absence of actual germs, they may be detected in the blood. Exoenzyme S may impede phagocytic cell activity in the bloodstream and internal organs, which might pave the path for *P. aeruginosa* invasion, according to one theory. Eukaryotic elongation factor 2 is ADP ribosylated by exotoxin A, which is a mechanism similar to that of diphtheria toxin. The result is a decrease in protein synthesis inside the specific cell. Its antigenic characteristics are distinct from diphtheria toxin, despite their shared resemblance. Unlike diphtheria toxin, it uses a different receptor on host cells to enter cells, but otherwise it follows the same enzymatic pathway. Iron from outside sources regulates exotoxin A production, although the exact processes used by *P. aeruginosa* and C. diphtheriae are quite different. Pathogenic mechanisms of *Pseudomonas aeruginosa*, both locally and systemically are both attributed to exotoxin A. It is thought to help in bacterial colonisation since it shows necrotizing effect when bacteria congregate.

High-Risk Individuals and Potential Vaccines

While the treatment of infectious diseases has been substantially improved by antibiotic therapy illnesses overall, some *P. aeruginosa* infections remain unresolved or persistent despite the use of anti-pseudomonal medications, resulting in the development of persistent infections. A good example is the colonisation of burn patients who manage to survive the initial burn injury by hospital-acquired, antibiotic-resistant *P. aeruginosa* strains. It is possible for bacteria with cystic fibrosis to become resistant to antibiotics if these patients use them too often. This, in turn, causes a faster deterioration in lung function compared to people infected with strains that are susceptible to antibiotics. Various antigens of *P. aeruginosa*, such as lipopolysaccharide, alginate polysaccharides, extracellular proteins, exotoxin A, and dead entire cell, are used in the production of vaccines. However, at this time, high-risk populations, like firefighters, immunocompromised patients, and people with cystic fibrosis, do not have access to any of these choices. Currently, many potential vaccines are undergoing phases one to three of exploratory clinical trials.

Addressing Infections

The use of antimicrobial topical creams

Scientific evidence has shown that the use of a potent topical antimicrobial medication significantly decreases the number of microorganisms present on the surface of an open burn site, hence lowering might potentially infect. Considering how often nosocomial infections are in burn units and the microorganisms detected in cultures taken from burn wound monitoring patients, the decision of topical antibiotic therapy should be made. Topical medications are often prescribed based on their pharmacokinetic characteristics and the particular formulation, such as ointment, cream, solution, or dressing. To lessen the likelihood of the development of antibiotic resistance, burn units may regularly switch up the topical antibacterial therapies they employ. It is recommended that the patient's bandages be treated with topical antibiotics before the burn site is treated. This helps to avoid the contamination of the container holding the agent by the bacteria present in the burn wound. Silver inhibits bacterial respiration by interacting strongly with thiol groups on respiratory enzymes inside the cell. Research has shown that silver may hinder DNA replication by interacting with structural proteins and specifically attaching to nucleic acid bases. A value of 48.46 is given. Silver is especially harmful to keratinocytes and fibroblasts, according to recent data. If silver is administered without discrimination to healing tissue sections that have been debrided, it may cause a delay in burn wound healing.

An effective antibacterial agent in cases of partial-thickness damage is wet exposure treatment, which uses a moisture-retentive substance, promote optimal moist wound healing, and enable fast autolysis debridement. The use of a moisture-retentive ointment led to faster healing of keratinocytes, better faster wound healing and less scarring. The efficacy of silver nitrate is highest prior to colonization of the burn site. After the burn site has been saturated with silver nitrate solution and a multi-layered dressing has been applied, it is necessary to wash the wound of emollients and other debris. To use this preparation effectively, it is necessary to consistently apply it along with other occlusive dressings, which might hinder the evaluation of the cut. The AgNO3 silver ion readily forms complexes with elemental chlorine ions, which may result in electrolyte imbalance such as hyponatremia and hypochloremia when this solution is applied repeatedly or over a broad surface area. The antibacterial action of silver nitrate is restricted to the surface of the burn site. Other genera, including as Klebsiella, providencia, and Enterobacter, are not inhibited by it. Due to its poor antifungal properties, silver nitrate should be used in conjunction with nystatin.

Silver Sulfadiazine

The chemical formula for this substance is sodium sulfadiazine + silver nitrate. The silver ion forms a chemical bond with the nucleic acid of the bacteria, causing the release of sulfadiazine. This subsequently disrupts the metabolism of the microbe. The use of this product is effortless and devoid of discomfort, and it may be used with or without a dressing. Repeated daily or twice-day use of the product has resulted in little systemic toxicity, save for a decrease in white blood cell count. Germs that do not have a gramme negative, such as *Pseudomonas aeruginosa* are among the many microorganisms that silver sulfadiazine effectively kills. The development of resistance to this chemical in some strains has, however, been reported recently. A combination of nystatin and silver sulfadiazine may greatly enhance the antifungal action, yet this medicine only partially works against Candida albicans. Despite the slower dissociation rate of silver sulfadiazine compared to silver nitrate, it still has limited ability to penetrate the wound. Some people with serious injuries may not be able to benefit from silver sulfadiazine since it can only be absorbed via the surface epidermal layer. European nations have utilized a mixture and silver sulfadiazine to combat this. As far as we can tell from the research, it may help with burn injury inflammation, bacterial colonization, and wound care by creating a solid eschar.

Mafenide Acetate

Mafenide acetate cream, when applied topically, permits the management of open burn wounds and makes it easier to monitor the burn wound surface on a regular basis, since it does not need the use of bandages. It has been shown that mafenide acetate may penetrate the burn eschar when applied topically at least twice a day. To achieve maximum effectiveness, please ensure that the gauze dressings are well saturated with the 5% solution. It is recommended to change these dressings every 8 hours. When used in this manner, it seems that the efficacy of mafenide acetate solution is comparable to that of the cream formulation. While mafenide acetate (Sulfamylon) creams work well against gram-negative bacteria, such as *Pseudomonas aeruginosa*, and gram-positive aerobic pathogens, such as Staphylococcus aureus, are difficult for them to eradicate. This medication also hinders the growth of anaerobic microorganisms like Clostridium spp. For the purpose of limiting the spread of fungi like Candida albicans, it is recommended to use mafenide acetate in conjunction with nystatin owing to its poor antifungal efficacy. Monoamide oxidase, a carbonic anhydrase inhibitor, converts this molecule into psulfamylvanzoic acid, leading to metabolic acidosis in burn patients. Repeated or extensive administration of mafenide acetate to a burn patient with inhalation damage and respiratory acidosis may be lethal. Additionally, mafenide acetate slows the healing process and decreases the tensile strength of wounds that have already healed.

Acticoat AB Dressing

The product is a specific kind of dressing that is made up consisting of two layers of HDPE mesh. These layers are covered with nanocrystalline silver, It has a rayon-polyester core and is an ionic silver form. By applying nanocrystalline silver to the area of a burn in a controlled and gradual manner, the need for frequent dressing changes is minimized. This in turn reduces the chances of tissue damage, hospital-acquired infections, patient pain, and the total expenses associated with topical treatment. Acticoat AB offers the most extensive and effective defense against several bacterial strains that often infect burn wounds, compared to other antimicrobial products available in the market. The dressings have possessed antibacterial characteristics that effectively inhibit the proliferation of aerobic gram-positive and gram-negative bacteria, including methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci, *Pseudomonas aeruginosa*, and antibiotic-resistant Escherichia coli. These

specialized dressings may keep killing germs for a long time if the burn wound surface doesn't leak much.

Antimicrobial Resistance

Resistance to antimicrobial agents that are applied topically

While Resistance to silver sulfadiazine has been reported. In *P. aeruginosa*, the specific mechanism behind this resistance remains unknown. Mutations in the outer membrane proteins that are responsible for transporting ions, including silver, across the bacterial membrane are thought to contribute to Pseudomonas' resistance to topical antimicrobials based on silver. Some studies have shown gentamicin-resistant *P. aeruginosa* strains in patients who have had burns. A tragic thirteen people lost their lives as a result of an epidemic of Enterobacter cloacae sepsis, according to research conducted in the United States. The germs tested showed susceptibility to silver sulfadiazine, whereas the strains taken from those who weren't burnt had three thousand micrograms per millilitre (MIC) values. Rosenkranz *et al*. identified and separated two strains of Entrobacter cloace that were resistant to silver sulfadiazine in a burn unit where silver sulfadiazine was being used. Results showed that the MIC was 400 µg/ml, suggesting that the bacteria were highly resistant to silver sulfadiazine. Not only that, but they showed resistance to both silver nitrate and silver benzoate. Recent research has shown that burn patients' *Pseudomonas aeruginosa* strains are resistant to silver sulfadiazine, even though the majority of these organisms were susceptible the solution of silver nitrate.

An Antibiotic Resistance Epidemic

Mutation-induced resistance

A variety of medications have been antibiotics that are used to treat infections caused by *Pseudomonas aeruginosa*, such as penicillins, polymyxins, cephalosporins, and fluoroquinolones. Most strains of *Pseudomonas aeruginosa* have been successfully treated with these medicines. However, all of them are susceptible to being weakened by mutational resistance. *Pseudomonas aeruginosa* is more prone to developing fluoroquinolone resistance via mutations in topoisomerases II and IV compared to Enterobacteriaceae. This is due to *P. aeruginosa* having a lower intrinsic susceptibility. The release of the AmpC β-lactamase gene from the chromosomes causes a decrease in susceptibility to penicillins and cephalosporins. In contrast to Enterobacter mutants, Resistance levels are affected by derepression. To a greater or lesser degree. MexAB-OprM overexpression impairs the effectiveness of fluoroquinolones, penicillins, cephalosporins, and partially meropenem (except imipenem). Additionally, it increases resistance to several other medicines that do not possess significant antipseudomonal action. A resistance to fluoroquinolones and some b-lactams develops when additional efflux systems, including MexCD-OprJ and MexEF-OprN, are activated. Additionally, aminoglycosides are affected by the overexpression of MexXY-OprM. There is more evidence indicating that enhanced impermeability serves as a mechanism for aminoglycoside resistance. This is evident in the occurrence of "small-colony variants" that are sometimes chosen during gentamycin treatment, as well as in isolates that show lower sensitivity to any and all carbapenems, fluoroquinolones, and aminoglycosides.

Mutations leading to multidrug resistance

There is there isn't a single mutation that can make all antipseudomonal medications useless. But because most b-lactams and fluoroquinolones have their efficacy reduced due to enhanced efflux, the only antibiotics left are aminoglycosides, which are not reliably effective as a single treatment against pseudomonas) and imipenem (which often develops resistance via mutations). The simultaneous increase in efflux, absence of OprD, and resistance to aminoglycosides undermines the effectiveness of all pharmacological categories, except for the polymyxins. The occurrence of each required mutation happens in approximately 1 cell out of every 107 to 109 cells. While it is highly unlikely for all mutations to arise simultaneously, it is unfortunately quite probable for them to occur sequentially. This is because when an infection becomes resistant to the first antibiotic used, it is often given a different antibiotic, and the cycle continues thereafter. Mutations that increase the activity of efflux mechanisms may have an additive impact with mutations affecting permeability, production of b-lactamase, or susceptibility of topoisomerases, leading to a worsening of resistance. The accumulation of repetitive mutations is accelerated in hypermutators because they either employ DNA polymerases with poor copying accuracy or are unable to perform DNA proofreading or mismatch repair. Because hypermutators are more prone to developing resistance, antibiotics may promote their selection, thereby increasing the likelihood of more resistance emerging.

Acquisition of Genes and Multidrug Resistance

Evidence suggests that *Pseudomonas aeruginosa* possesses an abundance of aminoglycoside-modifying enzymes and acquired beta-lactamases. It has been shown that resistance to oxyimino-aminothiazolyl cephalosporins, monobactams, penicillins, and potent aminoglycoside-modifying enzymes may develop, although resistance to carbapenems remains unaffected. The Metallo-β-lactamases enzyme exhibits fast hydrolysis of penicillins, cephalosporins, and carbapenems, but aztreonam is not affected. The presence of resistance is often associated with the manufacture of penicillins and cephalosporins. Canadian, French, Greek, South Korean, Italian, and Japanese enzymes have been found. In integrons, a natural recombination mechanism that organises a group of acquired genes under a single promoter, cassettes containing resistance genes are often carried. The ability to recombine genes is made possible by this design. An very important location for b-lactamase genes is close to the aac (6)-1b, an aminoglycoside 6-N acetyltransferase, factors.

How common is multidrug resistance?

The burn center's *Pseudomonas aeruginosa* strains are resistant to the majority of the drugs tested. Research conducted at Iran's Ghotbeddin Burn Hospital found that, with the exception of carbapenems, almost all *Pseudomonas aeruginosa* strains discovered in burn patients were resistant to the anti-Pseudomonal medications that were evaluated. In addition, additional Iranian articles have shown that burn patient isolates do, in fact, exhibit multidrug resistance. According to popular belief, the accumulation of mutations is the main culprit for multidrug resistance. Molecular investigations are still need to confirm this idea, however. Furthermore, additional places have reported significant rates of multidrug resistance connected with acquired

resistance genes. A 3 yearlong outbreak of a bacterium resistant to aztreonam, aminoglycosides, and ciprofloxacin was detected in a Greek hospital. A grand number of 1,211 samples of this strain were detected and collected over this period. To determine how often resistant P. aeurginosa strains are, researchers in South Korea set out to answer that question. In 9 out of 29 hospitals, the hydrolyzing enzyme was found in organisms. In addition, a comprehensive investigation at a single Korean hospital found that many Ph. aeruginosa family trees were disseminated.

Multi-Drug Resistance: How to Prevent and Manage It

The presence of mutants that are resistant, which is a potential danger in any treatment against Pseudomonas, depends on the specific antibiotic and its dose, as well as the location of the infection. The use of imipenem resulted in a risk of resistance selection that was twice as high compared to the use of ciprofloxacin, ceftazidime, or piperacillin. The prevailing belief is that combination treatment effectively inhibits the emergence of mutational resistance. However, there is little empirical support for this claim. Moreover, the presence of individual efflux mutations might have a detrimental impact with fluoroquinolone and b-lactam efficacy, therefore compromising the efficacy of therapeutic combos including both medications. Mutational resistance is more predictable, but multi-drug resistance, which is associated with plasmids and integrons, is less so. This is due to the fact that it is dependent on the random transfer of genes to DNA that may move. Nonetheless, resistance may arise from either the host strain or from other strains, and it can spread to additional patients. When treating severe Pseudomonal infections, most doctors prefer to utilise a synergistic combination. However, this may be problematic when various strains of the bacteria have distinct mutational or acquired resistances, which can severely restrict treatment choices. Ciprofloxacin remains the most effective fluoroquinolone against Pseudomonas bacteria, and no other fluoroquinolone can maintain its effectiveness against strains that are resistant to ciprofloxacin.

When fighting mutational resistance in *P. aeruginosa*, tobramycin and meropenem are the most effective treatments. This is due to the fact that the greatest intrinsic action against this bacterium is shown by aminoglycoside and beta-lactam antibiotics. Even though certain bacteria have become resistant to meropenem, penicillin's, and cephalosporins via efflux mechanisms, imipenem may be able to treat them. While meropenem often exhibits more activity as a carbapenem, it is important to constantly take this potential into account. The usage of polymyxins becomes necessary when other antibiotic choices, such as b-lactams, aminoglycosides, and quinolones, are exhausted. They have been utilized effectively despite their high toxicity. For infections caused by multidrug-resistant Pseudomonas and Acinetobacter, 35 out of 60 patients (58%) were effectively treated with intravenous polymyxin E (colistin) according to the research by Levin *et al*. However, when used to treat pneumonias, it had a failure rate of 75%. Especially concerning is the lack of novel pharmaceutical alternatives. Clinafloxacin had a somewhat higher level of activity compared to ciprofloxacin. However, its progress has been halted, and there are no other advanced-stage antiPseudomonal antibiotics now under research. Long-term usage of multi-drug efflux inhibitors shows promise in combination with fluoroquinolones or b-lactams, whereas laboratory work is now focused on metallo-b-lactamase inhibitors 105. Without the development of new medications, one cannot help but predict that the number of Pseudomonas strains resistant to several drugs will keep climbing, necessitating a growth in the usage of polymyxins despite their harmful effects.

Antibiotic treatment of biofilm infections

Bacterial biofilms are linked to many illnesses, infections may range from those connected to outside equipment, such catheters or artificial joints, to those that stay in the body, such as the CF lung infections. For the eradication of biofilms connected with devices, the most effective therapeutic option, if feasible, is the removal of the implant. However, when it comes to biofilms related with tissues or sputum, the only currently accessible treatment is antibiotic therapy. Nevertheless, even with prolonged antibiotic treatment targeting bacteria that demonstrate susceptibility in laboratory tests, biofilm-forming bacteria remain and cause destruction of the infected tissue because of a persistent inflammatory response, causing a persistent infection to develop. In this piece, we'll look at the mechanisms that make biofilms resistant to antibiotics and provide some solutions to make antibiotic treatment for biofilm infections work better.

Biofilm Infections: The Obstacles Facing Antimicrobial Treatment

***In vitro* and *in vivo* antimicrobial tolerance of biofilms**

Tolerance *in vitro*

As biofilms develop into adulthood, their intrinsic resistance to antimicrobials becomes increasingly noticeable. The growth pattern of biofilms is associated with their resistance to antimicrobials. Bacteria isolated from biofilms grown in a planktonic environment will be susceptible to these effects. Conversely, antibiotic-resistant microbes are not tied to the biofilm growth mode and is often seen in bacteria cultured in planktonic media. Antimicrobial tolerance, on the other hand, is associated with biofilms. The tolerance seen is influenced by several factors, including limited antibiotic penetration, restricted growth under low oxygen conditions, activation of genes relevant to biofilm development, along with the existence of per sisters.

Limited access. In some cases, biofilms may become more resistant to antimicrobials due to the low levels of these chemicals that penetrate the biofilm matrix. While biofilm matrices typically do not impede the spread of antibiotics, there may be instances where the antibiotics are hindered from penetrating through biofilms. Possible causes include antibiotic binding to biofilm matrix or bacterial membrane components or antibiotic ineffectiveness due to matrix enzymes.

Different physiological processes. The fact that bacteria inside biofilms exhibit a wide range of physiological activities is another factor that helps bring about antibiotic resistance. Research on *Pseudomonas aeruginosa* biofilms has shown that the bacteria exhibit elevated metabolic activity in the outer region of the biofilm, whereas metabolic activity is reduced in the interior region. The current body of knowledge suggests that the unique physiological responses seen in biofilms are due to the fact that bacterial consumption mediates the restriction of oxygen and nutrient transport inside the biofilm. Since several antibiotics specifically affect the activities that take place in actively

developing bacteria, such as replication and cell wall construction, biofilm bacteria with reduced metabolic activity exhibit heightened resistance to these types of antibiotics.

Unique patterns of gene expression in biofilms. Antimicrobial resistance mechanisms in biofilms could be linked to the bacterial production of a small set of genes. *Pseudomonas aeruginosa* PA14, which has the ndvB gene, is an example of this behaviour. The production of periplasmic glucans is dependent on enzymes encoded by this gene. By binding to the antibiotic tobramycin, these glucans prevent the antibiotic from killing the bacterial cells.

Cells that persist or remain in a certain state. Furthermore, the aforementioned method is also applicable to bacteria with slow cell division rates. Do not divide at all might impact biofilms' antimicrobial tolerance. The proportion of these persisted cells, often referred to as such, is typically minimal (<0.1%). It is important to differentiate them from the larger subset of bacteria in biofilms that are metabolically inactive. Persisted cells are postulated to arise from bacterial differentiation into a quiescent condition. Persister cells possess a reduced metabolic rate, which allows them to evade the effects of antibiotics that specifically attack essential cellular functions. Additionally, persister cells, in some instances, demonstrate resistance to antibiotics that eliminate non-dividing cells.

Tolerance *in vivo*

The unique complexity of *in vivo* circumstances gives patients an advantage over in models of biofilms *in vitro* with regard to the processes discovered. To reach the bacteria inside a biofilm, antibiotics must first overcome many obstacles, such as the immune system's presence, the biofilm's placement in an oxygen-poor environment, and the biofilm's several compartments. The study found that polymorphonuclear leukocytes (PMN) are detectable in CF sputum samples from individuals who have a recurrent *Pseudomonas aeruginosa* infection. Because these PMN cells use oxygen, the location of the biofilm infection becomes anaerobic, which inhibits bacterial growth. Biofilms may develop in non-oxygenated parts of an organism as a result of PMN inflammation or physiological variables, in addition to the limited development within the biofilm's innermost layer, as shown in laboratory models. Pneumonia, sinus secretions, wound tissue, bone, and cystic fibrosis (CF) sputum are among the biofilm infection sites where an oxygen deficiency has been identified. Bactericidal antibiotics are unable to kill bacteria when oxygen levels are low because reactive oxygen species (ROS) are unable to do their job. Additionally, oxygen is required for the translocation of many antibiotics across cell membranes, especially aminoglycosides. Also, it's not possible to measure how much antibiotic is really present in an *in vivo* biofilm. A new research, Cao *et al*. proposed the inclusion of biofilm as an additional compartment, alongside tissue and blood, that antibiotics need to penetrate to reach their target microorganisms.

An individual's medication metabolism, the biofilm's size and location, and other variables all play a role in determining the local biofilm antibiotic concentration. Dalbøge *et al*. found that people with cystic fibrosis (CF) had significantly different clarithromycin pharmacokinetics, which might potentially lead to therapeutic ineffectiveness in CF patients. Furthermore, the presence of antibiotics at lower concentrations antibiotic resistance might be more likely to develop at the location of biofilm infection. emergence because of the process of selection and heightened mutagenesis. Due to the multitude of variables influencing the local antibiotic concentration in biofilms, it is advisable to provide large doses of antibiotic combinations for extended durations during therapy.

Adaptive tolerance, which refers to the increase in resistance mechanisms without any genetic changes, occurs in biofilms when they are exposed to antibiotics. This tolerance is reduced when the antibiotic molecules are metabolized and eliminated from the infection site, contributing to the difficulty of treating biofilms with antibiotics. Biofilms persist due to the aforementioned tolerance mechanisms, which in turn foster an environment favourable to the development and natural selection of mutants resistant to antibiotics. This happens in the normal microbial population as well as at the location of the biofilm infection. According to Høiby *et al*., ciprofloxacin is quickly broken down by absorbs via perspiration and remains there for extended periods of time at low levels. which causes skin germs to quickly develop resistance. Thus, biofilms might be seen as a place where antibiotic-resistant bacteria can grow and spread, endangering both patients and the healthcare system as a whole.

Antimicrobial resistance development in biofilms

Bacterial biofilms may experience a considerable reduction in their ability to be affected by antimicrobial substances due to the acquisition of certain chromosomal mutations, in addition to their inherent resistance to antimicrobials. Resistance is acquired by *Pseudomonas aeruginosa* via chromosomal gene selection. Changing antibiotic targets, altering permeability, overexpressing efflux pumps, or derepressing chromosomal AmpC β-lactamase are all possible outcomes of mutations that may affect any drug.

The presence of hypermutator (or mutator) bacteria may promote the accumulation of successive mutations. Hypermutators have a significant increase in their spontaneous mutation rate, ranging from 100 to 1000 times higher than normal. This is caused by deficiencies in their DNA repair or error avoidance mechanisms. Deficits in these areas often affect mutU, mutS, and mutL, three crucial genes in the methyldirected mismatch repair system (MMRS). Mutator phenotypes may also result from mutations in genes that shield cells from the oxidative stress that reactive oxygen species (ROS) may induce, including as pfpI, oxyR, and mutY and mutT, respectively. Mutators in bacterial populations are considered advantageous for evolutionary purposes as they aid in adapting to challenging circumstances. *Pseudomonas aeruginosa*-induced chronic respiratory infections are characterised by the very high incidence of these mutator strains, in contrast to acute infections. This genetic variant is seen in 30-60% of cystic fibrosis (CF) patients. A 25-year research showed that the percentage of isolates with high mutation rates might go from 0% at the beginning to 65% at the chronic stages of the disease.

Research in both laboratory and living organisms has shown a clear link between the hypermutator phenotype and the processes of adaptation and antibiotic resistance. Many research recently have focused on hypermutation and the function it plays in biofilm development, as well as the link

between biofilms' elevated mutagenesis and diversity and adaptive mechanisms. The presence of limited oxygen and nutrients creates diverse microenvironments inside mature biofilms, It eventually results in the creation of specialized traits due to genetic variation. MMRS-deficient mutants are linked to the promotion of micro-colony growth and higher rates of phenotypic variation. Moreover, several studies have provided insight into the function of mutators in biofilms when subjected to antibiotic therapies. According to the research of Macia *et al*., mutational pathways are critical for biofilm resistance to evolve. Regarding biofilm, ciprofloxacin's bactericidal effect was significantly diminished because of the rapid and extensive proliferation of resistance mutants, despite sufficient drug exposure. When subjected to antibiotics, Mandsberg *et al*. found that GO system mutants showed higher antibiotic resistance than the wild type. Furthermore, the development of resistance in these mutants happened by pathways that were comparable to those seen in MMR mutants. Hence, the physiological and structural characteristics of biofilms seem to promote the slow emergence of resistance, particularly in mutator strains, hence providing a distinct advantage in terms of survival when subjected to selection pressure.

Conclusion

Pseudomonas aeruginosa burn infections remain a formidable clinical challenge, requiring comprehensive management strategies to mitigate its impact on patient morbidity and mortality. Continued research and innovation are essential to address the evolving threat of antibacterial resistance and improve the outcomes of patients affected by *P. aeruginosa* burn infections.

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