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Pathogenesis, Laboratory Diagnosis, Drug Resistance and Treatment of Pseudomonas Aeruginosa Infections: Review Article

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ABSTRACT

Background: An opportunistic pathogen, *Pseudomonas aeruginosa* is an aerobic gram-negative bacillus. It is a very adaptable microbe that can endure low oxygen levels. *P aeruginosa* can cause bacteremia, pneumonia, and urinary tract infections. It can also cause significant morbidity and death in cystic fibrosis patients because of persistent infections that lead to respiratory insufficiency and lung damage over time. *P aeruginosa* infections still provide a substantial problem for treatment.

Objective: This article aimed to review the pathogenesis, laboratory diagnosis, drug resistance and treatment of *P aeruginosa* infections.

Methods: We searched PubMed and Google, for *P aeruginosa*, diagnostic information, resistance and therapy drugs. The writers also assessed references from pertinent literature, although they only included the most recent or comprehensive study from April 2007 to April 2023. Documents in languages other than English have been disqualified due to lack of translation-related sources. Dissertations, oral presentations, unpublished manuscripts, conference abstracts, and other papers that did not pertain to significant scientific research were excluded.

Conclusion: One of the most frequent pathogens responsible for nosocomial infections is P aeruginosa. Since this organism is able to acquire antibiotic resistance, treating these infections is challenging and they have significant death and morbidity rates.

Keywords: *P Aeruginosa*, Pathogenesis, Diagnosis, Drug resistance, Treatment.

INTRODUCTION

The complex and diversified bacterial genus Pseudomonas inhabits a wide range of environmental settings. The most recognised species of gram-negative bacteria belong to this genus. With 18 subspecies identified, there are now more than 220 species, and the number of species is continually rising [(1)].

Pseudomonas is a genus of aerobic, non-spore-forming, straight or slightly curved, gram-negative rods that use one or more polar flagellae to move about and measure between 1.5 and 5 meters long and 0.5 to 1 meters broad. With an ideal development temperature of between 30 and 37 °C, the majority are mesophilic. Based on the 16S rRNA gene sequences, the genus has three major lineages, which are represented by the species *P aeruginosa*, *P fluorescens and P pertucinogena*. Despite the fact that the 16S rRNA gene serves as the fundamental component of the existing method for classifying bacteria, it is well known that closely related bacterial species cannot be distinguished on the basis of this gene [(2)]. The entire genome sequences of the species type strains are required since they serve as the species representatives when doing a phylogenomic study in bacterial taxonomy. For the genus Pseudomonas, this objective has been nearly entirely met in a number of papers [(3)].

Due to a single polar flagellum that is also glycosylated, *P aeruginosa* is able to move. Flagellin, which has two serotypes (type a and type b) is the main protein that makes up the flagellar filament. Flagella perform a variety of tasks, including bacterial adhesion and motility, and they can also activate the inflammatory response in the host via TLR5 [(4)].

On agar culture, *P aeruginosa* mucoid strains generate substantial quantities of an extracellular polysaccharide. The polysaccharide is chemically similar to alginic acid, which is a negatively charged co-polymerof 1, 4 linked Beta-D-mannuronic acid and Alpha-L-guluronic acid and is generally referred to as "alginate" proposed that alginate forms a "glycocalyx" or loose capsule in which microcolonies are entrapped [(4)]**.** *P aeruginosa* alginate is antigenic in both animals and humans. Antigenically, the polymer is conserved across all species. This alginate protects the cell from external environment, including complement and antibodies [(5)]**.**

- **Pathogenesis of *P aeruginosa*:**

P aeruginosa is a gram-negative rod that thrives in water, soil, and plants. It can grow and survive in practically any environment. It can occur in apparently healthy patients but is most usually linked to opportunistic infections. It is an opportunistic bacterium that preferentially affects immunocompromised and hospitalised individuals, where it produces more or less serious local to systemic infections that can be fatal. The dietary requirements of this virus are quite low, and it may adapt to a wide range of environmental factors [(6)].

Colonisation of altered epithelium by *P aeruginosa* is the initial stage of an infection. Up to 6% of oropharynxes are colonised by the pathogen. On the other hand, *P aeruginosa* colonisation represents a concern for up to 50% of hospitalised patients [(7)]. P aeruginosa's adhesion to epithelium is most likely mediated by type 4 pili that resemble Neisseria gonorrhea. Flagella, which are principally in charge of motility, can bind to epithelial cells and function as adhesions [(8)]. *P aeruginosa* generates a number of extracellular substances after colonisation that have the

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potential to extensively harm tissue, invade the circulation, and spread. Because *P aeruginosa* produces exotoxin A, exoenzyme S, alkaline protease, or elastase, all of which are necessary for the bacteria to be as virulent as possible [(9)].

- **Clinical significance of *P aeruginosa*:**

The spectrum of illnesses caused by nosocomial bacteria includes everything from minor skin infections to fulminant sepsis [(10)].

1. **Community acquired infections:** *P aeruginosa* infections occurs in an immune competent host *i.e.* not at significant risk for opportunistic pathogens tend to be localized and frequently associated with contaminated water or solution. They include: Mild skin infections, nail diseases (e.g. onycholysis), bacterial keratitis, otitis externa infections, endocarditis and tainted injectable medicines in intravenous drug users which may be associated with osteomyelitis of a variety of bones [(11)].
2. **Nosocomial infections:** In immunocompromised people, *P. aeruginosa* is a frequent cause of hospital acquired pneumonia. The contamination of medical equipment and/or cross-colonization from other patients start the colonisation of the respiratory system. *P aeruginosa* UTIs typically follow catheterization, instrumentation, or surgery. The most often isolated bacteria that causes serious burns and wound infections is P aeruginosa. MDR wound infections [(11)].
3. ***P aeruginosa* infections in patients with cystic fibrosis:** A gene mutation in the chloride channel protein CFTR, which is crucial in preserving homeostasis in epithelial tissues, is the cause of the illness. The control of chloride ion transport across the epithelia is disrupted by CFTR channel dysfunction, which causes sodium hyperabsorption and decreased mucociliary clearance [(11)].

- **Laboratory Diagnosis of *Pseudomonas aeruginosa* infections:**

P aeruginosa are being gram-negative rod shape, non-spore forming, non-capsulated and arranged in short chains orin small bundles**.** They are motile possessing a single polar flagellum but occasionally, some may have 2 or 3 flagellae [(12)].

Isolates of *P aeruginosa* can form a variety of colonies. Natural isolates from water or soil usually result in a sparse, tiny colony. It is assumed that the smooth and mucoid colonies contribute to colonisation and pathogenicity [(13)]. *P aeruginosa* grows well on all usual laboratory media as nutrient, blood and MacConkey's agars. Blood agar can be used to recover the organisms from clinical specimens such as cerebrospinal, joint or peritoneal dialysis fluids, where mixed flora are not anticipated. Clinical isolates are frequently β-hemolytic when grown on blood agar [(14)]. Isolation of *P. aeruginosa* from clinical samples with mixed flora is facilitated by use of selective media e.g. Cetrimide and irgasan-containing media [(15)].

Specific isolation of the organism can be performed on several specific media:

1. **Pseudomonas isolation agar (PIA):** The pigment-enhancers potassium sulphate and magnesium chloride boost P. aeruginosa's synthesis of blue or blue-green pigment, making it easier to identify. Energy is obtained from glycerol, which also encourages the formation of pyocyanin, a colour that is unique to Pseudomonas [(16)].
2. **Cetrimide Agar:** Additionally, pyocyanin and pyoverdine, two Pseudomonas pigments with distinctive blue-green and yellow-green hues, respectively, are produced in greater quantities thanks to cetrimide [(3)].

P. aeruginosa is often detectable using gram-negative bacilli standard testing. It does not digest sugars, it is oxidase positive but does not create indole or hydrogen sulphide, and has negative Voges-Proskauer and methyl red responses. *P. aeruginosa* has the ability to liquefy the gelatin, hydrolyze acetamide, and convert nitrate to nitrogen gas. When pyocyanin synthesis is missing or uncertain, these assays may be utilised to identify *P. aeruginosa* [(17)].

The immunological method that underpins ELISA uses enzyme-catalyzed reactions to increase the sensitivity of the particular antigen-antibody response. It is regarded as one of the most effective detection techniques during the past few decades and has been used extensively in the identification of infections. Anti-*Pseudomonas aeruginosa* antibodies can be utilised as markers for early diagnosis because this approach has a better sensitivity for detecting them [(18)].

Until the early stage of *P. aeruginosa* lung infection, the level of anti-LPS antibodies (IgG and IgA) significantly rose, and they continued to rise to very high levels until the late stage of infection. IgM levels rose during the initial stages of infection but did not rise further during the later stages of illness [(19)].

The easy operational procedures and high sensitivity and specificity of immunofluorescence techniques make them one of the most promising pathogen tests. A direct immunofluorescent antibody-staining technique that uses serum-specific monoclonal antibodies to identify *P. aeruginosa* in sputum samples and determine the severity of pulmonary infections [(19)].

One of the famous techniques for locating and identifying P. aeruginosa is PCR. The goal of this technique is to create a new DNA strand that is complementary to the desired template strand by primer-mediated enzymatic amplification of DNA. *P. aeruginosa* has been identified in clinical samples using several targeted genes [(20)].

- **Drug resistance in *P. aeruginosa***

Conventional antibiotics are inefficient for treating *P. aeruginosa* infections due to the variety of

antibiotic resistance pathways, which also contributes to the emergence of multidrug-resistant strains [21].

A. Intrinsic antibiotic resistance:

A bacterial species' inherent capacity to reduce an antibiotic's effectiveness through innate structural or functional traits is referred to as intrinsic antibiotic resistance [22]. The relative impermeability of P. aeruginosa's outer membrane to various antibiotics contributes to its intrinsic resistance. A combination of the outer membrane's limited permeability and the effective removal of antibiotic molecules by the activity of efflux pumps prevents antibiotics from building up inside the organism [23]. Membrane porins regulate the absorption of certain antibiotics, such as lactams, by cells. Modifications to the porin channel's size or conductance are examples of mutations. Reduction of the quantity of porins and full porin loss can happen as a key resistance mechanism. Loss of OprD, which results in carbapenem resistance, is the porin mutation for *P. aeruginosa* that has been most thoroughly studied. It seems that imipenem is most impacted, then meropenem. OprF expression reduction appears to affect β-lactam and fluoroquinolone permeability. Other changes in membrane properties can impact antibiotic efficacy in addition to porin mutations [24, 25].

One of the main mechanisms of intrinsic resistance in bacteria is the production of enzymes that inactivate or alter antibiotics. Many antibiotics have chemical bonds, such as amides and esters that can be hydrolyzed by P. aeruginosa-produced enzymes such as lactamases and enzymes that alter aminoglycosides [26].

P. aeruginosa has an inducible ampC gene, which encodes the hydrolytic enzyme lactamase, just as other gram-negative bacteria. The amide bond of the lactam ring can be broken by this enzyme, rendering lactam antibiotics inactive. Furthermore, depending on their amino acid sequences, lactamases may be categorised into four classes: A, B, C, and D. It has been demonstrated that P. aeruginosa's class C -lactamase inhibits antipseudomonal cephalosporins [21].

β-lactamase inhibitors including clavulanate, sulbactam, and tazobactam have been created and used in clinical practice to combat lactamase-mediated resistance, and it has been discovered that they significantly boost the efficacy of β -lactams in combination therapy [27].

B. Acquired antibiotic resistance:

Bacteria can develop resistance to antibiotics by mutational changes or through horizontal gene transfer. In addition to P. aeruginosa's high degree of inherent antibiotic resistance, acquired resistance plays a significant role in the formation of multidrug-resistant strains, which makes it harder to eradicate this microbe and causes more instances of chronic infection [26].

Reduced antibiotic absorption, altered antibiotic targets, increased production of efflux pumps and antibiotic-inactivating enzymes, and other mutational alterations can all help bacteria survive in the presence of antimicrobial compounds [26]. Mutations in the β-lactamase inducible gene ampC result in an overproduction of β-lactamases in certain *P. aeruginosa* clinical isolates, which significantly increases their resistance to cephalosporins [21].

The three primary methods by which bacteria acquire genetic material are transformation, transposition, and conjugation (collectively referred to as HGT), and the bacteria may also undergo alterations to its own chromosomal DNA. Antibiotic resistance genes can be carried on plasmids, transposons, integrons, and prophages, making it possible for bacteria to acquire these genes through horizontal gene transfer from either the same or different bacterial species. These genes have been demonstrated to be essential for the spread of antibiotic resistance in *P. aeruginosa* [22, 23].

C. Adaptive antibiotic resistance:

Due to temporary changes in gene and/or protein expression in response to an environmental stimulus, adaptive resistance makes bacteria more resistant to antibiotic assault and is reversible when the stimulus is withdrawn. The development of biofilm and the production of persister cells in *P. aeruginosa* are the mechanisms of adaptive resistance that are most understood, and they lead to chronic infection and a poor prognosis in CF patients [21].

An accumulation of microorganisms known as a biofilm is one that adheres to one another on a living or non-living surface and is encased in a self-produced matrix of EPSs, such as exopolysaccharides, proteins, metabolites, and eDNA [24]. Prevention of antibiotic penetration, changed microenvironment causing delayed development of biofilm cells, production of an adaptive stress response, and persister cell differentiation are the main mechanisms of biofilm-mediated resistance that shield bacteria from antibiotic assault [21]. About 1% of biofilm cells are persister cells, which are slow-growing, metabolically inert, and extremely resistant to antibiotics. Antibiotics have the ability to destroy the vast majority of *P. aeruginosa* cells. But because persisters have a latent condition that prevents the production of the antibiotic targets, they may survive and repopulate biofilms. Therefore, persistent infections that are difficult to cure are thought to be caused by the persister cells that are still present in biofilms [25].

- **Treatment of *P. aeruginosa* Infections**

Numerous medicines, including lactam antibiotics (penicillins, cephalosporins, monobactams, and carbapenems), aminoglycosides, and quinolones, are effective against *P. aeruginosa* [28]. The transpeptidase that is involved in cross-linking peptides to generate peptidoglycan is acylated by beta-lactam antibiotics, which prevents the last step in peptidoglycan formation. PBPs are what beta-lactam antibiotics use as their targets. Through autolytic mechanisms inside the bacterial cell, this binding subsequently stops the final

transpeptidation process and causes lysis and loss of viability [29].

Carboxypenicillins and ureidopenicillins are two families of broad-spectrum penicillins with antipseudomonal action that are frequently employed in the treatment of severe infections [29]. Similar to piperacillin and mezlocillin, which are both anti-Pseudomonal penicillins used intravenously to treat septicemia brought on by Pseudomonas infections, azocillin is a member of the uridopenicillins group and one of the important anti-Pseudomonal penicillins [18].

The beta-lactamase inhibitor tazobactam is most frequently used in conjunction with piperacillin (piperacillin/Tazobactam), which increases the efficiency of piperacillin by blocking several beta lactamases to which it is vulnerable. The combination of piperacillin and tazobactam has great action against Pseudomonas, but tazobactam alone has poor activity [7].

A third-generation cephalosporin antibiotic having bactericidal action, ceftazidime is a beta-lactam. The PBPs on the bacterial cell wall's inner membrane are attracted to and rendered inactive by ceftazidime. PBP inactivation prevents peptidoglycan chains from cross-linking, which is essential for the stiffness and strength of bacterial cell walls. The bacterial cell wall becomes more permeable as a result, leading to cell lysis. Ceftazidime is more effective against P aeruginosa than cefoperazone [3].

Aztreonam (ATM), a monobactam that makes an intriguing starting point for the creation of a novel antibiotic that targets Gram-negative bacteria [28]. Impenem, meropenem, ertapenem, and doripenem are all carbapenems. Among the B-lactam antibiotics, imipenem has the broadest spectrum action, active against both P. aeruginosa and all other prevalent bacterial species. Conversely, meropenem exhibits greater efficacy against Gram-negative bacteria, particularly P. aeruginosa. Doripenem, a recently developed antipseudomonal carbapenem, has been shown to be equally effective as meropenem and imipenem in the treatment of *P. aeruginosa* [29].

Clinicians need to be aware that, in addition to appropriate antibiotic coverage, additional elements such as ideal dose, interval between medication administrations, and length of therapy are crucial determinants of clinical outcomes [29].

CONCLUSION

It is acknowledged that *Pseudomonas aeruginosa* is a primary cause of illness and death. *P. aeruginosa* infections are becoming a serious worry when it comes to hospital-acquired infections, particularly in patients who are severely sick or have impaired immune systems.

In P. aeruginosa, antibiotic resistance can develop by innate, acquired, or adaptive pathways, making it multifactorial in nature. *P. aeruginosa* has been shown to produce antibiotic-inactivating enzymes such B-lactamases, have a restricted outer membrane permeability, efflux mechanisms that pump drugs out of the cell, and have high levels of inherent resistance to most antibiotics.

The proper use of antimicrobial agents entails making an accurate diagnosis, figuring out when antimicrobial therapy is necessary, understanding how dosing affects the antimicrobial activities of various agents, keeping the course of treatment as brief as possible, and switching to oral agents as soon as possible.

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