Chapter

Psuedomonas aeruginosa-Associated Acute and Chronic Pulmonary Infections

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Abstract

Pseudomonas aeruginosa is highly successful in colonizing in all types of environments. *P. aeruginosa* colonizing in adverse environment due to the presence of its virulence factors include production of toxins, proteases hemolysins, and formation of biofilms. In man, the most common opportunist pathogen is *P. aeruginosa*. Metabolically *P. aeruginosa* is versatile. Most of the antibiotics targeted metabolically active cells and bacteria could contribute to decrease in biofilm susceptibility to the antimicrobial agents. Scientists suggested about *Pseudomonas* that it can be catabolized any hydrocarbon in specific time along with availability of oxygen and nitrite. If bacteria are not susceptible to one agent in three or more, it is called as multidrug-resistance strains. The antimicrobial treatments were not suitable when microorganism presented *in vitro* microorganism resistance to antimicrobials used for treatment of the patient which lack of treatment for 24 h after diagnosis of microbial infections. Bacteria have developed resistance against commonly used antibiotics. Treatment of *Pseudomonas* infections is coming harder day by day as its resistance against most of the antibiotics. Because of resistance of bacteria antibiotics, alternative methods are in consideration. These methods include use of lactic acid bacteria (LAB) and most recently nano-particles. That is why they are used as antibacterial agents.

Keywords: *P. aeruginosa*, pulmonary infections, acute lung infections, cystic fibrosis, quorum sensing system, virulent genes, antibacterial agents, LAB

1. Introduction

P. aeruginosa contributes its pathogenicity onwards respiratory infections in the hospitalized patients. Dasenbrook et al. has reported two types of airways infection acute and chronic spread by hospital community *P. aeruginosa* is a bacterium that lives in versatile environments [1]. It is Gram negative bacterium, metabolically able to regulate its systems and highly resistance to antibiotic causing it to spread in diverse habitats mainly in hospitals. *P. aeruginosa* is recognized as a human adaptable pathogen causing acute infections (bacteremia, pneumonia and urinary tract infections) in individuals with HIV infections, surgical wounds, cancer, carrying catheters or burns, or an organ transplantations. *P. aeruginosa* is persistence in

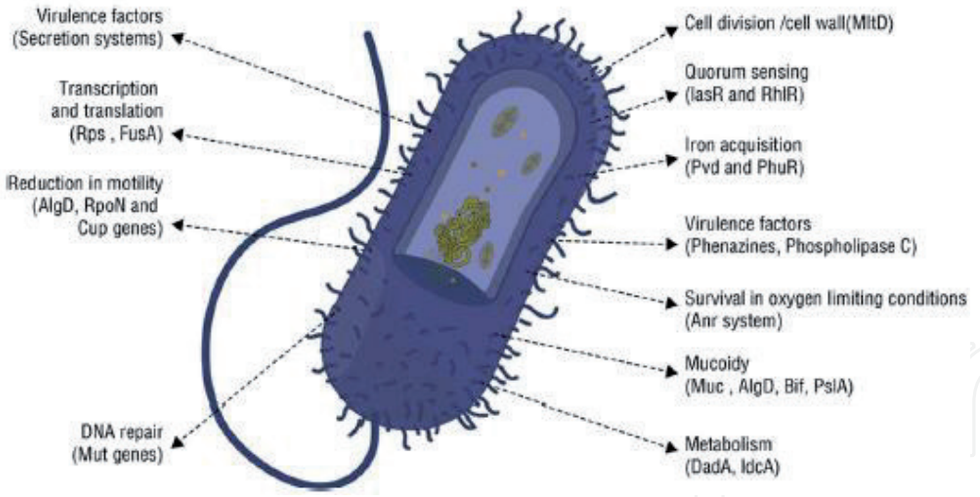

Figure 1.
P. aeruginosa *features relevant to pathogenicity and adaptation [2].*

chronic obstructive pulmonary diseases and chronic infections in individuals with cystic fibrosis (CF) [3, 4]. *P. aeruginosa* strains showed variation in their population as reported by characterization of the phenotypic clones. Four main clade of *P. aeruginosa* population are identified by the phylogenic analysis of single nucleotide polymorphisms (SNPs) which showed most different clade colonized by clonal outliers linked to the PA7 strain (**Figure 1**) [5].

2. Virulence factors

Acute infection usually observed in hospitalized patients having ventilator breathing. It is one of the main causative agents of hospital-acquired pneumonia and causing morbidity and mortality in the infected patients. Chastre and Fagon has reported 70–80% rate of mortality due to infection of *P. aeruginosa* in ventilator-associated pneumonia (Research has focused on type III secretion system (TTSS) secreting four exotoxins (ExoS, ExoT, ExoU and ExoY) [6]. ExoU is an effective virulence causing effector in TTSS. It is associated with morbidity and mortality in ventilator-associated pneumonia. Hogardt et al. reported that 40% of the isolates from such cases harbor the *exoU* gene [7].

3. Pathogenicity

The pathogenicity of *P. aeruginosa* makes its able to adhere and colonize in the presence of vast virulence factors and causing disease. Virulence factors used or synthesized by *P. aeruginosa* are enlisted in **Table 1**.

Virulence factor	Action
1. Colonization	
Flagella	Motility and invasion and adherence
Pili	Adhesion; transfer of secretions
Exopolysaccharides	Adherence and pathogen persistence
Lipopolysaccharide	Endotoxin; inflammatory agent; adherence and biofilm formation

Virulence factor	Action
2. Invasion	
Alkaline protease	Degrades immune system components
Elastase	Degrade elastin; disrupt membranes; impair
	Monocyte chemotaxis and degrade complement proteins
Lipase A and C	Involvement in degradation
Phospholipase C	Lung surfactant disruption
Protease	Degrades complement factors, plasmin, IgG, and fibrinogen
Pyocyanin	Inhibits lymphocyte proliferation; apoptosis of neutrophils
3. Pathogenesis	
Exotoxin A	Unknown role—possibly causes apoptosis of cells
Biofilm	Confers protection against biocides and immune system effectors as
	Impenetrable to antibodies (Ab), antibiotics, and biocides
Hydrogen cyanide	Unclear role, may be toxic agent.
Rhamnolipids	Dissolve phospholipids
Type III secretion	Exoenzyme (Exo) S, T and Y, and exotoxin U

Table 1.
Virulence factors produced and used by P. aeruginosa *[8–10].*

4. Biofilm

The pathogen colonized as planktonic form, and the cells convert to the sessile state to form biofilms. The hydrated structured matrices made up of exopolysaccharides and proteins, having 'slimy' characteristic can form on many surfaces from catheters to prokaryotic cells and eukaryotic. The main cause of persistent chronic infections is biofilm formation is essentially impenetrable inhabitants are protective for the bacterial strains from biocides [11]. The only one treatment to deal this situation is physical removal of the biofilm through surgery. Biofilms have heterogeneous populations of intra-species (phenotype and genotype, growth) and inter-species diversification. *P. aeruginosa* may be as dominant pathogen or with other pathogens such as Gram-negative *Burkholderia cenocepacia* and Gram-positive *S. aureus* [12]. The heterogeneous bacterial population of *P. aeruginosa* show distinct microenvironments for biofilms [13]. Metabolically active cells are at periphery and consume most of the oxygen, causing oxygen gradients in the biofilm [14]. The deeper layers of the biofilm have less metabolically active bacteria and are hypoxic. The actively growing peripheral bacterial cells of biofilms mostly susceptible to antibiotics or to the provided the drug which can penetrate slimy layer of the biofilm. Presence of a single polar flagellum made *P. aeruginosa* as motile. *P. aeruginosa is* exhibiting three distinctive types of motility and all of these types are required for development of biofilm which are;

- Swimming: It is aided by the flagellum
- Swarming: it requires both flagellum and type IV pili
- Twitching: it depends upon type IV pili

[15–20] (**Figure 2**).

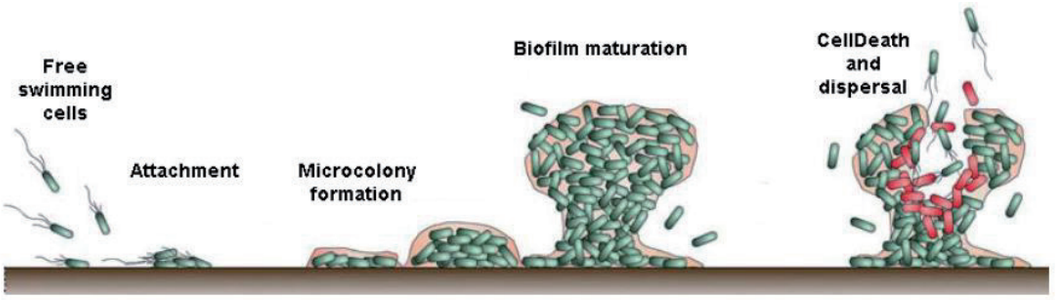

Figure 2.
Different stages of the biofilm development. Modified from [21].

5. Flagella

The bacterial flagellum is protrudes from the cell body in the form a long, thin filament that consists of the basal body, the hook and the filament. The basal body is rooted in the cytoplasmic membrane having three rings the outer membrane lipopolysaccharide (L) ring, the peptidoglycan (P) ring and the cytoplasmic membrane supra-membrane (MS) ring. The hook is exposed to the surface and is a flexible universal joint between the filament and basal body. The filament is made of polymerized flagellin monomers (up to 20,000 subunits) capped by the flagellar cap, FliD, which acts as mucin adhesion [22, 23].

The initial attachment of the bacteria needs flagella and have involvement in maturation of biofilm. Klausen et al. reported that the initial microcolony formation is occurred by clonal growth and flagella are not involved in biofilm development in *P. aeruginosa* during attachment [24].

6. Pili and type I fimbriae

The type IV pili are best characterized, which are composed of the Pil A subunit in a form of a helical polymer. Hahn and Solow reported that these IV pili are localized to the poles of the bacterial cells and facilitate the adhesive properties of *P. aeruginosa* [25]. Type IV pili appear to be required for biofilm formation and host colonization. Cell aggregation and formation of microcolonies are promoted by Type IV pili [26, 27]. *P. aeruginosa* having three sets of type I fimbriae (*CupA, CupB* and *CupC* fimbriae) which assembled by the chaperone usher pathway. CupA fimbriae demonstrated as important for adherence to abiotic surfaces causing biofilm formation and auto-aggregation of small colony variants (SCV) in *P. aeruginosa* [28].

7. Exopolysaccharides

Major components of the biofilm matrix are the exopolysachcarides produced by the *P. aeruginosa*. These exopolysaccharides include Alginate, and *Pel* polysaccharide and *Psl* polysaccharide. The *Pel* and *Psl* are associated with the non-mucoid strain [29] and Alginate is associated with the mucoid strains [30].

8. Alginate

P. aeruginosa produces alginate (an exopolysaccharide). This is a capsular polysaccharide and is overproduced in mucoid strains of *P. aeruginosa*. It is a high

molecular weight polymer composed of monomers of D-mannuronic acids and β-1, 4 linked L-guluronic which are not repeating. Mutations in the negative regulator *MucA* is mainly caused to isolate the alginate-producing variants from chronically infected CF lungs [31]. Bacteria prevent itself from phagocytosis by this polymer acts as a physical barrier and an adherence factor, it gets oxygen free radicals resulting in enhancement of resistance of the biofilm against the host immune defense and antimicrobial agents. The mucoid strains to remain persistent and establish chronic infections in the CF lung by the influence of alginate [32, 33]. Wozniak et al. has demonstrated that in non-mucoid *P. aeruginosa* strains (PAO1 and PA14) alginate is not the main component of the biofilm matrix [34].

9. *Pel* and *Psl* polysaccharides

The *pel* and *psl* operon are encoded polysaccharide associated in biofilm formation in PAO1, ZK2870 and PA14, the non-mucoid *P. aeruginosa* strains. The main components of the extracellular polysaccharide matrix are constituted by these polysaccharides [35].

The *pel* is an operon having 7 genes (PA3058 to PA3064), which encoding *Pel* polysaccharide biosynthetic proteins. The structure of *Pel* is unknown and it is a glucose-rich matrix polysaccharide and found to be involved in maintenance of biofilms and pellicle formation in *P. aeruginosa* PA14 strain. Sozzi and Smiley reported that biofilm formation is inversely regulated by cytoplasmic protein SadB1 result in altering the expression of *Pel* polysaccharide [36].

The *psl*, is an operon consist of 15 genes (PA2231 to PA2245), which encoding the *Psl* biosynthetic machinery. It is composed of a galactose-rich and mannose-rich polysaccharide. The exact structure of *Psl* has not been clarified yet. During attachment, *Psl* is holds and anchored bacteria during biofilm formation on the surface. *Psl* was associated in differentiation and maturation of *P. aeruginosa* biofilms in non-mucoid strains [37]. In *P. aeruginosa psl* and *pel* operons expresions are controlled by intracellular level of signaling molecule c-di-GMP (bis-(3′,5′)-cyclic-dimeric-guanosine monophosphate), the GacS/GacA/RsmZ and the Wsp chemosensory system [38–40].

10. Rhamnolipids

P. aeruginosa produce rhamnolipids, the biosurfactants. Enzymes of the *rhlABC* operon synthesized the rhamnolipids. Rhamnolipids are required for biofilm formation by promoting the formation of microcolony at the initial phase of the biofilm formation. These are associated with to maintain channels and void spaces in mature biofilms and also involved in biofilm dispersions [41–43].

11. Infections caused by *P. aeruginosa*

The ability of P. aeruginosa, to survive in indifferent environments including aquatic or marshes or even in low O_2 or in very high temperatures (42°C) [44] resulting to withstand and survive on dry surfaces for more than 16 months by this pathogen [45]. It can colonize on dialysis machines, 'in-dwelling' appliances, sinks, floors, and toilet surfaces [46]. Immuno compromised host can be infected by *P. aeruginosa* by causing various clinical conditions, such as pneumonia, cystic fibrosis (CF), urinary tract infections, complications in clinical burns, and wounds [47, 48].

Sr. no.	Disease caused by *P. aeruginosa*
1	Respiratory tract infections (RTI)
2	Bacteremia; septicemia
3	Otitis externa
4	Skin infection; ecthyma gangrenosum, pyoderma, folliculitis, acne vulgaris
5	Eyes infections
6	Rare conditions like meningitis, perirectal infections and specific forms of osteomyelitis

Table 2.
Infectious diseases caused by P. aeruginosa *[55].*

P. aeruginosa is a common isolate from the patients who are hospitalized for more than a week. It is associated with high rate of mortality within 24 hours, infection can results in pneumonia, septicemia and urinary tract infection.

In sever chronic infection especially in patients with cystic fibrosis (CF), *P. aeruginosa* is involved. The main cause of mortality is *P. aeruginosa* lung infection in CF patients [49]. Murray et al. reported transmissible epidemic strains of *P. aeruginosa* emerged within the CF community. In earlier reports, CF patients considered to having their own strain of *P. aeruginosa* from their environment not from other infected individuals [50]. It is known as the Liverpool epidemic strain (LES) in recent research of UK due to the most common isolate recovered from CF patients [51, 52]. *P. aeruginosa* has a major focus in research as it is reporting to transmitted from a CF patient to non-CF parents, and causing significant morbidity in infected patients. *P. aeruginosa* is considered an opportunistic pathogen and this is an unusual characteristic to infect healthy individuals. Manchester and Midlands 1, Clone C are considered as predominant epidemic strains of *P. aeruginosa* [53].

P. aeruginosa causes two types of respiratory infections. Acute (if patient have extended periods of ventilation) and chronic (if patient suffer from cystic fibrosis). Patients with these two types of infections in hospitalized settings are likely to be infected by this pathogen. Acute murine respiratory models used to identify a number of virulence factors in mutants of *P. aeruginosa*. The detailed studied is the TTSS proteins including *ExoS*, *ExoT*, *ExoU* and *ExoY*. Main contributor towards morbidity and mortality are *ExoS*, *ExoT* and *ExoU* as observed in a murein acute respiratory model (**Table 2**) [54].

The presence or absence of components of the TTSS can be correlated in human clinical results [55]. *P. aeruginosa* excreted a blue pigment Pyocyanin having antibacterial properties against other bacterial strains. The pyocyanin production also cause significant damage to lungs in murine acute respiratory infection, which demonstrated by an intranasal infection of adult CD-1 mice [49]. Quorum-sensing systems that are *LasI, LasR* [56], and *RhlI* [57] in *P. aeruginosa* contributing to acute infections. Intranasal infection in adult female Balb/c mice, and they also analyzed bacterial loads in lungs, liver and spleen after 16–18 hours of infection.

12. Cystic fibrosis

Cystic fibrosis trans-membrane conductance regulator (CFTR) mutation caused reduced chloride ion transport result in Cystic fibrosis (CF). It is a recessive genetic disorder. CF affected the development and functioning of various organs including immune system, pancreas and intestine, resulting a low life expectancy. The tissue damage is promoted in acute or chronic infections by constant stimulation of

immune system effectors. In young CF patient *P. aeruginosa* is an important pathogen which lasts later stages. CF lung is an enriched with the oxygen gradients and nutrient. The oxygen gradients contribute the uniqueness in development of mucus layer and excessive consumption of the epithelial cells in CF lungs. *P. aeruginosa is* adaptable to many phenotypes in these types of conditions. A single isolate genome showed 68 mutations over the period of 90 months. The pathology in the lung in acute infections is due to the presence of elastase, flagella, LPS with O-side chains proteases, and pyocyanin.

P. aeruginosa contributed biofilms formation, produce rough LPS (no O-side chains), lack flagella, and overproduction of alginate during chronic infections. In chronic infections typically mucoid phenotype with lesser production of pyocyanin, pyoverdine, and elastase was observed. *The antibiotic pressure* causes *P. aeruginosa* to mutate from non-mucoid form to mucoid form. Small colony variants after a continued antibiotic exposure resulted in production of mannose-rich (*psl*) or glucose- (*pel*) polysaccharides. These are hyperpiliated, which are characterized as persistent to this specific phenotype. Liverpool epidemic strain (LES) reported as *P. aeruginosa* strains by over-production of pyocyanin.

The isolates of *P. aeruginosa* form CF changed their genome to get rid form acute virulence factors [56]. The loss of *LasR* function is one of the main genetic changes observed in *P. aeruginosa* isolates from CF patients. Many other acute infection models shown that because of deficiency in quorum-sensing, *lasR* mutants are less virulent than wild-type. Bacteria used this selective pressure of genetic change in genome to escape from host immune system in acute virulence in CF airways. An effective chronic CF isolate would be one, that isolate must lose its ability to be an effective acute infection isolate. *lasR* mutants have been examined for its advantages. The *LasR* mutants can grow on selected carbon and nitrogen sources as compared to wild-type.

13. Innate immune system

Immune system is acting as natural defense mechanism to prevent the invasion of pathogens. PRRs (Porcine reproductive and respiratory syndrome) stimulus received by nonspecific innate system and respond the innate effectors responses;

- Phagocytosis by macrophages
- cell death or
- by the complement system with the membrane attack complex produced by the complement proteins or by natural killer (NK)

At the infection site the inflammatory response of effectors was induced by chemokines. Permanent tissue is damaged if continued stimulation of effectors by PAMPs (Pathogen associated molecular pattern) and virulence factors was induced [57].

14. Antibiotic resistance

P. aeruginosa is a free-living and aerobic bacillus that is isolated from soil and water in most of cases. Intrinsic resistance in *P. aeruginosa* causing high mortality due to a broad spectrum resistance of antibiotics and is able to quickly to acquire

resistance genes by horizontal gene transfer. Fluoroquinolones, gentamicin and imipenem are restricted antibiotics as effective against *P. aeruginosa* and susceptibility to these antibiotics can vary between different strains. Bacterial infections are cured traditionally with the use of antibiotics and immune system is unable to have or eradicate this use of antibiotics.

Fluoroquinolones (ciprofloxacin) prevented DNA repair and replication [58]. Aminoglycosides, Beta-lactams (imipenam but not penicillins), 3rd and 4th generation cephalosporins, and fluoroquinolones are anti-pseudomonal drugs [59].

Colistin, is a drug having lesser side-effect profile, and mainly used against multi drug resistant strains (MDR) *P. aeruginosa* strains these days. The pattern or the use of antibiotic treatment now bettered towards the treatment of specific diseases including CF. The transmission of *P. aeruginosa* reduced by separation of infected and susceptible one and use of strict hygiene procedures [60].

The unavailability of the effective therapeutic option, the treatment of infections with pseudomonas is becoming difficult to deal a very few anti-pseudomonal drugs are being considered good for the treatment of emerging resistance strain, these include aminoglycosides beta-lactams, and fluoroquinolones [61–63].

The bactericidal MoA (mechanism of action) is significant for the survival by selection pressure for the fittest one. Antibiotic resistant bacteria are selected and propagated very well in the absence of the environmental resources competitions. Specific antibiotic resistance can be void of by the use of an alternative group of antibiotic. Bacteria have established active defense mechanisms which lead to MDR species such as methicillin-resistant *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Acinetobacter baumannii (A. baumannii),* or *P. aeruginosa* are promoted as MDR strains and are difficult to eradicate this opportunistic pathogens [64].

15. *P. aeruginosa* and mechanisms of resistance

The mechanisms of resistance in *P. aeruginosa* against antibiotics can be intrinsic, adaptive, or acquired. Innately *P. aeruginosa* is resistant to many antibiotics. Intrinsically it has impermeable cell wall, outer membrane protein (Opr) channels, and multi-drug efflux pumps which give the bacteria resistance to certain antibiotics. Extended treatment and continuous use of higher therapeutic doses resulting in complete resistance [65].

16. Genomics of *P. aeruginosa*

The genome of a bacterium has two components first is the core genome and second the mobile genome. In a same species the core genome is common for all bacteria which include genes for the bacteria essential for development and the mobile genome can propagate within the whole genome [66, 67]. The mobile genome is varies between strains within a species. The mobile genome consists of a range of genetic elements such as insertion sequences, transposons, prophages, plasmids, and genomic islands. Horizontal gene transfer (HGT) processes such as conjugation, transformation, and transduction acquired the mobile genome. Genomic islands are the Clusters of genes in the mobile genome. Genomic islands encode gene products which enhance the fitness of a bacterium by survivability in new environment, increasing host range, and utilization of new nutrients. Genomic Island can be defined by various features [68].

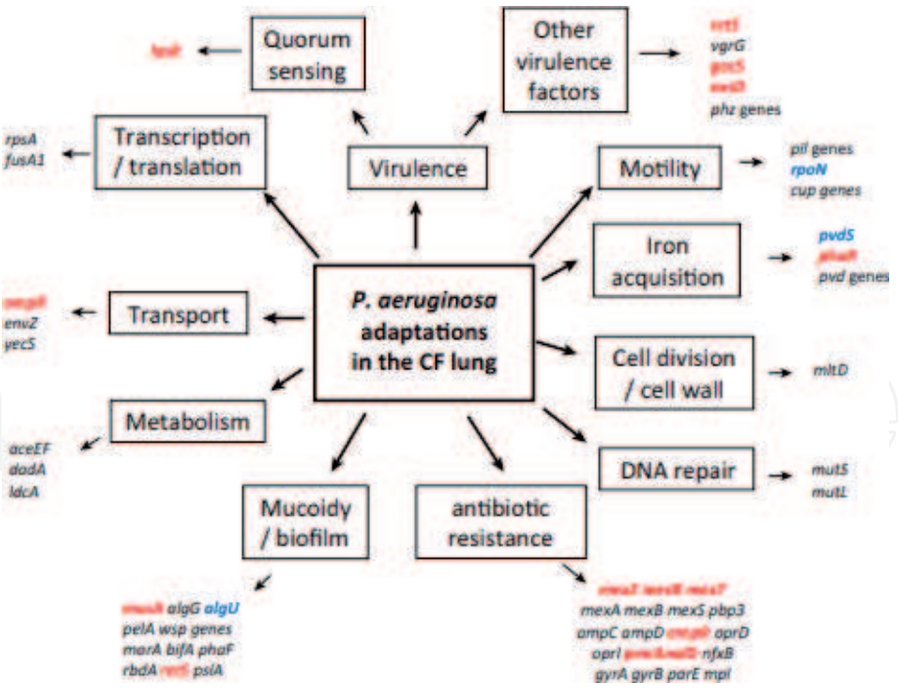

Figure 3.
The novel hypervirulent ExlA-ExlB system [69].

In *P. aeruginosa* classical clades, the most of homologous *exlB-exlA* loci are existing in *P. fluorescens, P. putida*, and *P. entomophila*, signifying this locus acquired by horizontal transfer in *Pseudomonas* spp. Strain toxicity data related to neutrophils and macrophages and ability of inflammatory cells to phagocytic *exlA*+ strains are not accessible for recognition the behavior of these strains *in vivo* in detail. Different mechanism for expression are used by bacteria for virulence effect to infect mammals and plants and that *ExlA* is not required for bacterial plants toxicity. It shows unequal level of virulence that could not be aid in one toxin, *Exl A* [70]. All strains have *lasB* gene which have *lasB* PAO1 sequence at same location and with the sequence identity up to 91–98%. Quorum sensing is regulatory for the expression of *LasB* and lacking of Quorum sensing genes affected the*lasB* expression. Quorum sensing genes are *lasR, lasI, vqsM, vqsR*, *rhlR, and rhlI* all existed at same locations in genomes of all the strains of *P. aeruginosa*. The sequences displayed identity of 98%, except for the strain PA39 (92%). Internally a frameshift mutation of *mvfR* gene, coding for an important regulator of the Quorum sensing, observed in PA7 genome [71]. Same mutation is also present in *mvfR* genes and absent in most of strains hence lacking *LasB* activity.It is reported that in the absence of *LasB* activity *lasB* sequences and Quorum sensing cannot explained.

PAO1 **Figure 3** (2001), PA14 (2005), PAS7 (2007) and LES (2008) are four complete sequenced genome available and *P. aeruginosa* is recent strain sequenced. Falagas et al. reported PAO1 had 44.2% of predicted ORFs in the class 4 with unknown function [72]. The labeling of pathogenicity islands in the genomic island must have virulence genes with known function other was this would be difficult to locate it (**Figure 4**).

P. aeruginosa PAO1 complete genome was available in 2001 and for remaining three strain genome sequences available in 2005–2008. The recent research data are based primarily on subtractive hybridization and microarray, for comparing the strains to reference strain that is PAO1 [73].

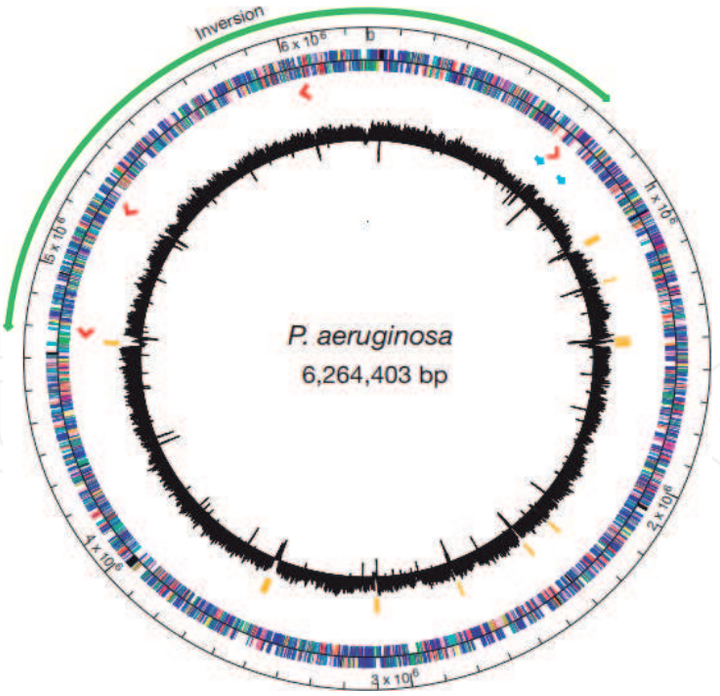

Figure 4.
Circular representation of the P. aeruginosa *genome.*

The differences in the genome targeted for further screening by using mutagenesis and virulence assays in models of *in vivo* infections. The current in vivo models commonly used for *P. aeruginosa* infection are:

- The nematode worm *Caenorhabditis elegans*
- The wax moth *Galleria mellonella* the plant *Arabidopsis thaliana*,
- The fruit fly *Drosophila melanogaster.*

Murine used for infection models that imitate the human mood of infection are not common [74].

The results of current studies of pathogenicity island mutant have explained the effectiveness in finding their influence towards virulence. PAI I_{536}–PAI V_{536} are five pathogenic island of *E. coli* and analyzed for their involvement in infection [75].

17. Quorum-sensing

Quorum-sensing systems controlled many virulent factors in *P. aeruginosa* such as the production of biofilm and the secretion of toxins [76]. Previously studies showed that insufficiency of the quorum-sensing systems can decrease the virulence in acute infection murine models. The use of insufficient quorum-sensing systems in a mutants murine burns injury model cause a decrease in mortality of the murine model. The ability of the bacteria to spread from the site of infection is also reduced. Lodise Jr. et al. reported the same results for a *LasR* mutant of PAO1 [77].

Quorum-sensing is a control system for coordination of gene expression in bacteria. As the level of an auto inducer goes to a threshold level, they caused the binding to specific bacterial receptors, in result gene transcription is initiated. In the result

majority of the bacteria in a population expressing the same phenotype. *P. aeruginosa* is having two quorum-sensing systems, the *Las* system and the *Rhl* system.

18. Recent antibacterial agents against respiratory infections

18.1 Lactic acid bacteria

Food borne pathogenic and spoilage microorganisms are affected by the lactic acid bacteria (LAB) [78, 79], for example the growth of *B. subtilis* inhibited as it spoils bread [80]. Studies showed that *Lactobacillus* strains reported an inhibitory activity against *E. coli* [81]. Proteolytic activities and lipolytic activities of psychrophilic *Pseudomonas* causing food spoilage [82]. *Lactobacillus* species produced hydrogen peroxide which inhibits the growth of *Pseudomonas* species [42].

Lactic acid functions as the natural compound having antimicrobial activity and generally recognized as safe. Lactic acid has ability to inhibit the growth of Gram-negative species of *Pseudomonadaceae* and *Enterobacteriaceae* [83]. Lactic acid is used as a bio-preservative in fermented products.

Ribosome synthesized the bacteriocin extracellularly and secreted peptide complexes or bioactive peptides havingbacteriostatic orbactericidal effect [84]. Smart et al. reported *Lactococcus lactis produced a bacteriocin called* Nisin, which is studied in detailed, and applied as stabilizer to certain foods worldwide [85]. Bacteriocins are harmless due to quick proteolytic degradation by the gastrointestinal tract enzymes [86, 87].

Four major classes of bacteriocins are

i. Lantibiotics: which are smaller and are heat stable peptides acting on membrane structures of the pathogens

ii. Non-lantibiotics: which are small are also heat stable peptides,

iii. Larger heat-labile proteins

iv. Complex bacteriocins [52, 88].

Most of bacteriocin are related to classes I and II. Proteinaceous compounds which are synthesized by ribosomes have bactericidal effect towards Gram-positive bacteria as compared to Gram-negative which have an additional layer composed of proteins, lipopolysaccharides and phospholipids [89–91].

Bacteriocins considered as potentially food-grade to increase food safety these can decrease the occurrence of foodborne diseases. These helped to lessen the use of chemical based preservatives and intensity of heat treatments for food preservations, resulting more naturally preserved food that is richer in nutritional and organoleptic properties [92].

Schillinger and Lucke has observed that the fact that most of bacteriocins have a narrower host range, which made them effective against closely related bacteria having same nutritive demands [93]. Lactic acid bacteria produced lactic acid functioning as a natural antimicrobial agent, having a generally known as safe to use. The growth of many Gram-negative species *Pseudomonadaceae* and *Enterobacteriaceae was* inhibited by the lactic acid bacteria. Forbio-preservation of the naturally fermented products lactic acid is used instead of other organic salts. Lactic acid is penetrates to cytoplasmic membrane of the organisms, which result in disruption of trans-membrane proton motive force and decrease in intracellular pH [94].

19. Antimicrobial property by hydrogen peroxide production

Bacterial enzymatic activity is destroyed by hydrogen peroxide which is a thermodynamically unstable and produced by Lactobacillus [95]. Other lactic-acid-producing bacteria and lactobacillus both are lacking heme and thus not utilizing the cytochrome systems for terminal oxidation. Flavoproteins are used by Lactobacilli, which convert oxygen to hydrogen peroxide and this mechanism, results in the formation of hydrogen peroxide in amounts which are degraded by the organism [96].

20. Antibacterial activity of nanoparticles (NPs)

The nanotechnology applications used in the food industry for food safety, disease treatment, for molecular and cellular biology as new tools, and for pathogen detection and protection [94]. NPs reported as applied in the nano tracer and nano-sensor fields in food industries [97, 98]. Nanotechnology used in food packaging to prevent contamination and to improve the shelf life of food [99, 100].

There are many types of NPs, and a variety of others are expected to introduce by the future researchers. Antibacterial agents are important and used in many industries, mainly in food industry. Cintas currently used antibacterial agents in the food industry are classified into two groups: inorganic agents and organic [101]. Inorganic antibacterial agents including NPs are used in food industry as they are stable under high pressures and temperatures conditions are required in food-processing, and regarded as safe to use for human and animals, as compared to organic materials [102, 103]. Studies showed that few NPs have selective toxicity to bacteria having lesser effects on human cells [104]. Foodborne outbreaks all over the world are increasing day by day and is important to control the causes. NPs are useful antibacterial agents that applied in the food industry. Silver (Ag) NPs are used in the medical and pharmaceutical industries. Ag NPs are very significant for the potential use in wide range of biological applications, as an antibacterial and antifungal agent for antibiotic resistant organisms to prevent infections. The concentration of NPs is linked with antibacterial activity. However, studies disagree with one another, indicating the mechanisms of NPs which causing antibacterial activity and toxicity to bacterial cells are very complex one [105–107]. Thus, it is challenging to classify the NPs as or adverse NPs or beneficial NPs towards bacteria. The tolerance property of bacteria having lesser growth rate is associated with the expression of genes related to stress-response [108].

After exposure to zinc oxide (ZnO) NP minimal inhibition concentration *Staphylococcus aureus (S. aureus) and Salmonella typhimurium* (S. *typhimurium*) were reduced to 0 within 4 and 8 h, respectively. Scanning electron micrographs of the targeted cell showed the completely lysis of the cells. *Pseudomonas* spp. were the most resistant and *Bacillus cereus* was the most sensitive among all of the studied strains against ZnO NPs.

Higher concentrations of Ag NPs showed the stronger antimicrobial activity. Ag NPs are used as antibacterial agents against *Escherichia coli (E. coli)* (Gram negative bacteria) [109].

The studied showed same results of Ag NPs between *S. aureus, E. coli* [110]. It was reported that smaller Ag NPs had effective antibacterial activity but having higher cytotoxicity. The antibacterial activity of Ag NPs is not only against the *S. aureus* and *E. coli* and, but also, *P. aeruginosa* [111]. ZnO NP inform of powders are widely used in coating electronic devices, cosmetics, catalysts and pigments. Instead of the extensive use of ZnO NPs, the safety of ZnO NPs for humans is not clear yet. In many studies the toxicity of metal oxide NPs and ZnO NPs towards

mammalian cell and organs reported [112, 113]. Concentrations of ZnO less than 100 g/mL caused a substantial decrease of mitochondrial function decreased up to substantial level by concentration of ZnO (less than 100 g/mL). Weiss and Takhistov reported that Ag NPs decreased the cell viability of epithelial cells in lung [114]. For extracellular biosynthesis of gold nanoparticles *P. aeruginosa* used.

Moraru (2003) observed the AuNPs were prepared by reduction of gold ion in bacterial cell supernatant solutions [115]. Silver nanoparticles showed excellent antibacterial effect against pathogenic bacteria, *Klebsiella pneumoniae and S. aureus* [116, 117].

21. Conclusion

From the total *P. aeruginosa* isolates 66 to >90% were from the Lahore region and showed *in vitro* resistance to many of the commercially available antibiotics tested. Meropenem, Piperacillin, and Amoxicillin were the drugs for which there was the greatest susceptibility and represent recommended treatments for infections due to *P. aeruginosa* in our region. A significant killing of these *resistant P. aeruginosa* strains by factors present in supernates of *Lactobacilli* spp. was observed, suggesting that the use of *Lactobacilli* spp. as probiotics may be of value for the treatment or prevention of *P. aeruginosa* colonization. We also found strong *in vitro* anti-bacterial efficacy of Ag, Zn and Fe3 oxide NPS against the local *P. aeruginosa* isolates, suggestive of additional research into their practical application in a healthcare department [117]. The differences in pathogenicity due to between the *P. aeruginosa* isolates, which could be due to genes involved in the quorum sensing and biofilm formation which having the ability to develop infections. The research could be important in future studies as the already reported isolates used are have same environmental conditions which having the multidrug resistant *P. aeruginosa* strains. The genomic variations between the isolates of *P. aeruginosa* are also observed for detection of virulence genes in strains of *P. aeruginosa* could highlight the link between acute and chronic respiratory infections. The collected and provided data could conclude that the virulence genes are important in severity of acute and chronic respiratory infections in human beings.

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