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ANTIBACTERIAL TARGETS IN PSEUDOMONAS AERUGINOSA

Shanooba M. Palamthodi, Vishnu J. Gaikwad,
Nitin V. Ghasghase, Sandip S. Patil

Department of Biotechnology, TKIET, Warananagar, Kolhapur, Maharashtra, India-416113

ABSTRACT:

Pseudomonas aeruginosa is a deadly opportunistic pathogen. It causes life-threatening infections in individuals with compromised immune systems, such as cancer patients undergoing chemotherapy or patients with cystic fibrosis. This bacterium is naturally resistant to many antimicrobials and with the overuse of antibiotics has become resistant to those it was once sensitive. Thus, there is a real need for new drugs and approaches to combat the myriad diseases caused by this pathogen. Computer aided drug design greatly facilitates the search for new antimicrobials. The various proteins that are essential for the pathogenesis of the organism can be the successful drug targets to facilitate the drug design processes. In this review we are discussing the various proteins involved in the pathogenesis of P. aeruginosa and their drugability. The identification of drug targets for a given human disease, whether it is mainly environmental or genetic in origin, depends on an understanding of the molecular chain of events that unfold in the disease process. Anatomic pathology, biochemistry, cellular physiology, and pharmacology constitute the main traditional approaches towards identifying potential therapeutic targets.

Keywords: Pseudomonas aeruginosa, opportunistic, pathogenesis, drugability, target

NEED OF NEW ANTIBACTERIAL DRUGS

Antimicrobial drug resistance constitutes a major problem all over the world. In fact, today patients are suffering from multi drug-resistant infections and are having higher levels of morbidity and mortality. Most importantly, the numbers of therapeutic options for serious, life-threatening bacterial infections are becoming more limited mainly due to the multi drug resistance. It is a common misconception that virtually all bacterial infections can be treated with the currently available antibacterial drugs. But in reality untreatable, drug-resistant bacterial infections do occur and are becoming increasingly common. Furthermore, deaths due to bacterial infections are on the rise even in patients who are infected with bacteria that are presumably treatable with currently available drugs [18, 20-21, 51, 54, 56].Many recent advances, which are in practice of medicine, are actually at serious risk. Multi drug-resistant bacteria interfere with the routine surgical procedures. Clearly, these novel therapies may become invalid because of untreatable, multi drug-resistant infections [2]. Therefore, there is a clear need of new antimicrobials to control these multi drug resistance infections [9, 33].

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa is a saprophytic organism that is widespread in nature, especially in moist environments. It is the most important pathogen of this genus and has one of the broadest ranges of infectivity of all microorganisms, causing disease in plants, insects, fish, amphibians, reptiles, birds, and mammals.

PATHOPHYSIOLOGY

P. aeruginosa is an opportunistic pathogen. It causes infection in immunocompromised persons. Most cases of *P. aeruginosa* infections are marked by lose of integrity of a physical barrier to infection (eg, skin, mucous membrane) or the presence of an underlying immune deficiency (eg, neutropenia, immunosuppression). The minimum nutritional requirements and its tolerance to wide range of physical conditions add to its pathogenicity [10 - 11].

The pathogenesis of pseudomonal infections is multifactorial and complex. The 3 stages of P. aeruginosa infection are bacterial attachment and colonization, local infection, and bloodstream

dissemination and systemic disease. The importance of colonization and adherence is most evident in the context of respiratory tract infection in patients with cystic fibrosis [13-15, 31]. Production of extracellular proteases enhances the virulence of this pathogen by assisting in bacterial adherence and invasion [37, 46, 55, 57].

PSEUDOMONAS AERUGINOSA INFECTIONS

P. aeruginosa causes life-threatening infections in individuals with compromised immune systems, such as cancer patients undergoing chemotherapy or patients with cystic fibrosis. Pseudomonas aeruginosa infections can involve any part of the body- respiratory tract, central nervous system, cardiovascular system, ear, eye, gastrointestinal system, urinary tract, skin and bones and joints [22, 27, 35].

In plants, *P. aeruginosa* induces symptoms of soft rot with *Arabidopsis thaliana*. It is a powerful pathogen with *Arabidopsis*. Pathogenesis in Arabidopsis involves the following steps: attachment to the leaf surface, congregation of bacteria at and invasion through stomata or wounds, colonization of intercellular spaces, and concomitant disruption of plant cell wall and membrane structures, basipetal movement along the vascular parenchyma, and maceration and rotting of the petiole and central bud [47].

Given the critical importance of *Pseudomonas aeruginosa* as an opportunistic pathogen, it is necessary to consider novel targets for therapeutic development. This is especially true as this bacterium is resistant to many antimicrobials [33, 48]. The resistance is mainly due to the low permeability of the bacterial cellular envelope [31]. Thus, there is a real need for new drugs and approaches to control the myriad of diseases caused by this pathogen [39].

TARGETS FOR DRUG DISCOVERY [36]

Exotoxin A

Exotoxin A is one of the major virulence factor in *P. aeruginosa* infections such as septicemia, corneal infections and lung infections [8, 50]. *P. aeruginosa* exotoxin A enhances the pathogenicity of this bacterium via inhibiting the production of pro-inflammatory cytokines. This indicates that Exotoxin A can be a potential target for the development of a novel antibiotic against *P. aeruginosa*.

Exoenzyme S

Exoenzyme S (ExoS) is a 49-kDa ADP-ribosyltransferase which plays an important role in *P. aeruginosa* pathogenesis [23]. Virtually all pneumonia and cystic fibrosis (CF) pulmonary *Pseudomonas* isolates produces *P. aeruginosa* virulence factor exoenzyme S [17]. Increased levels of ExoS correlate with increased pulmonary damage in animal models and CF patients [41-44]. Targeting ExoS may effectively reduce the *P. aeruginosa* infections.

Flagellin

Flagella *P. aeruginosa* contain the protein flagellin as a major structural component. Flagellin binds to the host cell membrane glycolipid. Binding to the glycolipid may change the transport properties of epithelial cells [19, 38]. Also the flagellum of Pseudomonas aeruginosa is essential for resistance to clearance by the surfactant protein A which is an important lung innate immune protein that kills microbial pathogens by opsonization and membrane permeabilization [43-44, 61]. Flagellin can be a successful drug target.

Pyocyanin

Pyocyanin is the blue phenazine pigment that is produced by *P. aeruginosa*. Pyocyanin slower the ciliary beat and disrupts the integrity of the epithelium *in vitro*. Also it results in slowing of mucociliary transport in guinea pig trachea *in vivo*. Pyocyanin-induced ciliary slowing is associated with a decrease in both intracellular cAMP and ATP. Studies revealed that the agents that raise intracellular cAMP levels inhibit the effect of pyocyanin on epithelium. This reflects that inhibition of pyocyanin may prevent the pathogenesis of p. aeruginosa.

Protease IV

Protease IV, is a 26 kDa serine endoprotease, secreted by most *P. aeruginosa* strains causing microbial keratitis. In association with other proteases protease IV has a major role in corneal virulence. The virulence of protease IV in ocular

infection has been attributed to the destruction of host proteins, including fibrinogen and components of the immune system [16]. Protease IV also degrades structural proteins such as elastin, facilitating bacterial adhesion and infection [53]. This leads to the conclusion that protease IV can be a suitable target in controlling *P. aeruginosa* pathogenesis.

Alkaline protease

Alkaline protease is one of the important enzymes in corneal opacity and neovascularization [32]. Also Pseudomonas aeruginosa alkaline protease degrades human gamma interferon and inhibits its bioactivity [26]. Studies showed that inhibition of this protein will help in reducing the harmful effects of *P. aeruginosa* infections. But since many virulence factors are involved in the pathogenesis loss of a single virulence factor such as alkaline protease does not significantly affect the ability of *P. aeruginosa* to cause disease in the eye. Therefore, any therapeutic intervention to prevent infection or limit tissue damage during infection from bacterial exoproteins should not be targeted towards a single protease or toxin. Instead combination therapy which will target more than one virulence factors will control the infection effectively [40].

FtsZ protein

FtsZ is the key protein in the cell division machinery. Polymerization of FtsZ into a macromolecular structure termed the Z ring is a key event in bacterial cell division. Its polymerization into Z ring and the GTPase activity are essential for the separation of daughter cells. Agents interfering with FtsZ polymerization potently inhibit septum formation and cell division. So this class of compounds can constitute a new class of antibiotics [6-7, 34].

Isocitrate lyase (ICL)

The glyoxylate pathway plays an important role in *P. aeruginosa* pathogenesis. Isocitrate lyase is the key enzyme in the glyoxylate pathway. ICL is essential for aerobic as well as anaerobic utilization of carbon sources. Studies using animal model systems of infection show that It is required for bacterial persistence [15]. Recent studies raise the possibility of controlling *P. aeruginosa* infections within the CF lung with the use of drugs that inhibit isocitrate lyase [52]. The apparent absence of this enzyme in humans makes it an attractive therapeutic target.

LpxC

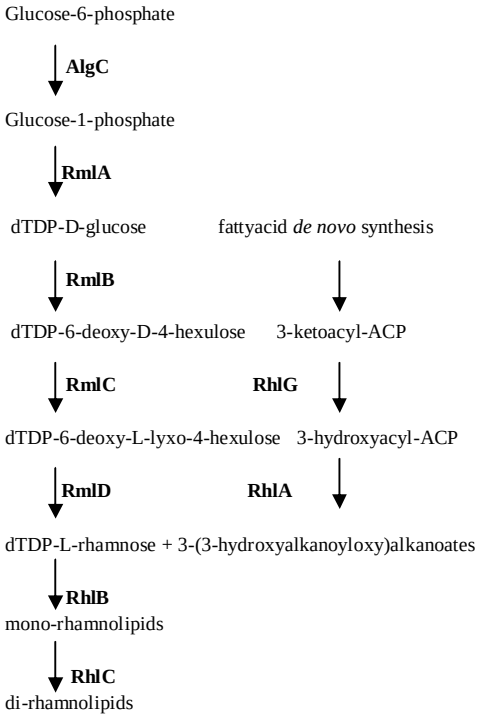
LpxC [UDP-(3-*O*-acyl)-N-acetyl glucosamine deacetylase] is a metalloenzyme that catalyzes the first step in the lipid A biosynthesis in bacteria [58]. Lipid A is a component of lipopolysaccharide which has a critical role in membrane integrity and host resistance. Therefore targeting the conserved lipopolysaccharide biosynthetic enzymes is attractive for design of novel antibacterial drugs. In *P. aeruginosa* LpxC is essential for growth of the organism. Therefore targeting LpxC is a good therapeutic approach to cure *P. aeruginosa* infections [12, 24, 28-29, 36, 59].

Cytotoxin

Pseudomonas aeruginosa produces a 29 kDa cytotoxin that causes the formation of pores with 2 nm diameter in the plasma membranes of many different eukaryotic cells [3-4]. *P. aeruginosa* may protect itself from such basic host defenses through the production of cytotoxin. *P. aeruginosa* cytotoxin, previously named leukocidin, inactivates eukaryotic cells by forming lesions or pores in the membrane of target cells of the immune system. This causes increased plasma membrane permeability to small molecules and ions [30]. Inhibition of this may prevent the pathogenesis of *p. aeruginosa*.

Rhamnolipids

Rhamnolipids act as virulence factors because they severely affect normal tracheal ciliary function , inhibit the phagocytic response of macrophages. Also they act as heat-stable hemolysins. Swarming motility of *P. aeruginosa* is affected by rhamnolipids by a reduction of surface tension which helps in the surface conditioning needed for efficient colonization [1, 25]. Therefore the enzymes involved in the rhanmolipid biosynthetic pathway (Figure: 1) can be good targets for drug discovery.



Figur 1: Rhamnolipids Biosynthetic Pathway

Esterase EstA

Esterase EstA is an autotransporter protein located in the outer membrane. Autotransporters predominantly show physiological functions related to the virulence of the corresponding organisms. EstA was found to be required for full virulence in a rat model of chronic respiratory infection. Inactivation of the *estA* gene not only resulted in rhamnolipid deficiency but also influenced other virulence-related functions like cellular motility, i.e., swimming, twitching motility, and swarming [1, 60]. So this protein can be a good drug target.

Polyphosphate Kinase

PPK is responsible for the synthesis of polyphosphate from ATP. It is required for motility and is essential for quorum sensing and virulence. Also inhibition of PPK prevents the formation of the biofilm. PPK is highly conserved in prokaryotes and is absent in eukaryotes. This all suggests that PPK is a therapeutic target to treat *P. aeruginosa* infections. Also since PPK is involved in the cellular metabolism rather than in an essential function, the chances to provoke resistance are less [45, 49].

REFERENCES

1. Al-Tahhan, R.A., Sandrin, T.R., Bodour, A.A., Maier, R.M. (2000). Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. *Applied and Environtal Microbiology*, 66:3262–3268.
2. Aparna., Madhu, S., Sarita, Y. (2008). Biofilms: microbes and disease. *The Brazilian Journal of Infectious Diseases*, 12:526-530.
3. Baltch, A. L., Obrig, T.G., Snith, R.P., Hammer, M.C., Conroy, J.V. (1987). Production of cytotoxin by clinical strains of Pseudomonas aeruginosa. *Canadian Journal of Microbiology*, 33: 104-111.
4. Baltch, A.L., Hammer, M.C., Smith, R.P., et al. (1985). Effects of Pseudomonas aeruginosa cytotoxin on human serum and granulocytes and their microbicidal, phagocytic, and chemotactic functions. *Infectious Immunology*, 48:498-506.
5. Bentzmann, S.D., Roger, P., Puchelle, E. (1996). *Pseudomonas aeruginosa* adherence to remodelling respiratory epithelium. *European Respiratory Journal* 9: 2145–2150.
6. Bleau, C., Beaumont, M., Sanschagrin, F., Voyer, N., Levesque, R.C. (2007). Parallel solid synthesis of inhibitors of the essential cell division FtsZ enzyme as a new potential class of antibacterial. *Bioorganic & Medicinal Chemistry*, 15:1330–1340.
7. Bleau, C.P., Sanschagrin, F., Levesque, R.C. (2004). Identification of Pseudomonas aeruginosa FtsZ peptide inhibitors as a tool for development of novel antimicrobials. *Journal of Antimicrobial Chemotherapy*, 40:235-238.
8. Burke, V., Robinson, J. O., Richardson, C. J. L. & Bundell, C. S. (1991). Longitudinal studies of virulence factors of *Pseudomonas aeruginosa* in cystic fibrosis. *Pathology* 23: 145-148.
9. Cegelski, L., Marshall, G.R., Eldridge, G.R., Hultgren, S.J. (2008). The biology and future prospects of antivirulence therapy. *Microbiology*, 6:17-27.
10. Celik, I., Cihangiroglu, M., Yilmaz, T., Kohle, U., Akbulut, A. (2006). The prevalence of bacteraemia-related retinal lesions in seriously ill patients. *Journal of infection*, 52:97–100.
11. Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay, R. (2006). Burn wound infections. *Clinical Microbiology Reviews*, 19:403-434.
12. Clements, J.M., Coignard, F., Johnson, I., Chandler, S., Palan, S., Waller, A., Wijkmans, J., Hunter, M.G. (2002). Antibacterial activities and characterization of novel inhibitors of LpxC. *Antimicrobial Agents and Chemotherapy*, 46: 1793–1799.
13. Cloete, T.E., Jacobs L. (2001). Surfactants and the attachment of Pseudomonas aeruginosa to 3CR12 stainless steel and glass. *Water SA* 27: 21-26.

14. Delden, C.V., Iglewski, B.H. (1998). Cell-to-cell signalling and *Pseudomonas aeruginosa* infections. *Emerging Infectious diseases*, 4:551-560.
15. Dıaz-Perez A.L., et al. (2007). Identi¢cation of the aceA gene encoding isocitrate lyase required for the growth ofPseudomonas aeruginosa onacetate, acyclic terpenes and leucine *Federation of European Microbiological Societies Microbiology Letters*, 269: 309–316.
16. Engel, L.S., Hill, J.M., Moreau, J.M., Green, L.C., Hobden, J.A., O'Callaghan, R.J. (1998). Pseudomonas aeruginosa protease IV produces corneal damage and contributes to bacterial virulence. Investigative Ophthalmology & Visual Science, 39: 662–665.
17. Epelman, S., Neely, G.G., Ling Ma, L., et al. (2002). Distinct fates of monocytes and T cells directly activated by *Pseudomonas aeruginosa* exoenzyme S Journal of Leukocyte Biology, 71: 458-468.
18. Falagas, M.E., Bliziotis, I.A., Kasiakou, S.K., Samonis, G., Athanassopoulou, P., Michalopoulos, A. (2005). Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria. *Infectious Diseases*, 5:24-29.
19. Feldman, M., Bryan, R., Rajan, S., Scheffler, L., Brunnert, S., et al. (1998). Role of flagella in pathogenesis of Pseudomonas aeruginosa pulmonary infection. *Infectious Immunology*, 66: 43–51.
20. Fischbach, M.A., Walsh, C.T. (2009). Antibiotics for emerging pathogens. *Science*, 325: 1089-1093.
21. Fish, D.N., Ohlinger, M.J. (2006). Antimicrobial resistance: factors and outcomes. *Critical care clinics*, 22:291-311.
22. Fleiszig, S.M., Evanes, D.J. (2002). The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa*. *Clinical and Experimental Optometry*, 85:271-278.
23. Ganesan, A.K., Vincent, T.S., Olson, J.C., Barbieri, J.T. (1999). *Pseudomonas aeruginosa* Exoenzyme S Disrupts Ras-mediated Signal Transduction by Inhibiting Guanine Nucleotide Exchange Factor-catalyzed Nucleotide Exchange. *The Journal Of Biological Chemistry*, 274: 21823–21829.
24. Heath, R.J., White, S.W., Rock, C.O. (2001). Lipid biosynthesis as a target for antibacterial agents. *Progress in Lipid Research*, 40:467–497.
25. Hori, K., Marsudi, S., Unno, H. (2002). Simultaneous production of polyhydroxyalkanoates and rhamnolipids by *Pseudomonas aeruginosa*. *Biotechnology and Bioengineering*, 78:699–707.
26. Horvat, R.T., Parmely, M.J. (1998). Pseudomonas aeruginosa alkaline protease degrades human gamma interferon and inhibits its bioactivity. *Infectious immunology*, 56: 2925-2932.
27. Hua Zhu., Bandara R., Conibear CR., Thuruthyil SJ., Rice SA., Kjelleberg S., Givskov M., Willcox, D.P. (2004). *Pseudomonas aeruginosa* with *LasI* Quorum-Sensing Deficiency during Corneal Infection. *Investigative Ophthalmology & Visual Science*, 45: 1897-1903.
28. Kadam, R.U., Chavan, A., Roy, N. (2007). Pharmacophoric features of *Pseudomonas aeruginosa* deacetylase LpxC inhibitors: An electronic and structural analysis. *Bioorganic & Medicinal Chemistry Letters*, 17:861–868.
29. Kadam, R.U., Roy, N. (2006). Cluster analysis and two-dimensional quantitative structure–activity relationship (2D-QSAR) of *Pseudomonas aeruginosa* deacetylase LpxC inhibitors. *Bioorganic & Medicinal Chemistry Letters*, 16:5136–5143.
30. Kluftinger, J.L., Lutz, F., Hancock, E.W. (1989). Pseudomonas aeruginosa Cytotoxin: Periplasmic Localization andInhibition of Macrophages. *Infection and Immunity*, 57: 882-886.
31. Kobayashi, H., Kobayashi, O., and Kawai, S. (2009). Pathogenesis and clinical manifestations of chronic colonization by *Pseudomonas aeruginosa* and its biofilms in the airway tract. *Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases*, 15:125-142.
32. Kreger, A.S., Gray, L.D. (1978). Purification of *Pseudomonas aeruginosa* proteases and microscopic characterization of pseudomonal protease-induced rabbit comeal damage. *Infectious Immunology*, 19: 630-648.
33. Lambert, P.A. (2005). Bacterial resistance to antibiotics: Modified target sites. *Advanced Drug Delivery Reviews*, 57:1471–1485
34. Lappchen, T., Pinas, V.A., Hartog, A.F., Koomen, G., Barbero, C.S., Andreu, J.M., Trambaiolo, D., Lowe, J., Juhem, A., Popov, A.V., Blaauwen, T. (2008). Probing FtsZ and tubulin with C8-substituted GTP analogs reveals differences in their nucleotide binding sites. *Chemistry & Biology*, 15: 93-94.
35. Ledbetter, E.C., Mun, J.J., Kowbel, D., Fleiszig, S.M. (2009). Pathogenic phenotype and genotype of *Pseudomonas aeruginosa* isolates from spontaneous canine ocular infections. *Investigative Ophthalmology & Visual Science*, 50:729-736.
36. Li, J.D., Dohrman, A.F., Gallup, M., Miyata, S., Gum, J.R., Kim, Y.S., Nadel, J.A., Prince, A., Carol B., Basbaum, C.B. (1997). Transcriptional activation of mucin by *Pseudomonas aeruginosa* lipopolysaccharide in the pathogenesis of cystic fibrosis lung disease. *Proceedings of the National Academy of Sciences*, 94:967-972.
37. Lyczak, J.B., Cannon, C.L., Pier, G.B. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes and Infection*, 2:1051–1060.
38. Mahenthiralingam, E., Campbell, M.E., Speert, D.P. (1994). Nonmotility and phagocytic resistance of Pseudomonas aeruginosa isolates from chronically colonized patients with cystic fibrosis. *Infectious Immunology*, 62: 596–605.
39. Moniri, R., Mosayebi, Z., Movahedian, A.H.,

Mossavi, G. (2006). Increasing trend of antimicrobial drug-resistance in *Pseudomonas aeruginosa* causing septicaemia. *Iranian Journal of Public Health*, 35:58-62.

40. Morihara, K.. (1964). Production of elastase and proteinase by Pseudomonas aeruginosa. *Journal of Bacteriology*, 88: 745-757.

41. Nicas, T.I., Frank, D.W., Lile, J.D., Iglewski, B.H. (1985). Role of exoenzyme S in chronic *Pseudomonas aeruginosa* lung infections. *European Journal of Clinical Microbiology*, 4: 175–179.

42. Olson, J.C., Fraylick, J.E., McGuffie, E.M., Dolan, K.M., Yahr, T.L., Frank, D.W., Vincent, T.S. (1999). Interruption of multiple cellular processes in HT-29 epithelial cells by *Pseudomonas aeruginosa* exoenzyme S. Infectious Immunology, 67: 2847–2854.

43. Overhage, J., Bains, M., Brazas, M.D., Hancock, R.E. (2008). Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *Journal of Bacteriology*, 190:2671–2679.

44. Overhage, J., Lewenza, S., Marr, A.K., Hancock, R. E. (2007). Identification of genes involved in swarming motility using a *Pseudomonas aeruginosa* PAO1 mini-Tn*5-lux* mutant library. Journal of Bacteriology, 189:2164–2169.

45. Parks, Q.M., Hobden, J.A. (2005). Polyphosphate Kinase 1 and the ocular virulence of *Pseudomonas aeruginosa*. *Investigative Ophthalmology & Visual Science*, 46:248-251.

46. Perumal, D., Lim, C.S., Vincent, T.K.C., Sakharkar, K.R. (2008). A combined computational-experimental analyses of selected metabolic enzymes in *Pseudomonas* species.*International Journal of Biological Sciences*, 4:309-317.

47. Pukatzki, S., Kessin, R.H., Mekalanos, J.J. (2002). The human pathogen Pseudomonas aeruginosa utilizes conserved virulence pathways to infect the social amoeba *Dictyostelium discoideum*. *Proceedings of the National Academy of Sciences*, 99:3159-3164.

48. Rashid, A., Chowdhury, A., Rahman, S.H., Begum, S.A., Muazzam, N. (2007). Infections by *Pseudomonas aeruginosa* and antibiotic resistance pattern of the isolates from Dhaka Medical College Hospital. *Bangladesh Journal of Medical Microbiology*, 1:48-51.

49. Rashid, M.H., Rumbaugh, K., Passador, L., Davies, D.G., Hamood, A.N., Iglewski, B.H., and Kornberg, A. (2000). Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences*, 97:9636–9641.

50. Sharma, S., Kaur, R.,Yadav, V., Harjai, K., Joshi, K. (2004). Contribution of Exotoxin A of Pseudomonas aeruginosa in acute and chronic experimental renal infection. *Japanese Journal of Infectious Diseases*, 57: 119-120.

51. Siegel, R.E. (2008). Emerging gram-negative antibiotic resistance: daunting challenges, declining sensitivities, and dire consequences. *Respiratory Care*, 53: 471-479.

52. Silo-Suh, L.A., Lindsey, L., Hagins, J.M., Pamela A., Soko, P.A. (2008). Virulence determinants from a cystic fibrosis isolate of *Pseudomonas aeruginosa* include isocitrate lyase. *Microbiology*, 154:1616–1627.

53. Smith,L., Rose, B. (2006). Protease IV production in Pseudomonas aeruginosa from the lungs of adults with cystic fibrosis. *Journal of Medical Microbiology*, 55: 1641–1644.

54. Srifuengfung, S., Tiensasitom, C., Yungyuen, T., Dhiraputra, C. (2004). Prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* mucoid and non-mucoid type. *Southeast Asian Journal of Tropical Medicine & Public Health*, 35:893-896.

55. Tan, M.W., Rahme, L.G., Sternberg, J.A., Tompkins, R.G., Ausubel, F.M. (1999). *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proceedings of the National Academy of Sciences*, 96:2408-2413.

56. Tenover, F.C. (2006). Mechanisms of Antimicrobial Resistance in Bacteria. *The American Journal of Medicine*, 119: S3–S10.

57. Vrane, J., Drinkovi, D.J., Zulj, I., Kruzic, V., Turkovic, B., Maric, S. (2004). Adherence ability of *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis to two different epithelial cells. *Collegium Antropologicum*, 2:675-680.

58. White, R.J., Margolis, P.S., Trias, J., Yuan, Z. (2003). Targeting metalloenzymes: a strategy that works. *Current Opinion in Pharmacology*, 3:502–507.

59. Witte, P.R., Kline, T., Barb, A.W., Erwin, A.L., Mansfield, B.E., McClerren, A.L., Pirrung, M.C., Tumey, L.N., Warrener, P., Raetz, C.R., and Stover, C.K. (2006). Molecular validation of LpxC as an antibacterial drug target in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 50:2178-2184.

60. Yang, L., Rybtke, M.T., Jakobsen, T.H., Hentzer, M., Bjarnsholt, T., Givskov, M., Nielsen, T.T. (2009). Computer-aided identification of recognized drugs as *Pseudomonas aeruginosa* quorum-sensing inhibitors. *Antimicrobial Agents and Chemotherapy*, 53:2432-2443.

61. Zhang, S., McCormack, F. ., Levesque, R.C., O'Toole, G.A., Lau, G.W. (2007). The Flagellum of Pseudomonas aeruginosa Is Required for Resistance to Clearance by Surfactant Protein A. *PLoS ONE*, 2: 1-11.