Evaluation of the Killing Virulence of Pigmented and Non-Pigmented Clinical Isolates of *Pseudomonas Aeruginosa* in Mice

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Abstract

*Pseudomonas aeruginosa* employ a large virulence armamentarium to overcome host defenses, including the production and dispersal of Pyocyanin exotoxin and other phinazine molecules that are toxic to their hosts. The aim of the present study is to evaluate the mice killing capacity of different clinical isolates of pigmented and non-pigmented *Pseudomonas aeruginosa*. Three reference isolates isolated previously from otitis media and otitis externa (pyocyanin highly producer, fluorescein highly producer, non-pigmented strain) where chosen to be inoculated intra peritoneally in mice. The results of the present study showed that the Mortality occurred within 24h in group one (pyocyanin producer) by 100% of mortality rate and within 48h in group two (fluorescin producer strains) by 100% of mortality rate whereas mortality occurred in group three (non-pigmented strains) at the end of 96h post infection by 66.6% of mice death when all compared with control group (Intra peritoneally saline injection). Our study concludes the highly significant mice killing capacity of highly pyocyanin *P. aeruginosa* producer when compared to other pigmented and non-pigmented and these different isolates retain the capability to develop otitis media.

Key words: *Pseudomonas aeruginosa*, Pyocyanin, Fluorescin, Killing Virulence, pigmented and non-pigmented, mice

Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes extensive morbidity and mortality in individuals who are immune compromised or have underlying medical conditions such as, urinary tract, respiratory tract and skin infections and primarily causes of nosocomial infections [1]. It's a non sporulating, gram negative, oxidase positive motile bacterium with a polar flagellum [2]. *P. aeruginosa* is a common nosocomial pathogen because it is capable of thriving in a wide variety of environmental niches [3]. It is a leading cause of hospital associated infections in the seriously ill, and the primary agent of chronic lung infections in cystic fibrosis patients [4]. They exist in very large numbers in the human environment and animal gut, they are capable of inhabiting/contaminating water, moist surface and sewage, hospital environment usually have resident *P. aeruginosa* [5].

Despite the apparent ubiquity of *P. aeruginosa* in the natural environment and the vast array of potential virulence factors, the incidence of community-acquired infections in healthy subjects is relatively low. However, in the hospital environment, particularly in immune suppressed, debilitated and burns patients, the incidence of *P. aeruginosa* infection is high [6].

It produces many numbers of extracellular toxins, which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phospholipase enterotoxin, exotoxin and slim [1]. *P. aeruginosa* grows well on media and most strains elaborate the blue phenazine pigment pyocyanin and fluorescein (yellow), which together impart the characteristic blue-green coloration to agar cultures [5]. Pyocyanin is a blue redox-active secondary metabolite [7], which induces rapid apoptosis of human neutrophils, with a10 fold acceleration of constitutive neutrophil apoptosis in vitro but no apoptosis of epithelial cell or macrophages [8]. The redox active exotoxin pyocyanin is produced in the concentration up to 100mol/l during the infection of CF patients and other bronchiectatic airways. The contributions of pyocyanin during infection of bronchiectatic airways are not appreciated [9]. Notably pyocyanin mediated ROS inhibit catalase activity, deplete cellular antioxidant reduced glutathione and increased the oxidized reduced glutathione in the bronchiolar epithelial cell [10,11]. Excessive and continuous production of ROS and inhibit of antioxidant mechanisms overwhelm the antioxidant capacity, leading to tissue damage, also pyocyanin inhibit ciliary beating of the airway epithelial
Experimental infection

Swiss albino mice treated with multiple previously referenced isolates of \textit{P. aeruginosa} (highly pyocyanin producer isolates, fluorescein producer and non-pigmented isolates). Bacterial culture adjusted to 0.5 Mcfarland and each mice (5 in each group) challenged in traperitoneally with 1ml of bacterial suspension and mortality rate calculated for 5 days and in compared with control (injected only with normal saline).

Result and Discussion

Effect of pigmented \textit{P. aeruginosa} on the laboratory animals

The results of the present study showed that the Mortality occurred within 24h in group one (pyocyanin producer) by 100% of mortality rate and within 48h in group two (fluorescein producer strains) by 100% of mortality rate whereas mortality occurred in group three (non-pigmented strains) at the end of 96h post infection by 66.6% of mortality rate when all compared with control group (Inrapерitoneally saline injection). The present results are in agreement with Al-shamaa et al. [19] that elucidate pyocyanin in is the important virulence factor among many virulence factors of \textit{P. aeruginosa} which caused the death of injured rat within 24h. Where aspyoverdin treated rat death within 4h, pyocyanin also alter specific immune defenses and potentiates and per pretauates harmful inflammatory reactions in the infected cystic fibrosis. O’Malley et al. [20] also recorded that pyocyanin exhibits paradoxical pro-oxidant property. Azwitter ion that can easily penetrate biological membranes, pyocyanin can directly accept electrons from reducing agent such as NADPH and reduced glutathione, then transfer the electrons to oxygen to generate ROS such as peroxide and single oxygen, also in harmony with Finlayson et al. [21] who elucidate pigmented strains of \textit{P. aeruginosa} were highly virulence than non pigmented strains. Furthermore, virulence factor is produced in large ratio than non pigmented strain in which pigmented strains produce significant more (P<0.05) DNase, elastase, protease and siderophore. Pyocyanin is the highest virulence factor which altered the host immune response in several ways to aid evasion of immune system and establish chronic infection, evidence suggest that pyocyanin could prevent the development of an-effective T-cell response against \textit{P. aeruginosa} and prevent activation of monocyte and macrophage [22], also pyocyanin in neutrophils induce a sustained increase in ROS and subsequent decrease in intracellular Camp, which triggers the time and concentration dependent acceleration of apoptosis [8]. As confirm in studies using wild type and isogenic pyocyanin deficient mutant \textit{P. aeruginosa}, pigment dependent acceleration of neutrophil apoptosis and admonished release of chemokine might represent an immune suppression mechanism of the pathogen [23]. The fundamental ability of pyocyanin to alter the redox cycle and increase oxidative stress appear central to its divers detrimental effect on host cell, for example pyocyan in disrupt \textit{Ca}^{2+} homeostasis in human airway epithelial cells by oxidant-dependent increases in inositol trisphosphate and...
abnormal releases of Ca\(^{2+}\) from intracellular stores, because Ca\(^{2+}\) is important for regulating ion transport, secretion and ciliary beat. These alterations probably have important ramifications for \(P.\ aeruginosa\) lung infection [24].

![Figure 1: Mortality rate in pyocyanin and fluorescein and non-pigmented strains treating mice within different times.](image)

Also pyocyanin function as inhibitor of ATPase and this explains the pyocyanin toxicity including ciliary dysmotility, disruption of calcium homeostasis and dimished apical membrane localization of the cystic fibrosis trans-membrane conductance regulator (CFTR) [25]. Other potential toxic effects of pyocyanin include pretubrance of cellular respiration, epidermal death inhibition, prostacyclin release from lung endothelial cell and alter balance of protease-antiprotease activity in the cystic fibrosis lung [10,11]. The pro-oxidant effect of pyocyan in can thus augment such innate immune response circuits, for example, pyocyanin increases the release of the neutrophil chemokine (IL-8) from lung epithelial cells and up regulates the expression of the neutrophil receptor intracellular adhesion molecule (ICAM-1) [26,27]. In spite of all above toxic effects of pyocyanin, pyocyanin producer strains show highly virulence because pyocyanin act as a signaling molecule for quorum sensing regulation, which is regulated virulence factor expression [10], in spite of also pyoverdin (PVD) importance virulence factor which is function as a powerful iron chelators solubilizing and transporting iron through the bacterial membrane via specific receptor process before it reaches its targets Oberhardt [29]. Elucidate that PVD is essential element in vivo iron gathering and virulence expression in \(P.\ aeruginosa\) who found that PVD deficient mutants demonstrated no virulence when injected into burned mice [27-32] (Figure 1).

References


Evaluation of the Killing Virulence of Pigmented and Non-Pigmented Clinical Isolates of Pseudomonas aeruginosa, on production of reactive nitrogen intermediates by murine alveolar macrophages. Infect Immun 60(9): 3913-3915.


