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

Pambuk CA¹*, Husein Al-Jubury SA² and Kamal MA²

¹College of Dentistry, Tikrit University, Iraq
²Biology Department, Tikrit University, Iraq

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*Corresponding author: Chateen I Ali Pambuk, College of Dentistry, Tikrit University, Iraq, Tel: 009647701808805, Email: dr.chatin2@yahoo.com

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 external (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 phanazine 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 a 10 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 bronchiecatic airways. The contributions of pyocyanin during infection of bronchietatic 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...
Evaluation of the Killing Virulence of Pigmented and Non-Pigmented Clinical Isolates of *P. aeruginosa* in Mice. Development of General Gompertz Models and Their Simplified Two-Parameter Forms Based on Specific Membrane Alterations in Porin Protein Channel Represent a Component of the Pathogen’s Virulence

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**Materials and Methods**

**Bacterial isolates**

Three reference isolates isolated previously from otitis media and otitis externa (*pyocyanin* highly producer, fluorescein highly producer, non-pigmented strain) were chosen to be inoculated in mice. All strains passed in mice to retain their virulence. Stock cultures were maintained at 70°C in brain heart infusion broth containing 5% glycerol.

**Laboratory animals**

Swiss albino male mice were purchased from the institute of biological and pharmaceutical research laboratory, Baghdad, aged 4-8 weeks, and a weight of 22-30g. These mice were housed at the College of Science, Tikrit University. All mice were kept at 22-25°C in plastic cages and fed pellets and water every day.

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**Experimental infection**

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

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**Result and Discussion**

**Effect of pigmented *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%). It was also found that pyocyanin deficient mutant *P. aeruginosa* caused the death of the infected rat within 24h. Whereas aspyoverdin treated rat death within 4h, pyocyanin also alter specific immune defenses and potentiates and pre-treats the disordered inflammatory reactions in the infected cystic fibrosis. O’Malley et al. [20] also reported 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 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 *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. OMalley et al. [20] also recorded that pyocyanin exhibits paradoxical pro-oxidant property.

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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 ramification for *P. aeruginosa* lung infection [24].

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 preturbance of cellular respiration, epidermal growth 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 pyocyanin 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).

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