Characterizing the Phenotypic Transition of *Pseudomonas aeruginosa* from the Hospital Environment to Nosocomial Infections

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**Abstract**

Combating nosocomial infections is a significant challenge facing modern medicine. In the Intensive Care Units (ICUs), pathogens, such as *Pseudomonas aeruginosa* (PA), thrive. However, the requirements for transitioning from environment to patient have been understudied. We isolated PA from both hospital sinks and infections and characterized the phenotypes of the isolates to determine which virulence factors promote pathogenesis. To study this phenomenon, we compared biofilm formation, quorum sensing activity, pyocyanin production, hemolysis, and protease activity of selected strains isolated from the Medical ICU produced higher levels of pyocyanin compared to isolates from the Burn ICU. Considering the documented ability of pyocyanin to damage host tissues, we theorized that increased pyocyanin production is beneficial for invading the more intact tissues of MICU patients but is less necessary for the rapid spread through the dead and damaged tissues of BICU patients. Through examination of the differential virulence of environmental and patient isolates, we found that biofilm biomass was higher for patient isolates than for environmental isolates. Similarly, both total quorum sensing and beta hemolysis of red blood cells were elevated in patient isolates. The most dramatic result was the remarkably higher protease activity among patient isolates compared with environmental isolates. Having determined the protease production of all isolates in vitro, we selected strains with the highest and lowest activity, from both patients and the environment, for comparison in vivo. In our murine chronic wound model, environmental isolates with high protease activity were more capable of establishing a wound infection and causing sepsis. Overall, we conclude that certain virulence factors significantly influence the ability of PA to cause infection. This knowledge may allow us to better predict and react to episodic outbreaks of PA in the hospital.

**Materials and Methods**

**Collection and isolation of *Pseudomonas aeruginosa* strains**

- PA isolates from 58 BICU, 20 MICU, 2 SICU, and 3 ET Patients were collected from wounds with PA and confirmed via PCR.
- Environmental PA isolates (31 MICU, 17 BICU, 17 SICU) were collected from sink drains via swabbing, plating on PA, and PCR.
- Patient samples were collected in accordance with IRB IR 119-117 and L19-053.

**Pyocyanin assay**

- OD adjusted cultures centrifuged to pellet the cells
- Chloroform layer was extracted with HCl
- The absorbance of extracted layer read at 530nm
- Pink layer containing pyocyanin toxins was removed

**Biofilm formation assay**

- 250ul of subculture was added to M9EC plate
- The M9EC plate incubated for 24hrs at 37ºC inside a humid chamber with shaking
- Biofilms stained with crystal violet and washed with 95% ethanol
- Absorbance of CV read at 590nm

**Pyocyanin production is higher in MICU patient PA isolates compared to BICU patient PA isolates**

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<th>MICU Unit</th>
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**Biofilm formation is higher in MICU patient PA isolates than environmental PA isolates**

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**Results**

**Quorum Sensing**

**AHL Extractions**

- Supplemented, frozen, and thawed with 50% acetic acid
- The organic phase was combined and dried with a centrifugal evaporator

**Bioreporter**

- QS bioreporter culture was pipetted into the wells
- Added AHL extracts dissolved in methanol to the wells containing bioreporters

**Blood Agar Hemolysis**

- Optical density adjusted and added to blood agar plates
- Incubate plates overnight at 37ºC for 72hrs

**Murine Chronic Wound Model**

- Wound covered with an adhesive OpSite® bandage and infected with an injection of an environmental or clinical strain of *P. aeruginosa*
- Wound tissue and spleens were surgically extracted and placed in a 2mL tube filled with 900µL of PBS
- The wound tissue and spleens were homogenized in PBS, serially diluted, and spot plated. CFUs were counted.

**Conclusions**

- Pyocyanin production is notably higher in MICU patient isolates compared to BICU patient isolates, suggesting that pyocyanin is important for the infection of intact skin and less necessary for spreading through the dead and damaged tissue of severe burn patients.
- Total quorum sensing is higher for patient isolates than environmental isolates. As QS controls virulence factor production, this result implies that patient isolates are overall more virulent than environmental isolates.
- Beta-hemolysis is higher for patient isolates, suggesting that hemolysis is beneficial for PA metabolism in hosts.
- Environmental isolates with high protease production are more pathogenic in a mouse wound infection model. Isolates with high protease production are more likely to establish a serious infection and cause sepsis, suggesting that protease production could be a vital first step in transitioning from an environmental strain to a successful human pathogenic strain.

**Acknowledgements**

- We would like to thank the Honors College Undergraduate Research Scholars Program, supported by the CT and Hellen Jones Foundations.
- We want to thank to the Texas Tech Health Sciences Clinical Research Institute for the patient *P. aeruginosa* sample and data collection.
- We would like to thank CSER Student Service Organization for their support.

**Future Investigations**

- Investigate the presence of other significant virulence factors produced by *P. aeruginosa*, including rhamnomolins and siderophores.
- Explore the relationship between virulence factor production in patient PA isolates from specific infection types.
- Further investigation of virulence factors in the murine chronic wound model to determine which phenotypic characteristics allow for a successful transition from survival in the hospital environment to infection of a human host.

**Hypothesis**

High virulence factor production correlates with infective potential of hospital environmental *P. aeruginosa*.

**Virulence Factors**

- Zwittertins that can pass through cellular membranes
- Inhibits cellular respiration, epithelial cell growth, and protease release
- Induces apoptosis of neutrophils
- Enzymes that form pores in cell membranes
- Elastase B & elastase C
- Invades host's cells and immune evasion
- Hemolysis
- Bactericidal activity
- Nuclease activity
- Inhibits oxidative burst
- Activates elastase
- Virulence factors

**Protease Assays**

**General Protease**

- Supernatant was removed and transferred to a tube
- The tubes were incubated in a rotator at 37°C for 15min

**Elastin Congo Red**

- Supernatant was removed and transferred to Congo Red and sodium phosphate buffer
- The tubes were incubated in a rotator at 37°C for 14hrs

**Elastase Activity**

- OD adjusted cultures centrifuged to pellet the cells
- The supernatant was removed, filtered, and centrifuged
- OD adjusted cultures centrifuged to pellet the cells
- OD adjusted cultures centrifuged to pellet the cells

**Murine Chronic Wound Model**

- Mouse anesthetized and dorsal surface shaved and administered a full-thickness surgical wound
- After 48 hrs, a biofilm-associated infection is visible in the wound-bed
- After 72 hrs, the mice were euthanized. The wound tissue and spleens were surgically extracted and placed in a 2mL tube filled with 900µL of PBS
- The wound tissue and spleens were homogenized in PBS, serially diluted, and spot plated. CFUs were counted.

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