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

Rebecca Schneider[1], Derek Fleming[2,3], Garrett Welch[2,3], Hui Hua[2,3], Angel Cueva[2,3], Lauren Choate[2], and Kendra Rumbaugh[2,3]

Texas Tech University, Lubbock, TX[1]

Department of Surgery[2], Burn Center for Research Excellence[3] , Texas Tech University Health Sciences Center, Lubbock, TX

Abstract

Combatting 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. Analysis by ICU type revealed that 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 greatly influence the ability of PA to cause infection. This knowledge may allow us to better predict and react to episodes of PA outbreak in the hospital.

P. aeruginosa in Nosocomial Infections:

P. aeruginosa, an opportunistic gram-negative bacteria, is one of the leading causative agents of hospital-acquired infections. Patients in the Intensive Care Units – particularly individuals with ventilators, medical implants, or extensive burns and surgical wounds – are susceptible to critical *P. aeruginosa* infections.

Virulence Factors

Pyocyanin	Hemolysis	Proteases
▪ Zwitterion toxin that can pass through cellular membranes ▪ Inhibits cellular respiration, epidermal cell growth, & prostacyclin release ▪ Induces apoptosis of neutrophils	▪ Enzymes that form pores in cell membranes ▪ Invasion of host's cells and immune evasion ▪ Allows for iron acquisition which is important for PA metabolism	• Elastase B: targets elastin • Alkaline protease: degrades host proteins including fibronectin and laminin • Protease IV: tissue invasion and vascular damage • MucD: interferes with functions of chemokines, cytokines, and neutrophils

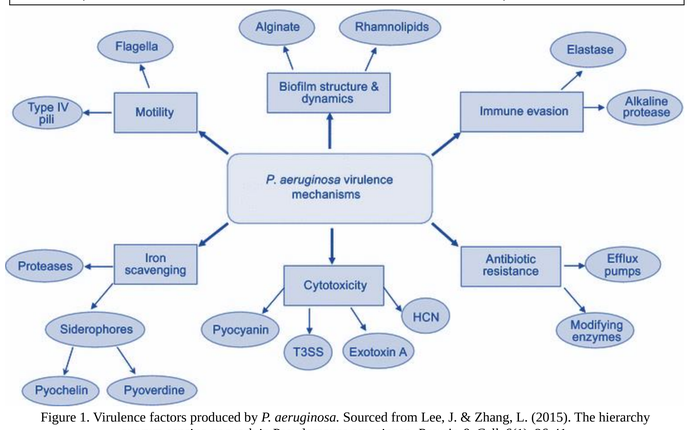


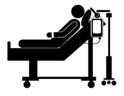
Figure 1. Virulence factors produced by *P. aeruginosa.* Sourced from Lee, J. & Zhang, L. (2015). The hierarchy quorum sensing network in Pseudomonas aeruginosa. *Protein & Cell*, 6(1), 26-41.

Hypothesis

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

Environmental Strains of PA in Hospital Sinks

Virulence Factors



PA Nosocomial Infections

Acknowledgements

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❖ Thanks to the Texas Tech Health Sciences Clinical Research Institute for patient *P. aeruginosa* sample and data collection
❖ We would like to thank CISER Student Service Organization for their support

Materials and Methods

Collection and Isolation of *Pseudomonas aeruginosa* strains

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

Pyocyanin Assay

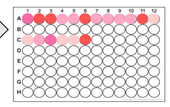
OD adjusted cultures centrifuged to pellet the cells

The supernatants were removed, filter sterilized, and extracted with chloroform

Chloroform layer was extracted with HCl

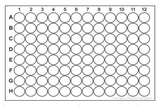
Pink layer containing pyocyanin toxins was removed

The absorbance of extracted layer read at 530nm



Biofilm Formation Assay

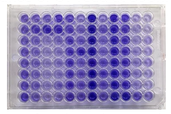
150µL of subculture was added to MBEC plate



The MBEC 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



OD_{600} of 24-hour bacterial growth in wells

Quorum Sensing

AHL Extractions

OD adjusted cultures were centrifuged to pellet the cells

Supernatants removed, filter sterilized, and extracted with of acidified ethyl acetate

The organic layer was collected

The organic phases were combined and dried with a centrifugal evaporator

Extractions stored at -20ºC

Bioreporter

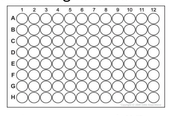
Overnight cultures of bioreporters

AHL extractions were dissolved in methanol (HPLC grade)

QS bioreporter culture was pipetted into the wells

Added AHL extracts dissolved in methanol to the wells containing bioreporters

The well plate was incubated with shaking for 3hrs

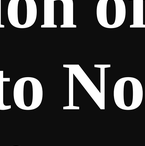


Luminescence and OD_{600}

Blood Agar Hemolysis

5mL subcultures were inoculated

Optical density adjusted and added to blood agar plates



Incubate plates upright at 37ºC for 72hrs

Evaluate plates for alpha (partial), beta (complete) & gamma (none) hemolysis

Protease Assays

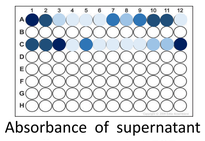
General Protease

OD adjusted cultures were centrifuged to pellet the cells

Supernatant was removed and transferred to Hide Powder Blue and sodium phosphate buffer

The tubes were incubated in a rotator at 37ºC for 15min

The tubes were centrifuged, and the supernatant was transferred to well plate



Absorbance of supernatant in wells measured at 595nm

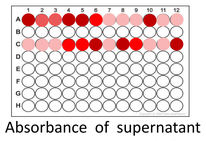
Elastin Congo Red

OD adjusted cultures were centrifuged to pellet the cells

Supernatant was removed and transferred to Congo Red and sodium phosphate buffer

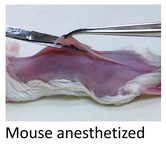
The tubes were incubated in a rotator at 37ºC for 14 hours

The tubes were centrifuged, and the supernatant was transferred to well plate

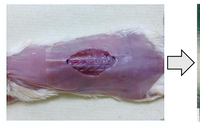


Absorbance of supernatant in wells measured at 400nm

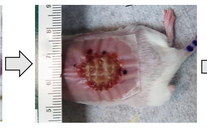
Murine Chronic Wound Model



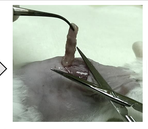
Mouse anesthetized and its Dorsal surface shaved and administered a full-thickness surgical wound



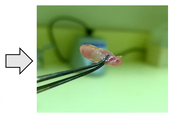
Wound covered with an adhesive OpSite® bandage and infected with an injection of an environmental or clinical strain of *P. aeruginosa*



After 48 hours, a biofilm-associated infection is visible in the wound-bed.



After 72 hours, 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.

Future Investigations

❖ **Investigate the presence of other significant virulence factors produced by *P. aeruginosa*, including rhamnolipids 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**

Results

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

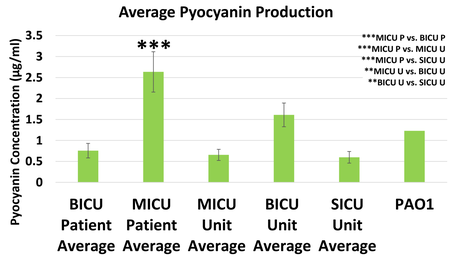


Figure 2. Comparison of average pyocyanin production (µg/mL) between patient PA isolates, environmental (unit) PA isolates, and PAO1 (reference strain).

Biofilm formation is higher in MICU patient PA isolates than environmental PA isolates

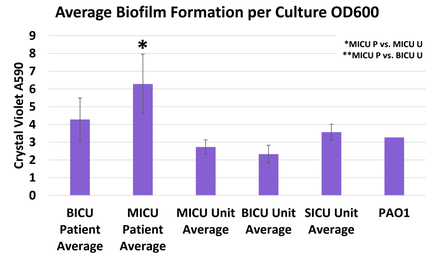


Figure 3. Comparison of average biofilm formation standardized by OD600 between patient PA isolates, environmental (unit) PA isolates, and PAO1.

Total Quorum Sensing is higher for patient PA than environmental PA

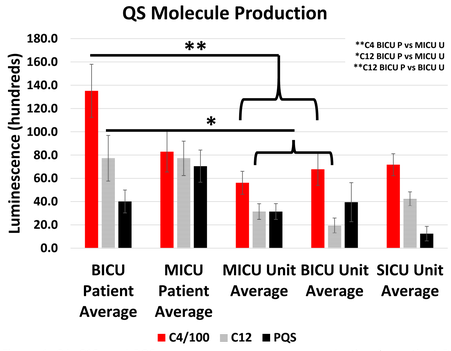


Figure 4. C4, C12, and PQS quorum sensing molecule production for patient PA isolates and environmental (unit) PA isolates.

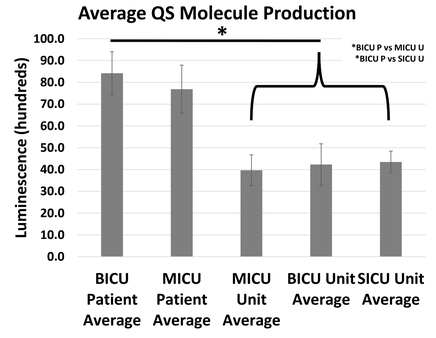


Figure 5. Average of C4, C12, and PQS quorum sensing molecule production for patient PA isolates and environmental (unit) isolates.

Beta Hemolysis is higher for patient PA than environmental PA

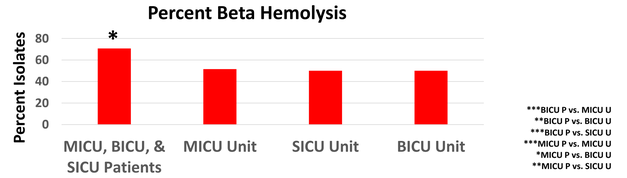


Figure 6. Beta hemolysis compared by percent of isolates for patient PA isolates and environmental (unit) PA isolates.

Protease activity is remarkably higher for BICU & MICU patient PA than environmental PA

High-protease environmental PA have higher wound CFU/g than low-protease environmental PA

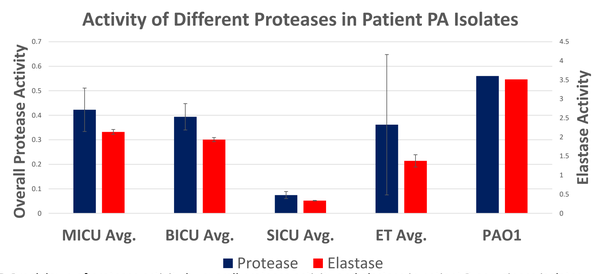


Figure 7. Breakdown of protease activity by overall protease activity and elastase in patient P. aeruginosa isolates.

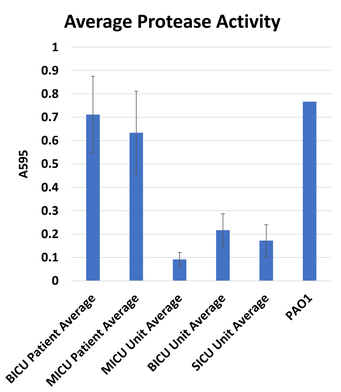
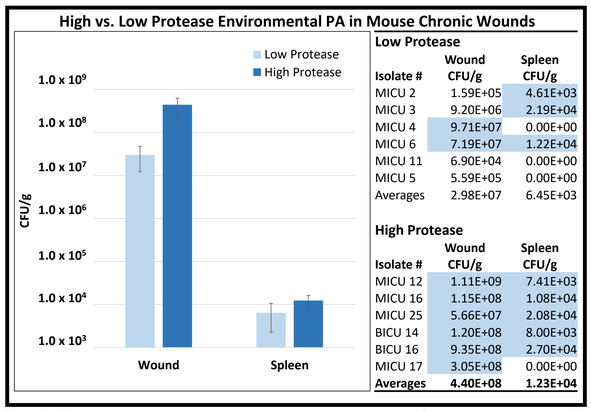


Figure 8. Average protease activity for patient PA isolates, environmental (unit) PA isolates, and PAO1.



Low Protease	Wound	Spleen
Isolate #	CFU/g	CFU/g
MICU 2	1.59E+05	4.61E+03
MICU 3	9.20E+06	2.19E+04
MICU 4	9.71E+07	0.00E+00
MICU 6	7.19E+07	1.22E+04
MICU 11	6.90E+04	0.00E+00
MICU 5	5.59E+05	0.00E+00
Averages	2.98E+07	6.45E+03

High Protease	Wound	Spleen
Isolate #	CFU/g	CFU/g
MICU 12	1.11E+09	7.41E+03
MICU 16	1.15E+08	1.08E+04
MICU 25	5.66E+07	2.08E+04
BICU 14	1.20E+08	8.00E+03
BICU 16	9.35E+08	2.70E+04
MICU 17	3.05E+08	0.00E+00
Averages	4.40E+08	1.23E+04

Figure 9. CFU/g for wound and spleen homogenates for high-protease envi. PA and low-protease envi. PA.

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 to cause sepsis, suggesting that protease production could be a vital first step in transitioning from an environmental strain to a successful human pathogenic strain.**