ABSTRACT

OXYGEN, NUTRIENTS, AND ANTIBOTICS: HOW THE CHRONIC INFECTION ENVIRONMENT SHAPES THE EVOLUTION OF *PSEUDOMONAS AERUGINOSA*

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Marquette University, 2026

Pseudomonas aeruginosa is one of the most concerning opportunistic pathogens and a common cause of nosocomial and chronic infections, where it establishes biofilm communities within oxygen-depleted, nutrient-rich microenvironments. Despite the clinical relevance of these anoxic niches, susceptibility testing and experimental studies are routinely conducted under oxic conditions that fail to reflect the metabolic reality of infection. This dissertation uses experimental evolution to examine how anaerobic metabolism, lifestyle, and nutrient environment shape *P. aeruginosa* adaptation.

Chapter 2 investigated how arginine, abundant in the host environment, and growth mode together shape evolutionary outcomes. Populations were evolved across five conditions varying oxygen availability, growth mode, and carbon source. Adaptation to arginine consistently selected for competitive fitness gains and increased biofilm formation. Whole-population genome sequencing identified parallel mutations in *fleQ* across all oxic arginine replicates, providing a genomic basis for convergent motility loss and biofilm increase. Twitching motility loss was most pronounced under the combination of arginine use and biofilm growth. A trade-off in anoxic biofilm capacity emerged and recovered only when selection acted simultaneously on anaerobic metabolism and biofilm lifestyle. This work highlights arginine as a clinically relevant selective pressure contributing to the persistence of chronic *P. aeruginosa* infection.

Chapter 3 examined how oxygen availability shapes antibiotic resistance evolution across eight conditions varying oxygen level, lifestyle, and tobramycin exposure. Subinhibitory antibiotic exposure alone drove resistance to clinically relevant levels. Anoxic populations consistently reached higher minimum inhibitory concentrations than oxic populations, reinforcing the concern that standard susceptibility testing underestimates resistance in oxygen-limited infections. Resistance mutations were condition-dependent: *amgS* dominated during oxic evolution, while *fusA1* and *ptsP* appeared across all antibiotic-exposed conditions. Mutations in *mexT*, connecting antibiotic resistance, virulence, and quorum sensing, were broadly selected under both tobramycin and anoxic exposure. Anoxic evolution also produced increased biofilm formation, type IV pilus gene mutations, reduced twitching motility, and high competitive fitness — phenotypes consistent with chronic infection adaptation.

Together, these studies demonstrate that oxygen availability, nutrient environment, and growth mode collectively shape *P. aeruginosa* evolution in ways that standard laboratory conditions fail to capture, highlighting the need for resistance research that reflects the environment where infections occur.