Pseudomonas aeruginosa diversification at early infection stages in cystic fibrosis lungs

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Cystic fibrosis (CF) is an autosomal recessive lung disease caused by a defect in the cystic fibrosis conductance regulator (CFTR) gene. The CFTR protein lack causes a defective chloride secretion creating an osmotic gradient that, consequently, provokes water hyper-reabsorption and abnormal thick and sticky sputum. The accumulated sputum is rich in nutrients being, thus, a good environment for microbial colonization. CF lungs are infected with a complex microbial flora, provoking acute and chronic infections that result in decline of the lung function and premature death of patients. The lungs colonization by *P. aeruginosa* in younger patients is less frequent however is directly associated with rapid lack of lung function and reduced chances of survival. While *P. aeruginosa* diversity and mechanisms of adaptation and evolution have been intensively studied at chronic stages, it is less clear the mechanisms used by *P. aeruginosa* to establish an infection in CF lungs and unclear the existence of bacterial diversification and its impact in infection establishment and progress.

This study aimed to investigate whether phenotypic diversity is present at early stages of CF infection and how it impacts in microevolution to chronic stages. Moreover, it was aimed to determine the role of early antibiotic treatments as a driven and selective force in *P. aeruginosa* populations towards diversification.

Three strains of P. *aeruginosa* were cultured in artificial sputum medium with and without sub-inhibitory concentrations of ciprofloxacin (CIP) for ten days. Afterwards, the diversity of the bacterial populations was assessed along time in terms of colony morphology. Each morphotype detected was further characterised regarding 6 virulence-associated traits and sensitivity to 10 clinical relevant antibiotics.

Results demonstrated the existence of population diversity at early stages of infection with and without antibiotic exposure. According the level of diversity, *P. aeruginosa* populations reacted differently to CIP concentrations. More diverse populations were able to resist to increased CIP concentrations in contrast with less diverse populations. Moreover, CIP treatments changed the population diversity and dynamics. CIP exposure favoured the emergence of mucoidy morphotypes (moist and mucoid variants) and small colony variants. The phenotypic diversity presents within *P. aeruginosa* populations was analyzed more in depth by isolating each colony morphology variant and measuring its antibiotic sensitivity, pyocyanin and hemolysin production, motility, auxotrophy and biofilm formation ability. The bacterial characteristics among CF early isolates significantly vary, however there is a trend towards high virulence potential. The examination of the virulence traits exhibited by colony morphotypes demonstrated that: bacteria had still limited ability to form biofilms, typical of early infection stages; hemolysin and pyocyanin production was variable during early infection; and bacteria still exhibited their ability to swim, swarm and twitch. Concerning antibiotic sensitivity, the majority of the colony variants that composed the bacterial populations exhibited sensitivity to antibiotics. Furthermore, it was observed that morphotypes have a degree of individuality, i.e., each morphotype gather particular combinations of characteristics no repeated in the population by other morphotype. According to the ecological model “insurance hypothesis”, this diversity ensures population to maintain or enhance their functioning against environmental fluctuations, typically antibiotic exposure, host immune defences, oxygen depletion and pH alterations.

In conclusion, according to these data *P. aeruginosa* diversification may exist in early in vivo CF infections. The functional importance and role within population of bacteria-associated colony morphology remains quite unexplored, however the findings of this study suggest that the level of bacterial diversification of populations at early stages can be an important indicator of the infection course and severity in CF airways.

Keywords: *Pseudomonas aeruginosa*; cystic fibrosis; clonal diversification, phenotypic variation

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