The role of antimicrobial stress on *Pseudomonas aeruginosa* colony morphology diversity, tolerance and virulence

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In natural environments, as well as in infections, bacteria faced several stresses like starvation, heat exposure, antimicrobials and host defense after entry in human body. The ability to quickly adapt to a new environment is critical to bacteria and the underlying mechanisms are not fully understood. One of the strategies adopted by bacteria is a high frequency of phenotype switching by a mechanism called phase variation. A sign of these bacterial changes is the altered colony morphology on solid media. Several colony morphologies have been isolated from clinical strains, being the best-studied the small colony variants, the rugose small colony variants and the mucoid phenotype.

It was aimed to study the prevalence and diversity of colony morphologies from planktonic and sessile *P. aeruginosa* (Pa) ATCC, chemically stressed, and to compare with the ones developed by a *P. aeruginosa* isolated from a medical device (Pa I). Pa is one of the most important opportunistic pathogen commonly found in clinical arena being often responsible for acute and chronic infections.

Planktonic and sessile Pa and Pa I were *in vitro* stressed by continuous exposure to benzalkonium chloride (BZK) and peroxide hydrogen and by attack with the same products. The stressed bacteria were collected, serial diluted and plated onto TSA to inspect colony morphology variants. Each predominant bacterial morphology was harvested and reserved for further phenotypic and motility characterization.

The results demonstrated that cells coming from biofilm and planktonic growth of Pa, regardless they were stressed or not or the type of stress implemented, develop colonies mostly with the same morphotype, type II, characterized by big and regular colony circumference, with small and dark center and wrinkled surface. This colony type showed to have a good ability to form biofilms, although the colonies from the stressed cultures developed biofilms with higher biomass accumulated.

The Pa I gave rise to high diversity of colony morphotypes, being 3 of them more prevalent and cataloged as type XVII, XXIII, XXVIII. The types XVII and XXVIII are characterized by regular colony circumferences with craters in the center. However their superficial area presented different colors. Type XXIII has irregular colony shape with craters in the center. These 3 morphotypes showed similar biofilm formation ability between them but lower than type II.

Nonetheless the phenotypic differences found between the several morphotypes, all of them generated biofilms with identical tolerance to antimicrobials (BZK and the fluoroquinolone antibiotic ciprofloxacin-CIP). However, the cells resulting from the planktonic growth of Pa I morphotypes demonstrated two-fold tolerance to BZK and CIP than their Pa counterparts.

Regarding bacteria motility, results highlighted that all Pa I morphotypes had impaired swimming motilities compared to type II. This result seems to indicate that the capacity of adhesion or invasion of Pa I morphotypes to, respectively, surfaces or tissues was compromised, which may interfere with their virulence. Although, the latter is not sustained by the susceptibility patterns, emphasizing the ambiguous relationship between virulence and antimicrobial resistance.

The morphologies described are not similar with previous reports and the colony morphologies more prevalent seemed to be less virulent than typical ones. Among the various colony morphologies detected, no Pa I morphotype match with Pa type. So, it can be concluded that phase variation is an adaptive strategy of bacteria to respond to fluctuating environment leading to mixed populations where the chances for survival is higher. The generation of varied bacterial phenotypes may be the sum of previous and successive adaptations suffer by Pa I as an attempt to adjust to adverse habitats.

Keywords: colony morphotypes, biofilm tolerance, antimicrobial stress, virulence, motility

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