

OPINION

By: Assoc.Prof. Stoyanka Stoitsova, PhD,

Member of the Scientific Jury (Order I-163/26.11.2024, issued by the Director of the Stephan Angeloff Institute of Microbiology), for evaluation of:

PhD Thesis of Dayana Borislavova Borisova

Title: "**Comparative investigations on *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients prior- and post- inhalatory tobramycin therapy**"

Professional field 4.3. Biological Sciences (Microbiology)

Supervisors: Assoc. Prof. Stoyanka Stoitsova, PhD, and Professor Tanya Strateva, PhD, DSc

Scientific relevance

The globally increasing problems related with the treatment and control of bacterial infections put forward numerous questions. Together with the clarification of the genetically determined antibiotic resistance, a recent focus was drawn by the phenomenon of drug tolerance. The latter combines sets of phenotypic adaptations that allow the bacteria, even if they are drug susceptible, to survive the treatment of the patients with antibiotics and become a source of chronic and persistent infections. Such sets of behavioral patterns are typical for *P. aeruginosa* strains causing infections of the patients with cystic fibrosis (CF). During the routine control procedures of these patients, bacterial strains from sequential phases of the disease can be collected. The studies of such sequential isolates from the same patient have lately provided novel data on the within-host evolution of the bacteria. Together with the specific microenvironment of the CF lungs and the patient's mechanisms of immune defense, antibiotic treatment is another pivotal selection factor driving the in-host evolution of the bacteria. In spite of this, the studies directed to the impact of antibiotics on the in-host changes of the bacteria are sparse. This determines the importance of the PhD research of Dayana Borisova, focused to the in host changes of *P. aeruginosa* driven by the inhalatory therapy of their hosts with tobramycin. The study includes 6 pairs of strains, each pair of strains being isolated from the same patient prior- and post-treatment. The strains were isolated by prof. T. Strateva, supervisor to the PhD student.

The Thesis and associated materials

The materials for the procedure are in accordance with the requirements of the respective rules and regulations for the acquisition of PhD. The thesis comprises 148 pages . The references review is based on 240 sources, 30% of which from the last 5 years. The thesis is illustrated by 48 figures and 15 tables. The results comprise 3 sections: (1) Molecular epidemiological part that shows clonal relatedness of 5 of the pre-treatment strains, and genetic diversification within the group of the post-tobramycin treatment isolates. (2) The phenotypic comparison between the strains comprises characteristics that have been considered in previous studies as adaptive for isolates from chronic infections. The analysis of growth parameters shows a trend for decrease of the growth rate and increase of the doubling time and the lag-phase of the post-treatment isolates when compared to their pre-treatment counterparts. This trend is more explicit when the cultivation is performed in minimal salt medium as well as for the strain pairs isolated from patients of higher age and, expectedly, more advanced stage of the infection. All strains are biofilm-forming, motile, and capable of invasion and intracellular reproduction in eukaryotic cells, however these characters do not follow a pattern related with the stage of isolation. (3) The phenotypic response (in growth, biofilm formation, bacterial viability, and motility) of the isolates when cultivated in the presence of sub-lethal amounts (1/4 or 1/2 MIC) of tobramycin. These results further characterise the phenotypic shift, and confirm a better fitness, resp. higher tolerance, of the post-treatment isolates in the presence of the antibiotic.

The results justify 12 conclusions, and the original contributions of the study are summarized as 4 items. Being one of the supervisors, I confirm that all the experimental results have been obtained by the PhD student herself.

Conclusion

The PhD thesis of Dayana Borisova summarizes results related with the adaptation of the opportunistic pathogen *P. aeruginosa* to chronic colonization within a peculiar microhabitat, the CF lung. The comparative study of strains isolated pre- and post-treatment with inhalatory tobramycin aims the collection of data on the adaptive traits of the bacteria for survival under the action of the antibiotic. This is an original approach to the topic, for which the contribution of the supervisor, Prof. T. Strateva should be acknowledged. During her PhD studies, Dayana Borisova learned and masters a variety of methodologies, and gained

experience in working with scientific literature. The PhD thesis, and all materials concerning both the research, and the educational programs of the PhD student, cover completely the requirements of the Law for the Development of Academics, and the Regulations for its application in the Republic of Bulgaria, BAS, and The Stephan Angeloff Institute of Microbiology. In support of this, I would like to recommend to the Scientific jury to vote positively for awarding the scientific and educational degree "PhD" to Diana Borislavova Borisova in the professional field 4.3. Biological Sciences.

03.02.2025 г.

Reviewer:

Sofia

/S. Stoitsova, PhD/