Evaluation of photoinactivation potential in the eradication of *Streptococcus agalactiae* carrier-state in the urogenital system: *in vitro* and *in vivo* studies

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According to the latest report of the European Medicines Agency (EMEA) and the European Centre for Disease Prevention and Control (ECDC), the introduction of antibiotics for routine treatment has led to revolutionary changes in the field of bacterial infections. Currently, the emerging and growing drug resistance among microorganisms is a huge threat to the health and lives of patients both in Europe and around the world. According to the EMEA/ECDC report work on the development and creation of alternative therapeutic options leading to a reduction in the use of antibiotics and a reduction in the rate of emerging drug resistance should form the fundamental direction of research. Prevention of perinatal infections caused by Group B Streptococcus (GBS) is based primarily on the use of intravenous beta-lactam antibiotics, which are not without effect on the human microbiota and induce the formation of drug-resistant strains of bacteria. Beta-lactam antibiotics, e.g. penicillin or cephalosporins, are an important group of antimicrobial drugs and the search for alternative therapeutic approaches leading to the reduction of their consumption and a slowdown in the acquisition of drug resistance should be a priority for direction of research.

Therefore, this project focuses on the following objectives:

- evaluation of the bactericidal activity of blue light treatment (405 nm) and photodynamic inactivation using photosensitizer compounds against different serotypes of *Streptococcus agalactiae* (both for planktonic and biofilm cultures);
- determination of the probability of the acquisition of resistance mechanisms for photodynamic inactivation after multiple phototreatment of *S. agalactiae* with lethal and sub-lethal doses of photoinactivation;
- evaluation of photo- and cytotoxicity of the used photosensitizing agents against human dermal fibroblasts;
- assessment of the mutagenicity of phototreatment in a prokaryotic (Ames test, rec assay) and a eukaryotic (comet assay) model;
- evaluation of the potential of photoinactivation in the decolonization of *S. agalactiae* in mouse vagina;
- assessment of the photoinactivation impact on the cultivable part of the normal flora of the mouse vagina.

In relation to one of the major human pathogens responsible for perinatal infections of newborns and infants, ie. *Streptococcus agalactiae* we will analyze Photodynamic Inactivation (PDI) using photosensitizing compounds (called Photosensitizers, PS) belonging to different chemical groups and blue light treatment using visible light (405 nm) without the use of exogenous photosensitizers. Preliminary studies by our team show that the proposed approach could lead to an effective eradication of *S. agalactiae* growing in planktonic and biofilm cultures. These antibacterial approaches will also be investigated against bacteria representing the natural vaginal flora, e.g. *Lactobacillus acidophilus* in order to determine a therapeutic window for which the effective eradication of *S. agalactiae* is possible without a substantial reduction in the viability of the bacteria belonging to the normal flora. In addition, in order to verify the obtained *in vitro* results, this project will assess the potential of photoinactivation in the eradication of *S. agalactiae in vivo* using a mouse model of vaginal colonization. The *in vivo* tests will show the impact of phototreatment on the cultivable part of the normal flora of the vagina. In addition, histopathological examination will evaluate the effect of photodynamic inactivation on the cell and tissue structure lining the vagina. We believe that this approach will allow us to evaluate the potential of photoinactivation in the eradication of *S. agalactiae* and confirm the safety of the proposed approach in relation to the cells and tissues of the host. In addition, using multiple treatment of *S. agalactiae* with lethal and sub-lethal doses of photoinactivation, we will evaluate the probability of the acquisition of the resistance mechanisms to phototreatment.