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# THE FORMATION OF BIOFILMS BY *PSEUDOMONAS AERUGINOSA* STRAINS, AND METHODS OF ITS CONTROL (REVIEW)

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The end of the twentieth century was marked by the introduction of specific form of human microflora existence as a well-organized association of microorganisms which cover surfaces of mucosa, skin, and human teeth [1]. By some estimate there are about $10^{14}$ microorganisms organized in such groups in human body. The effective form of microorganisms' existence is their attachment to solid surface compared to free way of life. Microorganisms combined on solid surface are united in so called biofilms balanced according to species composition and functions of association members. In nature biofilms are spread everywhere, and are formed under different conditions [2, 3].

The majority of natural bacterial populations exist in the form of biofilms, highly-structured multicellular communities incorporated in extracellular polymer matrix of own production. For a considerable part of clinically significant species this form of existence provides optimal conditions for reaching of pathogenic and colonizational potential, and also encourages saving of metabolically inactive part of population which is characterized by a low level of sensitivity to antibiotics impact. The latter plays a significant role in formation of chronic persistent infections resistant to antibiotics treatment [4].

The formation of parasitic association is caused by unreasonable use of vaccines, immune serums, gamma globulins, antibiotics, bacteriocins, and phages. Mechanisms of bacteria resistance to antibiotics in biofilms associated with reduced penetration of antibiotics through biofilms, limited nutrition and change of biofilm microenvironment lead to reduction of bacteria mitosis speed resulting in fewer targets for antibiotic action [1]. Bacteria in biofilm interact with one another through chemical constriction. In biofilm compared to pure cultures physiological processes including production of metabolites and biologically active substances happen differently. Microorganisms involved in biofilm composition have a kind of another phenotype which is manifested in change of growth parameters and specific genes production. Members of microbial group are united basing on the principle which excludes antagonism, determines their nutritional (trophic), energetic and another connections between them and environment. Such a connection of microorganisms community behavior received a special definition of "quorum sensing" [5]. Quorum sensing is an ability of some bacteria (probably, also another microorganisms) to communicate and coordinate their behavior through secretion of substances which are the signals for coordination of certain behavior or interaction between bacteria of the same type or subtype depending on their growth tightness.

When the concentration of excreted signaling agents reaches a borderline value the group of bacteria starts working as a single organism. Furthermore, signaling agents for Gram-negative and Gram-positive microorganisms differ [6]. For instance, for *Pseudomonas aeruginosa* microorganism a signaling molecule of quorum sensing is acyl homoserine lactone, and biofilm created by this pathogen produces pyocyanin which functions as an electronic transporter in current generation. Pyocyanin secreted by one bacterium may be used by another microorganisms to produce current though it also performs antibiotic function [7]. It is necessary to admit that a large-scale investigation of *P.aeruginosa* biofilm morphology, mechanisms of its production and degradation, peculiarities of formation under different conditions and impact of various factors are at the infancy stage [8, 9]. According to undivided opinion of scientists who investigate this problem the main source of nosocomial diseases and persistence factor of their causative agents in hospital ecosystems from air and water to inner surfaces of catheters and body systems are represented by biofilms [10, 11, 12].

Biofilm production provides bacterial population with new, sometimes unknown features which may not be manifested in planktonic form [13, 14, 15]. Increased interest to the study of biofilm of these clinically significant bacteria is also explained by the fact that *P. aeruginosa* is considered to be one of the major model objects for investigation of general principles of microorganisms functioning as a part of biofilm [16].

Bacteria of *Pseudomonas aeruginosa* type are human opportunistic pathogens which being a part of biofilm may cause different nosological forms of pyoinflammatory diseases with severe course and high fatality in immunocompromised patients [17, 18].

An apparent ability of *Pseudomonas aeruginosa* to form biofilms on biotic and abiotic surfaces combined with a limited sensitivity spectrum peculiar for strains circulating in hospital environment determines a number of clinical and epidemiological problems including incurable in fact pulmonary infections in patients suffering from mucoviscidosis and infections of urinary and respiratory tracts in patients admitted to intensive care unis; catheter-associated and burn infections with high risk of sepsis development; contamination of medical equipment, etc. [19, 20].

These microorganisms are able to contaminate external and internal surfaces of catheters, probes, respiratory tubes, lenses, and form biofilm on them. Being a part of biofilm, bacterial cells are protected from the action of defense immunological factors and also antimicrobial agents that is a significant problem in causal treatment of infectious complications [21].

The given information determines reasonability of searching the methods of control of biofilm production in *Pseudomonas aeruginosa* cultures which can be used to increase the effectiveness of antibiotic treatment in blue pus infection and/or decontamination of medical equipment and another objects of hospital environment.

One of the methods to control biofilms produced by *Pseudomonas aeruginosa* on abiotic surfaces from different materials is impact of antiseptics and decontaminants. Modern decontaminants are represented by a wide spectrum

of chemical compounds of different classes which have a common ability to destroy microorganisms even in low concentrations. However these agents are used only for processing of object surfaces but not human skin though medical staff of the hospital is one of the sources for hospital strains spread. Furthermore, the majority of decontaminant antimicrobial agents is applied for filmless microorganism cultures [22, 23].

According to results of comparative study of free living (planktonic) and fixed in biofilms microbial cells a number of authors admitted that in the first case bacteria demonstrated absolute sensitivity to 5 out of 6 test decontaminants, and in the second case there were certain differences. Only Welpas agent caused 100% of bactericidal effect. As for Isojin and ethanol death of microorganisms was admitted in 88.9% and 60.0% cases, respectively, Osvan only in 4.4%, and Hibitane and tego 51 demonstrated no bactericidal effect [24]. Clearance of bacteria was also studied by D.J.Jurgens and coauthors. Using PA14 test strain as an example they showed that chloramide in experimental concentrations at the time of water chlorination is ineffective regarding biofilms of this microorganism. Meanwhile this impact did not increase a level of *Pseudomonas aeruginosa* bacteria resistance to antibiotics [25].

At present the impact of bacteria on separate elements of biofilm formation is considered to be one of the most perspective targets for the action of new antimicrobial medicines. Numerous investigations of recent 40 years demonstrated that the process of biofilm formation is complex and multistage. There are five major stages in biofilm life cycle. They are adhesion, monolayer, microcolony, maturation, and degradation. All of these stages can be observed in formation of biofilm with any microorganism and each of them can be a target for impact of new antimicrobial medicines [26, 27, 28].

Factors providing resistance of Pseudomonas aeruginosa to modern antimicrobial medications are the following: enzymatic inactivation of medication, presence of alternative metabolic pathways, permeability disruption of external structure of bacterial cell, change of target of action, reflux mechanism activation, etc. [29].

Currently the principal directions of development of new antimicrobial medications are as follows: development of antiadhesive coatings and medications able to interrupt the function of eternal structures of cells in charge of adhesion (fimbriae, adhesins); development of medications that block synthesis or destabilize matrix of biofilm; development of medications that cause cell adhesion deficiency in microcolony, and block cell fission; development of medications that block intercellular signaling (quorum sensing), and cause disconnection of cells from biofilm and their transition into plankton existence or resist pathogenicity factor expression [30]. A number of authors identified significant capacity to inhibit the process of biofilm formation in bismuth complexes of synthetic and natural porphyrins [31]. It was found that amongst these compounds the bismuth complex meso-Tetra(4-N-methylpyridyl) porphyrin shows the most activity [32].

Nowadays the influence of certain physical factors on biological properties of biofilms is studied as well. No one can doubt the importance of study of electromagnetic radiation biological effect. Electromagnetic field (EM field) as a potential inductor of variability of microorganisms came to the front due to the study of meteorological conditions' role in initiation and progression of infectious diseases. Discussion of the matters of physical mechanisms of weak magnetic field effect on biological objects is most actively conducted lately [33, 34]. Effectiveness of low intensity electromagnetic fields utilization (EHF-radiation) in biotechnological processes is proved in a number of studies as well. The effects obtained in the process of microorganisms' radiation in the following may become the foundation of new methods of vaccine production, increase in process productivity of making antibiotics, etc. [35].

A number of scientists investigated influence of electromagnetic radiation of millimeter range on capacity of diphtheria causative agent to biofilm formation. It was found that millimeter waves in 61.1 GHz frequency range exert stimulating effect on capacity of pathogenic corynebacteria to biofilm formation. While studying influence of EHF-field of 42.2 GHz frequency range inhibition of test factor was identified [36].

Method research and study of influence of physical factors variety that inhibit biofilm formation are of current concern [37, 38]. At the present time the new technology that is photodynamic therapy is in intensive progress [39]. Over recent years high level of advancement was achieved in understanding of primary mechanisms underlying light emitting diode radiation effect on biological objects. Low intensity electromagnetic radiation has widespread application practically in all areas of medicine. In fact, under the influence of light emitting diode radiation metabolic and functional properties of biological system variety can be significantly changed. According to a number of investigators a direct method of light emitting diode radiation effect provides direct influence on cellular structure elements, moreover it has been proved that membrane structures of cell are the most sensitive to optical radiation effect [40, 41]. There is significant value of light emitting diode radiation effect on biofilm breakdown and biofilm formation prevention by *in vitro* microorganisms. It was found that influence effect of light of blue and purple spectrum involves their degradation with enzymatic activity inhibition [42]. There are data relative to study of light emitting diode radiation of blue and purple spectrum effect on *Pseudomonas aeruginosa* isolates. It was found that under influence of light emitting diode radiation a breakdown of daily biofilms with inhibition of plankton cells production capacity was observed. While identifying capacity for biofilm formation by *P.aeruginosa* plankton cells following light emitting diode radiation of purple and blue spectrum effect it was found that extracted plankton cells are not able to produce close biofilms that is important fact to prevent *P.aeruginosa*colonization and administer adequate complex antimicrobial therapy. Biodestructing effect of light emitting diode radiation on close biofilms of *P. aeruginosa* multiresistant strains was identified [43].

All the above allows to assess current problem of medical science and practice that is process of biofilm formation in microorganisms particularly in *Pseudomonas aeruginosa* strains. Review of literature sources shows practicability of research line of specific subject that is proved by crisis of antibiotic therapy observed for now and characterized not only by multitude resistant

microorganisms but also by absence of medications and infectious pathology therapeutic regimen that have assuring effectiveness and therefore search for alternative control methods is critical and challenging.

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application practically in all areas of medicine. In fact, under the influence of light emitting diode radiation metabolic and functional properties of biological system variety can be significantly changed. According to a number of investigators a direct method of light emitting diode radiation effect provides direct influence on cellular structure elements, moreover it has been proved that membrane structures of cell are the most sensitive to optical radiation effect. It was found that under influence of light emitting diode radiation a breakdown of daily biofilms with inhibition of plankton cells production capacity was observed. All the above allows to assess current problem of medical science and practice that is process of biofilm formation in microorganisms particularly in *Pseudomonas aeruginosa* strains. Analysis of literature sources shows practicability of research line of specific subject that is proved by crisis of antibiotic therapy observed for now and characterized not only by multitude of resistant microorganisms but also by absence of preparations and infection pathology therapeutic regimen that have assuring effectiveness and therefore search for alternative control methods is critical and challenging.

**Key words: *Pseudomonas aeruginosa*, formation of biofilms, antibiotics, physical factors, photodynamic therapy**