Resistance mechanisms in bacterial biofilm formations - Review Article

Nikiforos Rodis[1], Vasiliki Kalouda Tsapadikou[1], Charalampos Potsios[2], Panagiota Xaplanteri[3]

[1]Student in fifth year, Medical School, Democritus University of Thrace
[2]Department of Internal Medicine, University General Hospital of Patras
[3]Department of Microbiology, University General Hospital of Patras

Introduction

Bacteria survive in the environment in two forms, free or planktonic cells and adhered to living or non-living surfaces (sessile cells). Biofilms have a complex architectural structure formed by micro- and macro-colonies between which there are gaps that allow the passage of nutrients. Externally they are surrounded by a biopolymer matrix. These biopolymers (extracellular polymeric substances, EPS) are mainly polysaccharides, proteins, nucleic acids (extracellular DNA, eDNA) and lipids, and mediate cell-to-cell adhesion and cell-to-cell adhesion to the surface on which the cell grows.

Biomembrane-producing bacteria cause chronic and persistent infections with tissue damage. 65% of microbial infections are associated with biomembrane formation, such as periodontitis, endocarditis, chronic bronchopulmonary disease (*P. aeruginosa*) in patients with cystic fibrosis, otitis media in children (*H. influenzae*), chronic sinusitis, chronic osteomyelitis, surgical implant infections, intravenous catheters and stents (*S. aureus* and other Gram positive cocci), wound infections , urinary tract infections (*E. coli* and other pathogens).

Bacteria multiply protected by environmental pressures. The higher the density of a population of bacteria, the more resistant they are and the higher doses of antimicrobial are required to kill them. This is called the "inoculum effect" .

Resistant mechanisms

1. Host defense escape mechanisms
White blood cells and the various enzymes they produce cannot penetrate the biomembrane, while at the same time a decreased phagocytic capacity is observed. This phenomenon is called frustrated phagocytosis and macrophages and neutrophils cannot encapsulate the biomembrane bacteria, while at the same time their toxic derivatives destroy the surrounding healthy tissue.

2. Extracellular polymeric substances (EPS): Glycocalyx and biopolymer matrix
The glycocalyx consists of glycoproteins and polysaccharides and with the help of Van Der Waals forces and hydrogen bonds favors the adhesion of the biomembrane to solid surfaces. It helps in the maturation and survival of the biomembrane. Its absorbent surfaces restrict the transport of germicidal substances and serve to attach to exogenous enzymes that degrade sensitive antibiotics.
The eDNA in the extracellular polymer is derived endogenously not only as a result of cell lysis but also as a result of active secretion or exogenously by neutrophils in regions of inflammation. Its presence increases the resistance of biomembrane to specific antibiotics

3. Enzymes mediated resistance to antibiotics
Ionizing enzymes in the biomembrane convert the bactericidal agents into a non-toxic form, thereby conferring resistance. Enzymatic inactivation of antibiotics in the outer regions of the biomembrane prevents the antibiotic from reaching the deeper layers.

4. Heterogeneity in metabolism and growth rate
The rate of growth and metabolic activity in a biomembrane is influenced by the different availability of nutrients and oxygen. As a result, the cell's metabolic activities are enhanced in the periphery of the membrane, while in the interior are reduced.

5. Intercellular Communication Systems (Quorum Sensing-QS)
The formation of the biomembrane is due to the co-operation of the micro-organisms involved that seem to interact with different communication systems (Quorum Sensing-QS) and modify gene expression according to prevailing conditions. The function of these systems is density-dependent) and through them the bacteria regulate the expression of various genes and the production of virulence factors such as extracellular enzymes and lysines which are necessary for the pathogenesis of infections, but also antibiotic resistance, inflammatory response and biomembrane development.

6. The persister cells
Persisters are defined as the population of bacterial cells that are resistant to antimicrobials and cause chronic infections. In the biomembranes there are such cells that survive in the presence of germicidal substances at deadly concentrations greater than in planktonic cells. The resulting resistance is not related to genetic or inherited changes (phenotypic switch). At the end of antibiotic treatment, these cells begin to proliferate and re-form the biomembrane. The presence of extracellular polysaccharide protects persisters cells from the host's immune system.

7. Genetic adaptation and mutations
Genetic adaptation within the biomembrane is necessary to reduce susceptibility and to adopt a durable phenotype. The rate of biomembrane cell translocation compared to plankton cells is significantly higher and greater horizontal gene transfer and plasmid transfer is accelerated due to proximity of cells. Another factor that appears to enhance mutations is oxidative stress within the biomembrane. This is due to the increased production of endogenous reactive oxygen species (ROS) by activated neutrophils and a defective antioxidant system. As a result, genetic adaptation and evolutionary changes are increasing.

8. Efflux Pumps
Bacteria use specialized proteins to eliminate various substances from the cytoplasm called efflux pumps. They are associated with endogenous and induced resistance to antibiotics. Genes that control their production are based on plasmids.

9. Response to stress
The stress caused by lack of nutrients, extreme temperatures, hyperosmolarity and acidic pH leads to changes in the morphology and physiology of the biomembrane cells with the ultimate goal of increasing endurance and preventing cell damage.

10. Structure of the outer membrane
Deprivation or overexpression of outer membrane proteins is associated with resistance to certain antibiotics

11. Bacteriophages
Phages contribute to cell death and eDNA release, and thus contribute to the development of resistance. In addition, their presence pushes P. aeruginosa to organize into a bio-membrane of particular architecture (liquid crystalline biofilms) that confer resistance to tobramycin. Pf phages are negatively charged and bind tobramycin to the extracellular polymeric membrane.

12. Interactions among different species
Biomembranes consisting of more than one species of microbes demonstrates a wider range of resistance compared to biomembranes consisting of only one species of microorganism.

13. Various other mechanisms
tssC1: Type VI secretion systems -T6SS in Gram-negative bacteria serve as secretory pathways that facilitate protein transport and are involved in antibiotic resistance of biomembrane bacteria. **PA2070 and PA5033:**
The PA2070 and PA5033 regions are associated with resistance to tobramycin and gentamicin in P. aeruginosa biomembranes by unknown mechanism.

Conclusions

During their evolution, the bacteria develop strategies that ensure their survival. One such is the creation of biomembrane. The increased resistance to the antimicrobial agents observed in the biomembranes is due to a combination of mechanisms such as decreased cross-linking, binding of the antibiotic to the extracellular polymer, enzyme-mediated resistance, level of metabolic activity, efflux pumps, persister cells and the structure of extrapolysaccharide. Changes in the level of expression of various genes in response to protect surface-bound bacteria.
Which mechanism will be used depends on the type of micro-organism. In addition, the genetic and biochemical properties of the biomembrane need to be further investigated in vivo with the aim of producing antimicrobial agents capable of providing effective protection. The importance of this event is fundamental in prosthetic and catheter infections, as the biomembrane bacteria multiply and influence the patient's treatment as well as the treatment of persisters and the reduction of relapses.

References
1. Paraje MG. Antimicrobial resistance in biofilms. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Editor), pages 736-744. Προσβάσιμο στο: www.formatex.info/microbiology3/book/736-744.pdf, τελευταία πρόσβαση Σεπτέμβριος, 9, 2018.
2. de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock REW. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Current Opinion in Microbiology, 2013;16(5):580-589.
3. Singh S, Singh SK, Chowdhury I, Singh R. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. The Open Microbiology Journal, 2017;11:53-62.
4. Abee T, Kovacs AT, Kuipers OP, van der Veen S. Biofilm formation and dispersal in Gram-positive bacteria. Current Opinion in Biotechnology, 2011; 22:172–179.
5. Rafii F, Hart ME. Antimicrobial resistance in clinically important biofilms. World J Pharmacol, 2015; 4(1): 31-46.
6. Dibartola AC, Swearingen MC, Granger JF, Stoodley P, Dusane DH. Biofilms in orthopedic infections: a review of laboratory methods. APMIS, 2017; 125: 418–428.
7. Vega NM , Gore J. Collective Antibiotic Resistance: Mechanisms and Implications. Current Opinion in Microbiology, 2014; 21: 28–34. doi:10.1016/j.mib.2014.09.003.
8. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International Journal of Antimicrobial Agents, 2010; 35: 322–332.
9. Emami S, Eftekhar F. The Correlation between Biofilm Formation and Drug Resistance in Nosocomial Isolates of Acinetobacter baumanii. Avicenna J Clin Microb Infec, 2015;2(2): e23954. DOI: 10.17795
10. Solano C, Echeverz M, Lasa I. Biofilm dispersion and quorum sensing. Current Opinion in Microbiology, 2014; 18:96–104.
11. Stewart P. Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol., 2002; 292:107-113.
12. Leid JG. Bacterial Biofilms Resist Key Host Defenses. Microbe, 2009;4(2):66-70.
13. Myszka K & Czaczyk K. Mechanisms Determining Bacterial Biofilm Resistance to Antimicrobial Factors. Στο βιβλίο Antimicrobial Agents, 2012, chapter 10, IntechOpen, pages: 215-216. doi.org/10.5772/33048
14. Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev., 2017;41(3):276-301. doi: 10.1093/femsre/fux010.
15. (Romeo T. (Editor). Bacterial Biofilms e-ISBN 978-3-540-75418-3 DOI 10.1007/978-3-540-75418-3, 2008 Springer, page 87. Available at: https://www.springer.com/gp/book/9783540754176).
16. Taraszkiewicz A, Fila G, Grinholc M, Nakonieczna J. Innovative strategies to overcome biofilm resistance. BioMed Research International, Volume 2013, Article ID 150653,13 pages http://dx.doi.org/10.1155/2013/150653
17. Johnson L, Mulcahy H, Kanevets U, Shi Y, Lewenza S. Surface-localized spermidine protects the Pseudomonas aeruginosa outer membrane from antibiotic treatment and oxidative stress. J Bacteriol. 2012 Feb;194(4):813-26. doi: 10.1128/JB.05230-11.
18. Petropoulos AD, Xaplanteri MA, Dinos GP, Wilson DN, Kalpaxis DL. Polyamines affect diversely the antibiotic potency: insight gained from kinetic studies of the blasticidin S AND spiramycin interactions with functional ribosomes. J Biol Chem. 2004;279(25):26518-25.
19. Cloete T.E. Resistance mechanisms of bacteria to antimicrobial compounds, International Biodeterioration & Biodegradation, 2003;51(4):277-282.
20. Hazan R, Que YA, Maura D et al. Auto poisoning of the respiratory chain by a quorum-sensing-regulated molecule favors biofilm formation and antibiotic tolerance. Curr Biol 2016;26:195–206.
21. Liang TJ. Drug resistance mechanism of Staphylococcus aureus bacterial biofilm. Journal of Clinical and Nursing Research, 2017; 1(1): 62-65
22. Admassie M. Current Review on Molecular and Phenotypic Mechanism of Bacterial Resistance to Antibiotic. Science Journal of Clinical Medicine, 2018;7(2):13-19. doi: 10.11648/j.sjcm.20180702.11
23. Conlon BP, Rowe SE, Gandt AB, Nuxoll AS, Donegan NP, Zalis EA, et al. Persister formation in Staphylococcus aureus is associated with ATP depletion. Nat Microbiol. 2016;1: . doi:10.1038/nmicrobiol.2016.51.
24. Van Acker H & Coenye T. The Role of Efflux and Physiological Adaptation in Biofilm Tolerance and Resistance. The Journal of Biological Chemistry, 2016;291(24):12565–12572.
25. Balcázar JL, Subirats J, Borrego CM. The role of biofilms as environmental reservoirs of antibiotic resistance. Front Microbiol. 2015; 6: article 1216. doi: 10.3389/fmicb.2015.01216