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ISOLATION AND DETECTION OF BIOFILM-PRODUCING BACTERIA FROM TAP WATER

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Abstract

Biofilm is a collection of usually more than species of microorganisms that are enclosed by a layer called an exopolysaccharide membrane. The whole biofilm act as a multicellular system. It is the dominant form of microbial life as compare to free-floating bacteria. Quorum sensing and some genetic control signals induce the production of biofilm. It is matured in five developmental stages. Biofilm causes some serious diseases in humans such as Nosocomial infections, Dental caries, musculoskeletal infections, and Cystic fibrosis pneumonia. The most prominent bacteria which produce a film in tape water are Staphylococcus aureus, Burkholderia cepacia, Pseudomonas aeruginosa, etc;. biofilm-producing bacteria are more resistance to antibiotics as compare to free-living bacteria. The antibiotics resistance due to the poor penetration of antibiotics into the biofilm. It is very difficult for any solutes and drugs to cross the exopolysaccharide layer of biofilm. Besides this outer layer, bacteria inside the biofilm can produce some vital enzymes and other metabolites through which antibiotics can be neutralized. Few factors such as oxygen concentration, nutrient level, temperature, and microbial community affect the formation of biofilm. Not only causing infection in humans but also disrupt the quality of water. Through releasing some toxic metabolites, the color and smell of water change. Biofilm also damages the surface of the substrate such as pipes and walls of the water container. Many developed countries consume a very high amount of money on the treatment and removal of biofilm for drinking water to ensure the safety level of drinking water.

Keywords: *Antibiotic resistance of biofilm, Drinking water contamination, S-aureus biofilm*

INTRODUCTION

Instead of the free-floating phase, bacteria often in nature are found attached to any substrate. Most types of bacteria come together in colony form and attach themselves to a surface. This colonial form of bacteria is called biofilm. As a protective layer, the Exopolysaccharide matrices covered the whole biofilm (1). This matrix is a self-produced polymorphic of an adherent bacterial cell. Bacteria inside the exopolysaccharide matrix are present in a highly organized manner, acts like a multi cellular structure, and coordinate all the functions such as the exchange of different metabolites and nutrients between the cell and surrounds (2).

Throughout their transformation from the planktonic to a culture attached to the shore, numerous changes occur. New phenotypic characteristics in these bacteria adapt to certain environmental signals (3). Usually, a biofilm contains more than one species of bacteria while the presence of single species of bacteria in the biofilm has also been reported (2). Dental plaque, biofilm in the mouth cavity, is a solid example of a bacterial biofilm community in which more than a hundred species are present. Bacteria secrete different types of chemical signal which mediate the communication between the different species of bacteria in biofilms. Bacteria in biofilms create a big clinical problem in that the bacteria inside the biofilm are highly resistant to antibiotics (4).

Bacteria in biofilm are considered the dominant form of microbial life. Inside the biofilm bacterial cell secrete some valuable chemical molecule like digestive enzyme and protein metrics polysaccharides and nucleic acids (5). Biofilm formation and auto aggregation is facilitated by outer surface component of bacterial cell with the combination of environmental factor (6).

Therefore, to control the biofilm formations, it is very crucial to known about the mechanism of formation of biofilm production. For fitness and best survival of a living organism, genetic adaption is the basic factor which causes due to mutation during DNA recombination. Mutation in bacteria during gene expression plays a vital role in adaptation in a rapid changing environment (7).

It has been found the poor growth environment and deficient metabolism of the bacteria can provoke the ability of aggregation in those bacteria that are living free floating in nature. On this behalf, the aggregation capacity of bacteria is the way to adopt the stress environment for survival (8). The biofilm consists of layers of cell clusters embedded in an extracellular polysaccharide matrix, called intercellular polysaccharide adhesive (PIA), consisting of b-1,6-N-acetyl glucosamine and synthesized by N-acetylglucosaminyl transferase (5). Biofilms are also the site affecting their creation for quorum sensing (9). There are several factors which affect the formation of biofilm such as bacterial mobility, surface attachment and nutrientional availability. A scientist named Dr. "Stanley wall" has examined the bacterial species, *Bacillus subtilis*, he found that a protein "Deg U" is responsible for the deciding either to create the biofilm or not (9).

Henrici probably gave the first recorded biofilm discovery in 1933, observing that water bacteria do not float freely, but emerge on immersed materials. The regulation of biofilm development includes certain surface proteins, extracellular proteins, capsular polysaccharides, adhesions, and autolysin. The gene codes for intracellular adhesion and may also code for biofilm development and is needed for it (3). Two methods are widely used for detecting biofilm forming ability: phenotypic and genotypic. Phenotypic method mainly contains congo red agar process, micro titer process and Christensen test tub method. Through Christensen test tub method the capacity of bacteria to stick with surface is described while congo red agar process identify the slim matrix production of the biofilm producing bacteria (9).

Biofilm formation is now recognized as a main component of bacterial infections, containing ocular implant infections, Infective endocarditis, infections associated with medical devices, osteomyelitis, dental caries, otaitis media and lung infections in patients with cystic fibrosis. Biofilms bacteria need 10-1000 times more concentrated antimicrobial agent which is used for the killing of planktons. Planktons bacteria that are genetically similar, and are also extremely phagocytosis resistant, biofilms are extraordinarily hard to eliminate from living hosts (10). The creation of biolfim in the laboratory can be initiated by the reversible attachment of planktonic bacteria to the surface of substrate. At this stage bacteria are susceptible to most antibiotic so the chance of postoperative infections is decreased. After the attachment of planktonic cell, bacterial cells start irreversible attachment to the surface of substratum and produce matrix which results the formation of small colony of bacterial species (11).

The high clinical impact of biofilm producing bacteria attracts many scientists to find out the regulatory mechanism of biofilm development for the purpose to design the antimicrobial agent. A variety of gene-directed target studies, as well as global proteomics and genomics studies have resulted in the discovery of a multitude of genes associated with the production of biofilms. Nevertheless, figuring out the functions of all these genes is an incredibly complex task (7, 12).

COMPONENTS INVOLVED IN BIOFILM FORMATION

Inside the biofilm, bacterial cells are enclosed in an exopolysaccharide matrix which is polymers protective matrix formed by the bacteria themselves. Intercellular communication through quorum sensing involves the highest level of dynamic and managed social integration of bacteria. Bacterial aggregates often bind to a solid-liquid interface before adsorption to a thin film of organic molecules that constitutes the site of adhesion[12]. Five developmental stages occur in biofilm formation; initial attachment, irreversible attachment, maturation 1, maturation 2, and dispersion (13). Cell can be transported through passive transport or intrinsic motility of planktonic cell to this interface. The bacterial interface aggregation is achieved in two phases; (a) non-specific reversible attachment mediated by hydrophobic and electrostatic attraction between the cell and substrate (b) irreversible adhesion between the micro colony of bacteria. During the starting phase of biofilm formation on the surface, surface bacterial constituents containing pili, fimbriae and lipopolysaccharide play very important roles in mechanical process. The developmental

phases of biofilm, maturation, and disassembly initially based on the synthesis of biopolymers such as protein and extracellular DNA. This biopolymer provides immobility to matrix of microbial cells (6).

QUORUM SENSING

Most of the bacteria have capacity to develop a biofilm and interact with specific signal. Bacteria interact with signaling molecules by synthesizing and reacting. Quorum sensing enables the bacterial species to detect the concentration of other microbes in a limited microenvironment and to respond by activating several genes which then produce toxins (14). In several Gram-positive bacteria, these QS molecules may be peptides while the well-described QS molecules in Gram-negative bacteria are N-acyl-L-homoserine lactones. QS is also present in fungi-forming biofilms such as Aspergillus species and Candida albicans where alcohols farnesol and tyrosol are morphologically influential QS molecules etc. Bioluminescence activation in *Aliivibrio fischeri* is the classical example of QS, and most of the AHL-producing bacteria are connected to higher species, such as plants and animals, where they function as symbionts or pathogens (15).

BIOFILM PRODUCIING BACTERIA IN WATER

All those bacteria are capable of producing biofilm which has three basic properties; producing signaling molecules, regulation of signaling molecules with gene regulation, and recognition of population density of bacterial cells (16). The signal produces either due to the cell density or directly produced by bacteria at different stages of growth. The main difference between these signals and signals produce by quorum sensing is that these are dependent on cell to cell communication and cell density while the quorum sensing is auto inducer which is independent to any environmental signals [4]. There are many bacterial species that the ability to form biofilm in tape water as shown in Table I.

Table I. The list of bacterial species involved in biofilm production in tape water

S#	Bacterial species	References
1	*Staphylococcus Aureus*	17
2	*Burkholderia Cepacia*	18
3	*Pseudomonas Aeruginosa*	19
4	*Clostridium Difficile*	20
5	*Klebsiella Pneumonia*	21
6	*Escherichia Coli*	21
7	*Acinetobacter Baumann*	22
8	*Neisseria Gonorrhea*	23
9	*Streptococcus Pyroxenes*	19
10	*Salmonella*	17

FACTORS AFFECTING THE PRODUCTION OF BIOFILM

It is well recognized that the environmental conditions in which bacteria grow and develop can have a significant influence on planktonic cell behavior: development, resistance, production of toxins, etc. some of the factors which affect the biofilm production are nutrients, temperature, water activity, metals and redox potential (24). Some environmental factors also modify the physiological condition of the bacterial cell in the biofilm. These factors can also affect the substratum' to which bacterial cells get attached. The main influencing factors which affect the biofilm production discussing in this review are fellow (25).

FACONCENTRATION OF OXYGEN

All the living microorganisms are linked with host substratum either in hydrated or free-living in the aquatic environment, as these organisms experience the amount of oxygen that diffuses in the environment. A mature bacterial biofilm contains a high amount of nutrients that need to be metabolized by bacteria. Oxygen is considered the final electron chain acceptor in metabolism. So the amount of oxygen is directly proportional to the development of biofilm (26).

NUTRIENTS

Extracellular polymers concentrate the trace organic molecules, waste products, on its surface to acquire the nutrient. Bacteria inside the biofilm use some enzymes to breakdown the food supply chain. The surface of the outer matrix is usually negatively charged unlike the nutrient which is positively charged, so in this way, the matrix and nutrient attract each other. Sometimes when the nutrients have negatively charged then the ions exchange occurs between the outer surface of biofilm, and nutrients. Through these ions exchange method the biofilm gets enriched from nutrients as compare to surround (27).

ANTIBIOGRAM OF BIOFILM BACTERIA

The main cause of antimicrobial resistance of biofilm producing bacteria is that the most of antibiotics cannot get access to the depth of biofilm, cannot cross the layer of biofilm. The exopolysaccharide layer of biofilm makes obstacles for the diffusion of antibiotics to the bacteria inside the biofilm (18). Overall, the solutes diffuse inside biofilms at slower rates than they occur in water. According to several reports, some antibiotics have the ability to cross the layer of biofilim while other has the poor ability to cross the layer during antibiotic therapy. It depends on the nature of biofilim and structure of antibiotics. Mathematical models predict that if the antimicrobial agent is deactivated in the outer layers of the biofilm faster than it diffuses, a formidable penetration barrier should be built (29).

A second hypothesis to explain the decreased resistance of biofilms to antibiotics suggests that at least some of the cells in a biofilm have nutritional deficiencies and therefore live in a slow-growing or hungry state. Slowly developing cells or no growing cells are not very susceptible to many antimicrobials. This biofilm heterogeneity is an essential survival tactic since at least some of the cells are almost guaranteed to survive any metabolically guided attack, representing a wide range of different metabolic states (30).

The third process of decreased susceptibility to biofilm is that at least some of the biofilm cells adopt a distinct and safe biofilm phenotype. This phenotype is not a response to nutrient limitation; it is a surface growth response that is biologically programmed (29).

Table II. Biofilm bacteria resistant to different antimicrobial agents

Antibiotics	Resistant strain	References
Penicillin	Staphylococcus Aureus	32
	Burkholderia Cepacia	33
	Pseudomonas Aeruginosa	34
	Acinetobacter Baumannii	
Ciprofloxacin	Burkholderia Cepacia	33
	Pseudomonas Aeruginosa	31
	Clostridium Difficile	35
	Klebsiella Pneumonia	33
	Escherichia Coli	34
	Acinetobacter Baumannii	32
	Neisseria Gonorrhea	35
Erythromycin	Pseudomonas Aeruginosa	23
	Clostridium Difficile	33
	Klebsiella Pneumonia	31
	Escherichia Coli	35
	Burkholderia Cepacia	33
Rifampicin	Clostridium Difficile	34
	Acinetobacter Baumannii	32
	Streptococcus Pyroxenes	35
	Salmonella	23

Antibiotic therapy generally improves the acute clinical symptoms which lead to the release of planktonic cells from the biofilm but cannot fully destroy the bacteria in the biofilm (31). On medical devices such as urinary catheters, prosthetic joints, and heart valves, biofilm-related infections are caused by

bacteria. These biofilm-forming bacteria as shown in table 1.2 are up to 1000 times more antibiotic-resistant than planktonic cells (29). This antibiotic resistance may be attributable to increased transmission of resistance markers, efflux pumps and the resistance gained (2).

BIOFILM IN INFECTIOUS DISEASE

Biofilms are a serious cause of human infections (14). Biofilms are responsible for most hospital-acquired infections because they can be life-threatening colonies of biomedical equipment. Indeed, a significant number of hospital-acquired infections are correlated with pathogen colonization on the surface of implanted medical devices such as respirators, catheters, heart valves, and orthopedic devices. Also, a urinary catheter is associated with 95% of urinary tract infections, mechanical ventilation is associated with 80% of pneumonia, and intravascular devices are associated with 87% of bloodstream infections (34).

The prevalent bacterial species related to device-associated infections are coagulase-negative Staphylococci and *Staphylococcus aureus*. CoNS are a significant cause of intravascular catheter endogenous infection, either by tracking along its outer surface or by entering via the catheter hub during manipulation by healthcare staff (35).

Enterococci are among the most prominent sources of nosocomial infections, especially in intensive care units where enterococci can be transmitted through the hands of clinical personnel from one patient to another as shown in Table III. The main contamination sites are the urinary tract and soft tissue corresponding to the intestinal flora, where enterococci reside. The biggest risk is for patients undergoing comprehensive abdominal surgery, indwelling devices, or abdominal procedures. Resistance to antibiotics, particularly resistance to vancomycin, has become a serious problem in enterococcus infections (36).

Table III. List of diseases caused by biofilm bacteria

Diseases caused	Bacterial species	References
Dental caries	Acidogenic Gram-positive cocci	30
Periodontitis	Gram-negative anaerobic oral bacteria	29
Otitis media	Non-typable strains of *Homophiles influenza*	30
Musculoskeletal infections	Gram-positive cocci (e.g., staphylococci)	32
Penile prostheses	*S. aureus* and *S. epidermites*	37
Orthopedic devices	*S. aureus* and *S. epidermites*	38
Endotracheal tubes	A variety of bacteria and fungi	29
Native valve endocarditis	E. coli and other Gram-negative bacteria	2
Osteomyelitis	Various bacterial and fungal species—often mixed	39
Meloidosis	Pseudomonas	29
Nosocomial infections	*Staphylococcus epidermites* and *S. aureus*	40
Central venous catheters	*S. epidermites* and others	32
Vascular grafts	Gram-positive cocci	34
Peritoneal dialysis	A variety of bacteria and fungi	29
Cystic fibrosis pneumonia	*P. aeruginosa* and *Burkholderia cepacia*	30

BIOFILMS IMPACT ON DRINKING WATER QUALITY

The presence of biofilms in water delivery and storage systems has been shown to cause a worsening of the water quality and also cause the corrosion of the substratum surface. The most important factor is that it harbors some pathogenic microorganisms such as bacteria, viruses, and protozoans which

can be lethal to human health. Many research reports that most of the health-related to water is due to the contamination of biofilm-producing bacteria (41).

The adverse effects of chemicals, primarily toxins and other inorganic compounds created by microorganisms living in the matrix of biofilms and water sediments may also include the impact of biofilms and biofouling on drinking water delivery systems. Multiple volatile molecules, absorbed as a result of microbial metabolism or degradation of particulates, such as organic and inorganic acids, enzymes, and metallic oxides, may cause water problems with aesthetic and organoleptic characteristics, often changes in color, odors, and degradation of taste, which may have a significant impact on consumer and end-user acceptance of water (42). Peter (2008) examined the causes of taste and odor in drinking water to establish appropriate mitigation strategies and discovered that low chlorine residues, stagnant water, plastic pipes, and particle aggregation in delivery systems contribute to increased taste and odor production as a result of high microbial activity. The oxidation and reduction of soluble metals by microorganisms associated with biofilms might create metal oxides, contributing to metallic taste and stained water color (43).

THE ECONOMIC LOSSES CAUSED BY BIOFILMS

Access to clean drinking water is necessary for health and is a fundamental human need and must be considered as a vital component of every government health care policy. The main disturbing effects of biofouling in drinking water delivery systems are the economic cost of wastewater disposal, head loss filtration, and energy depletion for government and industry alike. In particular, biofilms cost the UK billions of pounds in energy losses, product pollution, damage to facilities, and medical infections every year. It is said that the expenditures from energy losses alone are overwhelming (2). Fuel consumption of large ships with clean hulls, for example, is low compared to those whose hulls are polluted by biofouling of microbial biofilm colonies. The cost of the impact of biofilm is directly proportional to the cost of fuel. More specifically, some quarters have claimed that nearly 5% of the UK's carbon production originates via shipping, so higher fuel consumption would undoubtedly contribute to climate change. This in itself has an environmental price as well (29).

The decreased effectiveness of antimicrobial agents induced by bio-fouling of surfaces is a problem for many third world countries. To date, antimicrobial agents that have been effective in regulating and inhibiting bacterial growth in vivo have been less effective in biofilm-related infections. Consequently, O'Toole (2002) reported that the economic cost of biofilm infections in the United States is $6 billion per year. Scientific groups and the healthcare industry are just beginning to recognize the extent of the effect of biofilms on the cost of healthcare (30). According to a U.S. report, the data available on biofilms and related microbial resistance from the Government Accounting Office (GAO) is insufficient to determine costs to public health systems (35).

CONCLUSION

Biofilm is the aggregation of bacterial cells at the same place. Usually, bacteria cannot float free. Quorum sensing and some other signals induce the production of biofilm. More than one species can reside in biofilm. The biofilm is covered with an exopolysaccharide layer. This layer enhances the antibiotic resistance of bacteria residing within the biofilm. The main reason for the antibiotic resistance of biofilm-producing bacteria is the poor penetration of antibiotics to biofilm. As compared to free-floating bacteria, biofilm-producing bacteria are more persistent in causing infection. Other than causing infection, it also deteriorates the quality of drinking water, color, and odor, by producing toxic metabolites in the water container. The surface of the substrate, to which biofilm is attached may be damaged. Some developmental countries utilize a very high amount of budget on the treatment and removal of biofilm from water to ensure the safety level of drinking water. To control biofilm production in tap water or industrial water, those factors which influence biofilm production such as oxygen concentration, nutrient, and temperature, must be controlled.

References:

1. Simões LC, Simões M, Vieira MJ. Biofilm interactions between distinct bacterial genera isolated from drinking water. Appl Environ Microbiol. 2007;73(19):6192-6200.
2. Banerjee D, Shivapriya PM, Gautam PK, Misra K, Sahoo AK, Samanta SK. A Review on Basic Biology of Bacterial Biofilm Infections and Their Treatments by Nanotechnology-Based Approaches. Proc Natl Acad Sci India Sect B - Biol Sci. 2020;90(2):243-259.
3. Bose S, Khodke M, Basak S, Mallick SK. Detection of biofilm producing staphylococci: Need of the hour. J Clin Diagnostic Res. 2009;3(6):1915-1920.
4. Jayaraman A, Wood TK. Bacterial Quorum Sensing: Signals, Circuits, and Implications for Biofilms and Disease. Annu Rev Biomed Eng. 2008;10(1):145-167.
5. Nadell CD, Drescher K, Wingreen NS, Bassler BL. Extracellular matrix structure governs invasion resistance in bacterial biofilms. 2015:1700-1709.
6. Bogino PC, De M, Oliva M, Sorroche FG. The Role of Bacterial Biofilms and Surface Components in Plant-Bacterial Associations. 2013:15838-15859.
7. Jefferson KK. What drives bacteria to produce a biofilm? FEMS Microbiol Lett. 2004;236(2):163-173.
8. Oliveira A, Cunha MDLRS. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. BMC Res Notes. 2010;3.
9. Růžička F, Holá V, Votava M, et al. Biofilm detection and the clinical significance of Staphylococcus epidermidis isolates. Folia Microbiol (Praha). 2004;49(5):596-600.
10. Schnurr PJ, Allen DG. Factors affecting algae bio fi lm growth and lipid production : A review. 2015;52:418-429.
11. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35(4):322-332.
12. Klausen M, Gjermansen M, Kreft JU, Tolker-Nielsen T. Dynamics of development and dispersal in sessile microbial communities: Examples from Pseudomonas aeruginosa and Pseudomonas putida model biofilms. FEMS Microbiol Lett. 2006;261(1):1-11.
13. Monroe D. Looking for chinks in the armor of bacterial biofilms. PLoS Biol. 2007;5(11):2458-2461.
14. Fuqua C, Parsek MR, Greenberg EP. Regulation of Gene Expression by Cell-to-Cell Communication: Acyl-Homoserine Lactone Quorum Sensing. Annu Rev Genet. 2001;35(1):439-468.
15. Høiby N, Ciofu O, Johansen HK, et al. The clinical impact of bacterial biofilms. Int J Oral Sci. 2011;3(2):55-65.
16. Chen X, Zhang L, Zhang M, Liu H, Lu P, Lin K. Quorum sensing inhibitors: a patent review (2014–2018). Expert Opin Ther Pat. 2018;28(12):849-865.
17. Rewatkar AR, Wadher BJ. Staphylococcus aureus and Pseudomonas aeruginosa- Biofilm formation Methods. 2013;8(5):36-40.
18. Huber B, Riedel K, Hentzer M, et al. The cep quorum-sensing system of Burkholderia cepacia H111 controls biofilm formation and swarming motility. 2017;(2001):2517-2528.
19. Houston P, Rowe SE, Pozzi C, Waters EM, Gara JPO. Essential Role for the Major Autolysin in the Fibronectin-Binding Protein-Mediated Staphylococcus aureus Biofilm Phenotype □. 2011;79(3):1153-1165.
20. Đapa T, Leuzzi R, Ng YK, et al. Multiple Factors Modulate Biofilm Formation by the Anaerobic Pathogen Clostridium difficile. 2013;195(3):545-555.
21. Rijavec M, Mu M, Zakotnik B. Virulence factors and biofilm production among Escherichia coli strains causing bacteraemia of urinary tract origin. 2019;(2008):1329-1334.
22. Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. 2014:119-127.
23. Greiner LL, Edwards JL, Shao J, Rabinak C, Entz D, Apicella MA. Biofilm Formation by Neisseria gonorrhoeae. 2005;73(4):1964-1970.
24. Ahmad I, Husain FM. Factors Affecting Biofilm Formation in in vitro and in the Rhizosphere Factors Affecting Biofilm Formation in in vitro and in the Rhizosphere. 2017;(August 2018).
25. Ollos PJ, Huck PM, Slawson RM. in Model Distribution Systems. 2003;95(1):87-97.
26. García-gonzalo D, Pagán R. Influence of Environmental Factors on Bacterial Biofilm Formation in the Food Industry : A Review Influence of Environmental Factors on Bacterial Biofilm Formation in the Food Industry : A Review. 2015;(September):2-13.
27. Ansari MI, Schiwon K, Malik A. Biofilm Formation by Environmental Bacteria Chapter 11 Biofilm Formation by Environmental Bacteria.; 1970. 2
28. Rinaudi L V., Giordano W. An integrated view of biofilm formation in rhizobia. FEMS Microbiol Lett. 2010;304(1):1-11.

29. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science (80-). 1999;284(5418):1318-1322.
30. Parsek MR, Singh PK. Bacterial Biofilms: An Emerging Link to Disease Pathogenesis. Annu Rev Microbiol. 2003;57:677-701.
31. Noiri Y, Ehara A, Kawahara T, Takemura N. Participation of Bacterial Biofilms in Refractory and Chronic Periapical Periodontitis. 2002;(Table 1):10-14.
32. Ang JY, Ezike E, Asmar BI. Antibacterial Resistance. Indian J Pediatr. 2004;71(3):229-239.
33. Baquero F, Baraibar R, Campos J, et al. Antibiotic resistance in Spain: What can be done? Clin Infect Dis. 1996;23(4):819-826.
34. Normark BH, Novak R, Örtqvist Å, Källenius G, Tuomanen E, Normark S. Clinical isolates of Streptococcus pneumoniae that exhibit tolerance of vancomycin. Clin Infect Dis. 2001;32(4):552-558.
35. Richet HM, Mohammed J, McDonald LC, Jarvis WR. Building communication networks: International network for the study and prevention of emerging antimicrobial resistance. Emerg Infect Dis. 2001;7(2):319-322.
36. Dufour D, Leung V, Lévesque CM. Bacterial biofilm : structure , function , and antimicrobial resistance. 2012:2-16.
37. Böswald M, Lugauer S, Regenfus A, et al. Reduced rates of catheter-associated infection by use of a new silver-impregnated central venous catheter. Infection. 1999;27(SUPPL. 1):56-60.
38. D'Agata EMC. Antimicrobial-Resistant, Gram-Positive Bacteria among Patients Undergoing Chronic Hemodialysis. Clin Infect Dis. 2002;35(10):1212-1218.
39. Torretta S, Drago L, Marchisio P, et al. Recurrences in chronic tonsillitis substained by tonsillar biofilm-producing bacteria in children. Relationship with the grade of tonsillar hyperplasy. Int J Pediatr Otorhinolaryngol. 2013;77(2):200-204.
40. Raza A, Muhammad G, Sharif S, Atta A. Biofilm Producing Staphylococcus aureus and Bovine Mastitis: A Review. Mol Microbiol Res. 2013;3(1).
41. Drinking P, Systems W. P Rivate D Rinking W Ater. Small. 2009;(1):1-4.
42. Videla HA, Characklis WG. Biofouling and microbially influenced corrosion. Int Biodeterior Biodegrad. 1992;29(3-4):195-212.
43. Nya EJ. Biofilms and biofouling: cost and effect on drinking water quality for human development. J Glob Biosci. 2015;4(2):1571-1578.
44. Monroe, D. (2007). Looking for chinks in the armor of bacterial biofilms. PLoS Biol, 5(11), e307.