Bacterial Biofilms; Links to Pathogenesis and Résistance Mechanism

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Abstract

Biofilms are usually defined as surface-associated microbial communities, surrounded by an extracellular polymeric substance matrix. There are three major steps that are observed in biofilm formation: initial attachment events, microcolony formation and construction of mushroom-like structure with secretion of extracelluar polymeric substances. These substances can be considered a mechanism to protect the bacterial community from external insults.

Biofilms, significantly increase the ability of the pathogen to evade both host defenses and antibiotics and they are being implicated in the pathogenesis and also clinical manifestation of

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several infections. They cause a variety of persistent infections, such as native valve endocarditis, osteomyelitis, dental caries, middle ear infections, ocular implant infections, urinary tract infections and cystic fibrosis. Established biofilms can tolerate antimicrobial agents at concentrations of 10–1000-times that needed to kill genetically equivalent planktonic bacteria, and are also resistant to phagocytosis, making biofilms extremely difficult to eradicate from living hosts. Consequently, biofilm-related infections that appear to respond to a therapeutic course of antibiotics may relapse weeks or even months later, making surgical removal and replacement of the infected tissue or medical device a frequent and unfortunate necessity.

Several pathogens associated with chronic infections, including *Pseudomonas aeruginosa* in cystic fibrosis pneumonia, *Haemophilus influenzae* and *Streptococcus pneumoniae* in chronic otitis media and Enteropathogenic *Escherichia coli* in recurrent urinary tract infections, are linked to biofilm formation..

Key word; biofilm, biofilm formation organism, Mechanism of Biofilm

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1. INTRODUCTION

Biofilm were first described and named in 1978 (1). Biofilms are multicellular communities held together by a self-produced extracellular polymeric substance (EPS) matrix. The mechanisms that different bacteria employ to form biofilms vary, frequently depending on environmental conditions and specific strain attributes (2). Biofilms are usually defined as surface-associated microbial communities, surrounded by an EPS matrix (3). Microorganisms undergo profound changes during their transition from planktonic (free-swimming) organisms to cells that are part of a complex, surface-attached community. These changes are reflected in the new phenotypic characteristics developed by biofilm (4). Biofilm formation has been demonstrated for numerous pathogens and an important microbial survival strategy (3) and the ability to form biofilms is a universal attribute of bacteria. Biofilms consist of communities or groups of microorganisms that attach to the surfaces of animate objects such as heart valves, bones, or tissues, or to inanimate objects such as artificial heart valves, prosthetic implants, or catheters (5).

Biofilm development on surfaces is a dynamic stepwise process involving adhesion, growth, motility and extracellular polysaccharide production. These represent microbial societies with their own defense and communication system. Transitioning from acute to chronic infection is frequently associated with biofilm formation. The presence of indwelling medical devices increases the risk for biofilm formation and subsequent infection (6). Bacterial attachment is mediated by fimbriae, pilli, flagella and EPS that from a bridge between bacteria and the biofilm (7). Production of an extracellular mixture of sugar polymers called exopolysaccharide is characteristic and critical for biofilm formation (8). In addition to its roles in aggregation and biofilm structure, EPS plays a part in defense, enabling biofilms to resist shear forces and phagocytosis by inflammatory cells (9).

In the human body, bacteria are present in biofilms in essentially every niche that they colonize. These include both pathogenic and nonpathogenic skin flora, pathogenic and nonpathogenic oropharyngeal and nose flora, commensal and pathogenic intestinal flora, and bacteria adherent to endovascular structures such as native and prosthetic heart valves, central venous catheters, and endovascular thromboses. In each of these environments, the bacteria are guided to or away from the biofilm by environmental signals (10) so biofilm formation is an important aspect of

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many most bacterial diseases, including native valve endocarditis, osteomyelitis, dental caries, middle ear infections, medical device-related infections, ocular implant infections, and chronic lung infections in cystic fibrosis patients (9).

Disease-related biofilms can be multi-species or even multi-kingdom, such as the biofilms involved in tooth decay, or single-species such as those involved in endocarditis (7). Established biofilms can tolerate antimicrobial agents at concentrations of 10–1000-times that needed to kill genetically equivalent planktonic bacteria, and are also resistant to phagocytosis, making biofilms extremely difficult to eradicate from living hosts (2).

2. MECHANISM OF BIOFILM FORMATION

There are three major steps that are observed in biofilm formation: initial attachment events, the growth of complex biofilms, and detachment events (figure 1) by clumps of bacteria or by a 'swarming' phenomenon within the interior of bacterial clusters, resulting in so-called 'seeding dispersal' (11). Once a biofilm has fully formed, it often contains channels in which nutrients can circulate. Cells in different regions of a biofilm also exhibit different patterns of gene expression. Because biofilms often develop their own metabolism, they are sometimes compared to the tissues of higher organisms, in which closely packed cells work together and create a network in which minerals can flow (12).

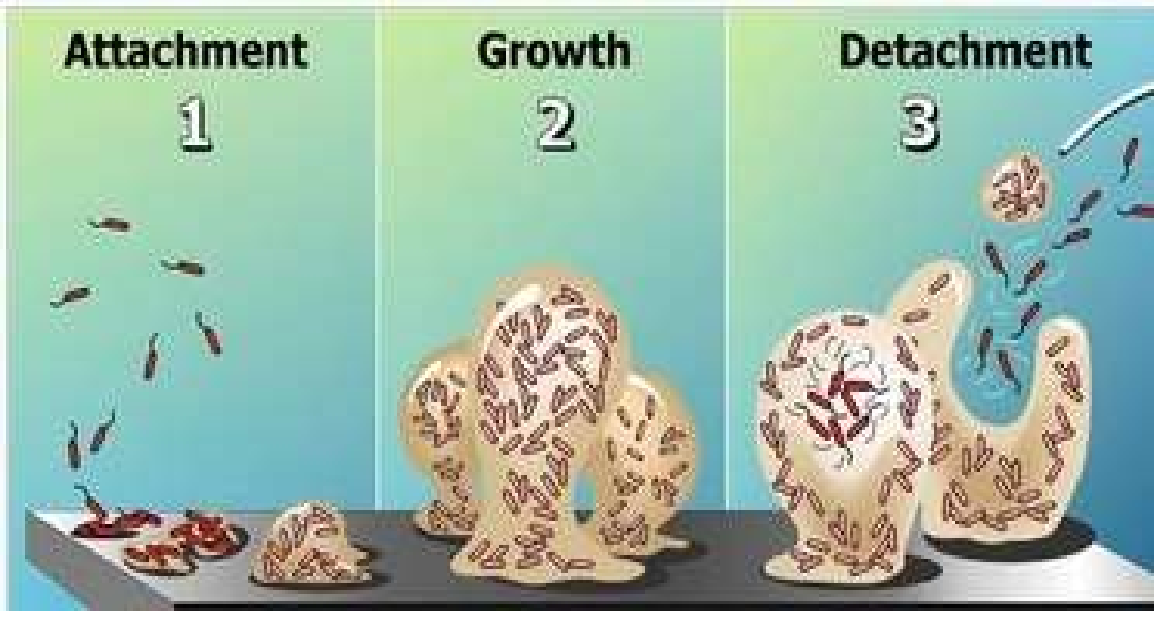

Figure 1*:* Mechanism of biofilm formation

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The biofilm life cycle in three steps: attachement, growth of colonies (development, and periodic detachement of planktonic cells: (1) Free-floating, or planktonic bacteria encounter a submerged surface and within minutes can become attached. They begin to produce slimy EPS and to colonize the surface. (2) EPS production allows the emerging biofilm community to develop a complex, three-dimensional structure that is influenced by a variety of environmental factors. Biofilm communities can develop within hours. (3) Biofilms can propagate through detachment of small or large clumps of cells, or by a type of "seeding dispersal" that releases individual cells. Either type of detachment allows bacteria to attach to a surface or to a biofilm downstream of the original community (11).

3. STRUCTURE AND COMPONENTS OF A BIOFILM

The molecular mechanisms that regulate biofilm formation vary greatly among different species, and even vary between different strains of the same species. However, some features are recognized as general attributes of biofilm formation (13). For instance, all biofilms contain an extracellular matrix that holds cells together. This matrix is often composed of a polysaccharide biopolymer along with other components such as proteins or DNA (14). The biofilm matrix contains EPS, proteins and DNA; EPS constituents 50 % to 90 % of the organic carbon in the matrix. Many of the stalks and mushrooms together result in an architecture with water channels between the bacterial clusters. The water channels have been likened to a primitive circulatory system which protects cell bacteria against buildup of toxic metabolites and starvation while providing a source of nutrients (15). The nature of the matrix exopolysaccharide greatly varies depending on growth conditions, medium, and substrates.

Depending on the surrounding environment, biofilms can contain bacterial clusters (or microcolonies), channels or pores through which water can flow (carrying nutrients through the biofilm), void areas within biofilms (no longer) populated with bacteria, and streamers created by bulk fluid flow (figure 2).

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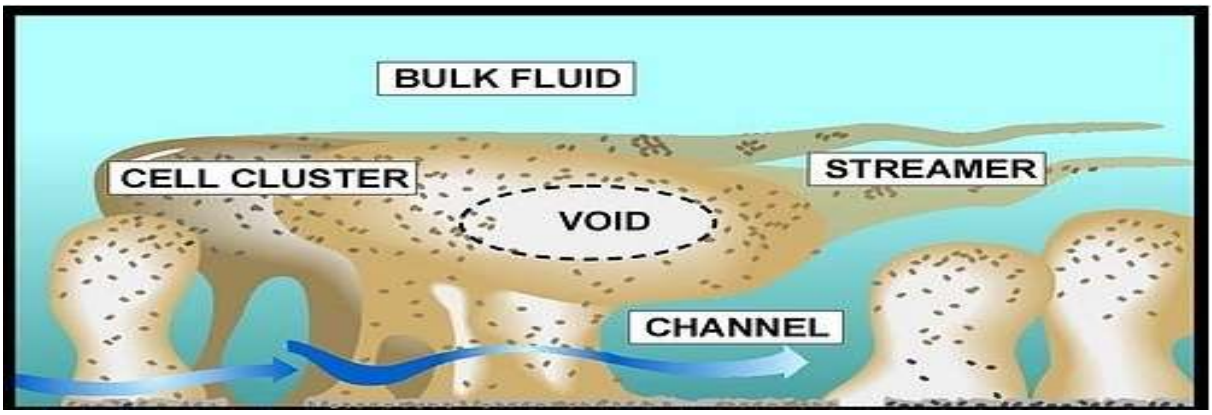

Figure 2: Biofilm structure with labels (16).

4. THE ROLE OF BIOFILM FORMATION IN BACTERIAL COMMUNITIES

The formation of a biofilm can be considered a mechanism to protect the bacterial community from external insults, it seems reasonable that specific extracellular cues regulate activation of the metabolic pathways that lead to biofilm formation. These external cues come from diverse sources. Signals can be produced and secreted by the bacterial community itself, in which case the molecules are termed auto inducers. Auto inducers accumulate extracellularly and the concentration of auto inducer can be correlated with population density. At high concentrations, auto inducers trigger signal transduction cascades that lead to multi-cellular responses in the bacterial population. This mechanism of cell–cell communication in bacteria (termed quorum sensing) controls a large number of developmental processes included those related to biofilm formation (17). Communities of bacteria are different from their planktonic counterparts in very important ways. Like, when bacteria live as a community, they become much less susceptible to antibiotics, even if highly susceptible as individual cells. Thus, when microorganisms form a community, they are protected against a variety of antibiotics that clinicians commonly prescribe for their patients. And also these communities of microorganisms resist attack and killing by the host immune system. In addition, biofilms are resistant to physical forces such as the shear forces produced by blood flow and the washing action of saliva. Organisms within biofilms can withstand nutrient deprivation, pH changes, oxygen radicals, disinfectants, than planktonic organisms (7).

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4.1 To protect from immune response

There is a never-ending race between the development of the host immune system and the progression of bacterial strategies to evade it. The body is inhabited by a large number of commensals, many of which exist as biofilms. Bacteria embedded within biofilms are resistant to immunological and non- specific defence mechanisms of the body. Bacteria have a number of strategies to ensure that they remain fixed in the human body. Bacterial surface proteins that bind to host extracellular matrix proteins such as fibronectin, fibrinogen, vitronectin, and elastin are referred to as MSCRAMMs (microbial surface component recognizing adhesive matrix molecules) and often play a key role in initial adherence of bacteria to solid surfaces within the host (18). Contact with a solid surface triggers the expression of a panel of bacterial enzymes which catalyze the formation of sticky polysaccharides that promote colonization and protection. Phagocytes are unable to effectively engulf a bacterium growing within a complex polysaccharide matrix attached to a solid surface. This causes the phagocyte to release large amounts of pro-inflammatory enzymes and cytokines, leading to inflammation and destruction of nearby tissues (18).

One key antibacterial mechanism within the innate immune system depends on phagocytes, including neutrophils and macrophages, engulfing and killing microorganisms. This defensive mechanism is very effective against many types of pathogens when they live as planktonic, individual organisms. However, this process is less effective when phagocytes encounter bacteria in biofilms—a phenomenon called frustrated phagocytosis (Fig. 3). When such "frustrated" macrophages and neutrophils encounter but cannot engulf bacteria in biofilms, they are activated and secrete toxic compounds that damage nearby healthy host tissues.

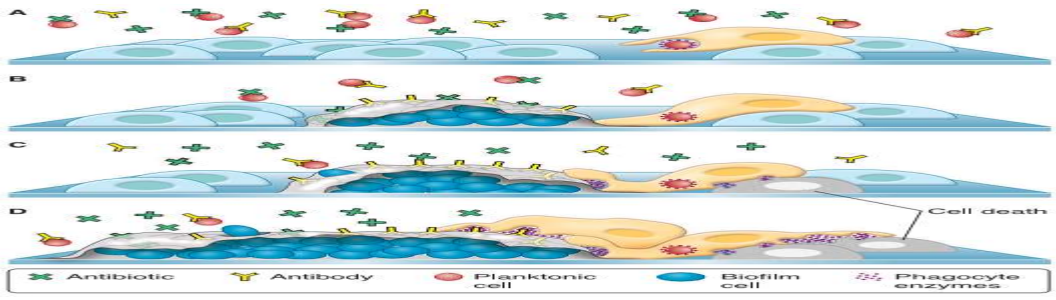

Figure 3: Antibacterial mechanism within the innate immune system

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Frustrated phagocytosis occurring as phagocytes responds to biofiolm organisms. (A) Representation of a human phagocyte responding to a planktonic infection red bacteria. Host antibodies, antibiotics, and the phagocyte cell are well equipped to kill these types of cells. As a biofilm develops (B-D), antibodies, antibiotics and even host phagocytes gain access to the bacteria within these communities, but the host phagocytes cannot engulf and kill the bacteria. Nonetheless, the biofilm pathogens are recognized by the phagocyte, which induces the release of enzymes and other toxic compounds, causing death of the healthy host cells (C-D). The necrotic debris can then become additional substrate for the biofilm community to expand (19).

4.2. The role of biofilms to antibiotic resistance

The development of a biofilm allows for the cells inside becoming more resistant to the body's natural antimicrobials as well as the antibiotics administered in a standard fashion. In fact, depending on the organism and type of antimicrobial and experimental system, biofilm bacteria can be up to a thousand times more resistant to antimicrobial stress than free-swimming bacteria of the same species (20). Bacteria in biofilms resist antibiotics via several mechanisms, including decreased penetration or diffusion of antimicrobial agents into biofilms, increased activity of multidrug efflux pumps, nutrient limitation and slow growth, involvement of quorum sensing systems, starvation or stress responses, and genetic switches that turn susceptible planktonic cells into antibiotic-resistant persisters (21).

5. BIOFILMS AND PATHOGENICITY

Pathogenic bacteria species that are found in the environment can form complex multicellular structures on surfaces known as biofilms. *Pseudomonas aeruginosa, Vibrio cholerae* and certain species of non-tuberculosis mycobacteria are examples of human pathogens that form biofilms in natural aquatic environments. The researcher findings show that the dynamics of biofilm formation facilitates the transmission of pathogens by providing a stable protective environment and for the dissemination of large numbers of microorganisms; both as detached biofilm clumps and by the fluid-driven dispersal of biofilm clusters along surfaces. The researcher also recommend that emerging evidence indicates that biofilm formation conveys a selective advantage to certain pathogens by increasing their ability to persist under diverse environmental conditions (22).

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Microbial biofilms constitute a major reason for infections to occur and persist at various sites in the human body, especially in association with medical devices. Antibiotic resistance of bacteria in the biofilm mode of growth contributes to the chronicity of infections such as those associated with implanted medical devices (20).

Medical devices such as intravascular catheters, Urinary catheters, prosthetic vascular grafts, cardiac devices, prosthetic joints, and shunts are reported to contribute in biofilm formation (23). Most potentially pathogenic bacteria tend to grow on indwelling medical devices. For example, *Staphylococcus aureus, S.epidermidis, P.aeruginosa, and K.pneumoniae* tend to grow on intravenous catheters(1, 9, 24), S *.epidermidis, Enterococcuss fecalis, E.coli, Proteus mirabilis, P,aeroginosa, K.pneumoniae* tend to grow on Urinary catheters (20). On the other hand, *Staphylococcus aureus, Enterococcus faecalis, Streptococcus, E. coli, Klebsiella, Pseudomonas, S. epidermidis* tend to grow on artificial joints and mechanical heart valves (25, 26). These organisms may originate from the skin of patient, health care worker, tap water to which entry ports are exposed or other source in the environment (27).

Biofilms add to the virulence of the pathogen. It has been estimated that the frequency of infections caused by biofilms, especially in the developed world, lies between 65% as reports from Centres for Disease Control and 80 % as reports from Prevention and National Institutes of Health (28). Such common infections like urinary tract infections caused by *E. coli* and other pathogens, catheter infections caused by *Staphylococcus aureus* and other gram-positive pathogens, child middle-ear infections caused by *Haemophilus influenzae*, common dental plaque formation, and gingivitis, all of which are caused by biofilms. Especially, endocarditis caused by *S. aureus* and infections in cystic fibrosis patients caused by *P. aeruginosa are* biofilm infections that cause serious morbidity and mortality (2). Among hospitalized patients, 8–10 % are susceptible to infection by opportunistic pathogenic bacteria such as *P. aeruginosa* and *S. aureus*, which are notorious for forming chronic, biofilm-based infections in their hosts (29).

Biofilms are also important as environmental reservoirs for pathogens, and the biofilm growth mode may provide organisms with survival advantages in natural environments and increase their virulence by different pathogenic mechanisms. These include: Allow attachment to a solid surface and "Division of labor" increases metabolic efficiency of the community, evade host

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defenses such as phagocytosis and obtain a high density of microorganisms. Exchange genes that can result in more virulent strains of microorganisms produce a large concentration of toxins, Protect from antimicrobial agents. Detachment of microbial aggregates transmits microorganisms to other sites (6). These biofilm activity has been recorded in various infections like native valve endocarditis, otitis media, eye infections, osteomyelitis, chronic wound infection, urinary tract infections, dental caries and Cystic fibrosis (9, 28).

5.1. Endocarditis

Inflammation of the smooth membranes which line the inside of the heart is caused by a complex biofilm composed of both bacterial and host components (30). The primary infectious lesion in endocarditis is a complex biofilm composed of both bacterial and host components located on a cardiac valve. This biofilm known as vegetation causes disease by three basic mechanisms. First, the vegetation physically disrupts valve function, causing leakage when the valve is closed and turbulence and diminished flow when the valve is open. Second, the vegetation provides a source for near-continuous infection of the bloodstream that persists even during antibiotic treatment. This causes recurrent fever, chronic systemic inflammation, and other infections. Third, pieces of the infected vegetation can break off and be carried to a terminal point in the circulation (a process known as embolization). The brain, kidney, and extremities are particularly vulnerable to emboli. Despite the fact that most cases are caused by antibiotic-susceptible organisms, successful treatment requires prolonged administration of intravenous antibiotics and, sometimes, surgical excision and replacement of the infected valve (31).

5.2.Inner ear infections

The majority of ear infections are caused by biofilm bacteria (3). These infections, which can be either acute or chronic, are referred to collectively as otitis media (OM). Biofilm formation is an important factor in the pathogenesis of chronic otitis media with effusion (32).

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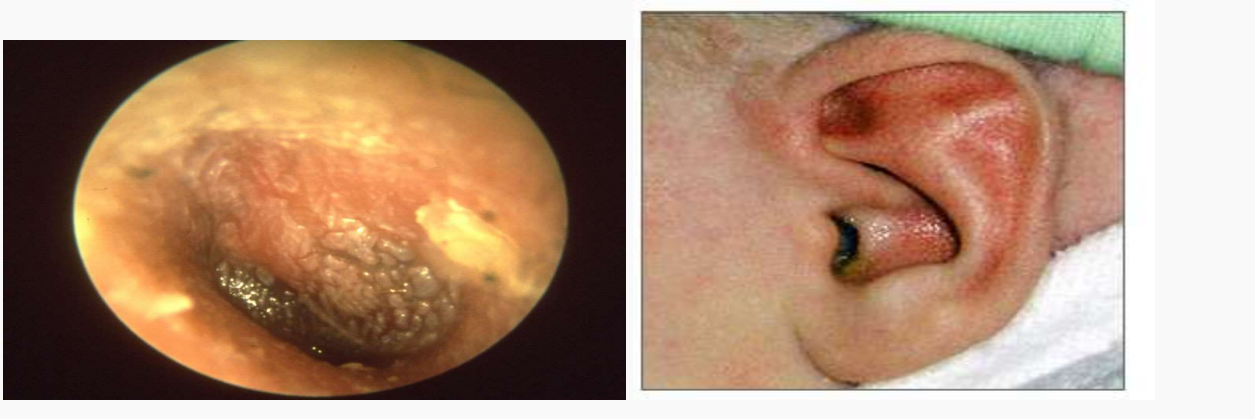

Figure 4: Otitis media or inflammation of the inner ear caused by biofilm (33, 34).

5.3.Chronic wound infection.

Chronic wounds including diabetic foot ulcers, pressure ulcers, and venous leg ulcers are a worldwide health problem. It has been speculated that bacteria colonizing chronic wounds exist as highly persistent biofilm communities. The sequential stages involved in the formation of a biofilm in a wound include the initial conditioning of the wound surface, irreversible adhesion of pioneering microorganisms via microbial adhesions and wound bed receptors, co-adhesion of secondary and tertiary colonizers and the development of a matrix of EPS which encases the attached or 'sessile' bacteria (35). Conditions within the chronic wound biofilm which can affect its balance include the interactions that occur with other microorganisms, as well as changes in pH, temperature, nutrient levels and the host's immune response. This enhanced growth of opportunistic pathogenic microorganisms within the wound biofilm. It represents an ecological shift in the wound microbiology resulting in an alteration of the once stable climax community. The microbiological shift will alter the homeostasis of the biofilm leading to potential overgrowth of problematic opportunistic wound pathogens. Many different species of bacteria have been cultured from chronic wounds including *Klebsiella* sp., *Enterococcus* sp., *Proteus* sp. and *Enterobacter cloacae* (36, 37). In addition to these, non-sporing Gram-negative anaerobic bacteria are often found in abundance in chronic wounds (38). Anaerobic bacteria that are commonly cultured have included *Peptostreptococcus*, *Bacteroides*, *Prevotella*, *Finegoldia*, *Peptoniphilus* and *Porphyromonas* species (34, 37).

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5.4.Urinary tract infections

Bacterial urinary tract infections represent the most common type of nosocomial infection. In many cases, the ability of bacteria to both establish and maintain these infections is directly related to biofilm formation on indwelling devices like Urinary catheters or within the urinary tract itself (39). Urinary catheters are tubular latex or silicone device that inserted through urethra into the bladder to measure the urine output and collect urine during surgery. Catheters may be open or closed system. In open system catheter gets contaminates and develop Urinary tract infections (UTIs) (40). One study (41) found that the percentage of patients undergoing indwelling urinary catheterization was 13.2 % for hospital patients, 4.9 % for nursing homes, and 3.9 % for patients receiving home care. The organisms commonly contaminating these device and developing biofilms are *S.epidermidis, Enterococcuss fecalis, , Proteus mirabilis, P,aeroginosa, K.pneumoniae* and strains of uropathogenic *Escherichia coli* (UPEC) increase urinary PH result enhancement of bacterial attachment (42). Among these, UPEC are the principal causative agents of UTIs, accounting for most community (up to 95 %) and hospital acquired (~50 %) infections (43-45).

5.5.Dental caries

Biofilm which accumulates on our teeth and which if left undisturbed can result in loss of tooth enamel and the formation of dental caries (cavities). The mouth is the natural habitat of over 500 identified species of microorganisms and a large number of these engage in forming the gummy coating on our teeth called plaque (6, 46). After a good dental cleaning, tooth enamel becomes coated with a variety of proteins and glycoproteins of host origin. This coating is called as acquired pellicle. Then the primary colonizers, first *streptococci* and later actinomycetes, colonize the surface of the teeth by adhesion molecules and pilli. Within the incredibly complex bioilm produced on teeth are microorganisms such as *Streptococcus mutans* which ferment sugars to lactic acid which causes cavities (6). The bacteria on the pellicle undergo cell to cell interaction via quorum sensing. A number of *streptococci*, including *Streptococcus mutans* and related organisms, begin to synthesize insoluble glucan via glucan binding protein. Bridge bacteria (members of the genus fusobacterium) form aggregates with primary colonisers. The late colonisers form aggregate with bridge bacteria. At this point of time, the biofilm consists primarily of non-pathogen bacteria. However, in the presence of dietary sucrose and other

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carbohydrate, acids are produced via fermentation, which leads to demineralization of the tooth enamel, over the time, caries. If the plaque is allowed to remain undisturbed on the teeth for several days, the microbial flora continues to change. The last colonisers of the biofilm are considered pathogenic because of their role in periodontal disease. The most important pathogens include *Porphyromonas gingivalis, Bacteriodes forsythus* and *Treponema denticola* (6, 46).

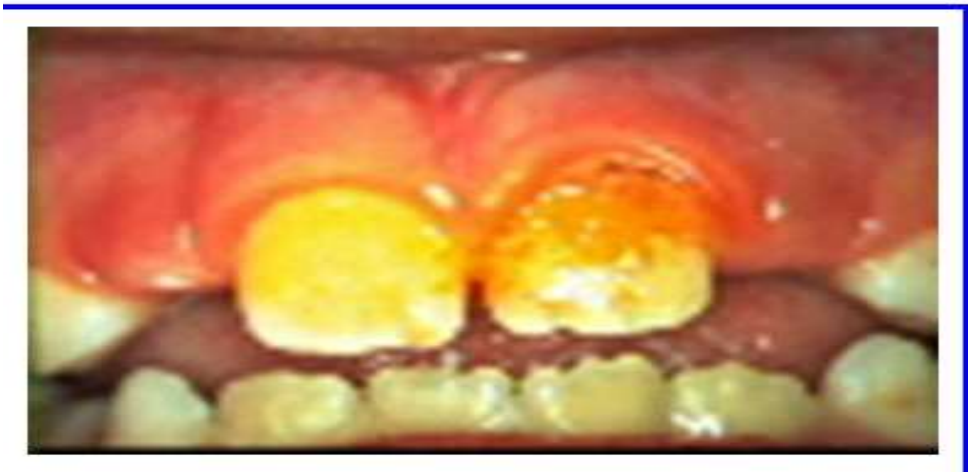

Figure 5: Dental plague (47).

5.6. Cystic fibrosis

Pulmonary colonization of the lower respiratory tract of Cystic fibrosis (CF) patients begins in infancy or early childhood, most commonly by *S. aureus* and *Haemophilus influenzae*. However, by adolescence and early adulthood most CF patients have become colonized with *P. aeruginosa* (48). Once within the lungs, these bacteria adapt to the environment and develop resistance to commonly used antibiotics. *Pseudomonas* can develop special characteristics that allow the formation of large colonies, known as "mucoid" *Pseudomonas*, which are rarely seen in people that do not have Cystic fibrosis (49). Infection by the bacterium *Pseudomonas aeruginosa* is the main cause of morbidity and mortality among patients with cystic fibrosis (50) .

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6. BIOFILM FORMATION BY DIFFERENT BACTERIAL PATHOGEN

6.1.*Staphylococcal* Biofilms

The genetic and molecular basis of biofilm formation in *staphylococci* is multifaceted. The ability to form a biofilm affords at least two properties: the adherence of cells to a surface and accumulation to form multilayered cell clusters. A trade mark is the production of the slime substance polysaccharide intercellular adhesin (PIA) , a polysaccharide composed of beta - 1,6 - linked N-acetyl glucosamines with partly diacetylated residues, in which the cells are embedded and protected against the host's immune defense and antibiotic treatment (51). S*taphylococci* are frequent commensal bacteria on the human skin and mucous surfaces (and those of many other mammals). Thus, *Staphylococci* are among the most likely germs to infect any medical device that penetrates those surfaces mediated by PIA, such as when being inserted during surgery. Therefore *Staphylococci* are recognized as the most frequent causes of biofilm-associated infections (52). It is estimated that ≤60 % of nosocomial infections are derived from biofilm-related infections, many of which are caused by coagulase-negative *Staphylococci* (4). *Staphylococcus epidermidis* and *S. aureus* are the most frequent causes of nosocomial infections and infections on indwelling medical devices, which characteristically involve biofilms (53).

6.1.1. *Staphylococcus epidermidis* biofilms

Staphylococcus epidermidis is an opportunistic pathogen associated with foreign body infections and nosocomial sepsis. The pathogenicity of *S. epidermidis* is mostly due to its ability to colonize indwelling polymeric devices and form a thick, multilayered biofilm. Biofilm formation is a major problem in treating *S. epidermidis* infection as biofilms provide significant resistance to antibiotics and to components of the innate host defenses. Various cell surface associated bacterial factors play a role in adherence and accumulation of the biofilm such as the PIA and the autolysin AtlE. Furthermore, QS system have an important function in the regulation of biofilm formation (54).

The opportunistic human pathogen *Staphylococcus epidermidis* has become the most important cause of nosocomial infections in recent years. Its pathogenicity is mainly due to the ability to form biofilms on indwelling medical devices. In a biofilm, *S. epidermidis* is protected against

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attacks from the immune system and against antibiotic treatment, making *S. epidermidis* infections difficult to eradicate (52).

6.1.2. *Staphylococcus aureus* biofilms

Staphylococcus aureus is an opportunistic pathogen, which forms biofilms on medical devices and causes pneumonia, meningitis, endocarditis, osteomyelitis and septicemia (51). The biofilm formation by *S. aureus* involves complex processes. The biofilm cells are held together and exhibit an altered phenotype with respect to bacterial physiology, metabolism and gene transcription (9). Most *S. aureus* strains that have been reported so far contain the *ica* operon. The expression of the *ica* operon and subsequent biofilm formation is strongly influenced by a variety of external conditions, including nutrient supply, osmolarity, temperature and sub-inhibitory concentrations of certain antibiotics (55).

Staphylococcus aureus biofilm mode of growth is tightly regulated by complex genetic factors. Host immune responses against persistent biofilm infections are largely ineffective and lead to chronic disease. In the human population, approximately 20–25 % have become persistently colonized and 75–80 % intermittently or never colonized (56). Invading *staphylococci* are then either removed by the host innate immune response or attach to host extracellular matrix proteins and form a biofilm. The cellular physiology is then quickly transformed into one reflective of a biofilm. Owing to the escalating involvement of *Staphylococcus aureus* in foreign body-related infections, the swift development and exhibition of multiple-antibiotic resistance, and their predilection to transform from an acute infection to one that is persistent, chronic and recurrent infection (57).

Staphylococcus aureus has re-emerged as a clinically relevant pathogen due to its resistance to antibiotics and the increased use of indwelling medical devices. Infections associated with *S. aureus* in the US have a crude mortality rate of 25 % along with hospitalizations resulting in approximately twice the length of stay, deaths and medical costs of typical hospitalizations (58). *S. aureus* biofilms, once established, are recalcitrant to antimicrobial treatment and the host response, and therefore are the etiological agent of many recurrent infections (55).

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6.2. *Enterococci* biofilm

Enterococci are an important global cause of nosocomial infections, being increasingly associated with urinary tract infections, endocarditis, intra-abdominal and pelvic infections, catheter-related infections, surgical wound infections, and central nervous system infections. The two most common enterococci species are *Enterococcus faecalis* and *Enterococcus faecium*. Both are capable of producing biofilms, which consist of a population of cells attached irreversibly on various biotic and abiotic surfaces, encased in a hydrated matrix of exopolymeric substances. Many environmental and genetic factors are associated or have been proposed to be associated with the production of biofilm (48).

Enterococci, recognized as opportunistic pathogens, are natural inhabitants of the oral cavity, normal intestinal microflora, and female genital tract of both human and animals. They are common nosocomial agents that infect the urinary tract, bloodstream, intra-abdominal and pelvic regions, surgical sites and central nervous system (59). *Enterococcus faecalis* is the most common enterococci species, and it is responsible for 80–90 % of human enterococcal infections (60).

6.3. *Escherichia coli* biofilm

Biofilm production in *E. coli* may promote colonization and lead to increased rate of UTIs. Such infections may be difficult to treat as they exhibit multi-drug resistance. (61).

Urinary tract infections pose a serious health threat with respect to antibiotic resistance and high recurrence rates. *Escherichia coli* is the predominant organism causing urinary tract infections. UPEC forms intracellular bacterial communities with many biofilm-like properties within the bladder epithelium (62). These intracellular biofilm-like pods allow bacteria to outlast a strong host immune response to establish a dormant reservoir of pathogens inside the bladder cells. Re-emergence of bacteria from this reservoir might be source of recurrent infections (61).

6.4. *Streptococcus pneumonia* biofilm

S. pneumoniae is the main cause of community-acquired pneumonia and meningitis in children and the elderly, and of septicemia in HIV-infected persons. When *S. pneumonia* grows in biofilms, genes are specifically expressed that respond to oxidative stress and induce

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competence. Formation of a biofilm depends on competence stimulating peptide (CSP). CSP also functions as a quorum-sensing peptide. It not only induces biofilm formation, but also increases virulence in pneumonia and meningitis (63).

It has been proposed that competence development and biofilm formation is an adaptation of *S. pneumoniae* to survive the defenses of the host (64). In particular, the host's polymorphonuclear leukocytes produce an oxidative burst to defend against the invading bacteria, and this response can kill bacteria by damaging their DNA. Competent *S. pneumoniae* in a biofilm have the survival advantage that they can more easily take up transforming DNA from nearby cells in the biofilm to use for recombinational repair of oxidative damages in their DNA. Competent *S. pneumoniae* can also secrete an enzyme (murein hydrolase) that destroys non-competent cells causing DNA to be released into the surrounding medium for potential use by the competent cells (65).

6.5. *Pseudomonas aeruginosa* Biofilms:

Pseudomonas aeruginosa has been shown to form biofilms on a number of surfaces, including the tissues of the CF lung and on abiotic surfaces such as contact lenses and catheter lines. This ubiquitous organism is also the cause of nosocomial infections in immunocompromised patients and individuals with severe burns (66).

Pulmonary colonization of the lower respiratory tract of CF patients begins in infancy or early childhood, most commonly by *S. aureus* and *Haemophilus influenzae*. However, by adolescence and early adulthood most CF patients have become colonized with *P. aeruginosa* (48).

P. aeruginosa is the principal pathogen in the lungs of patients with CF. Chronic colonization by this bacteria leads to progressive lung damage and eventually respiratory failure and death in most CF patients (6). As shown on figure 6, *Pseudomonas* is able to set up permanent residence in the lungs of patients with CF. Eventually, chronic inflammation produced by the immune system in response to *Pseudomonas* destroys the lung and causes respiratory failure. In the permanent infection phase, *P. aeruginosa* biofilms are thought to be present in the airway, although much about the infection pathogenesis remains unclear.

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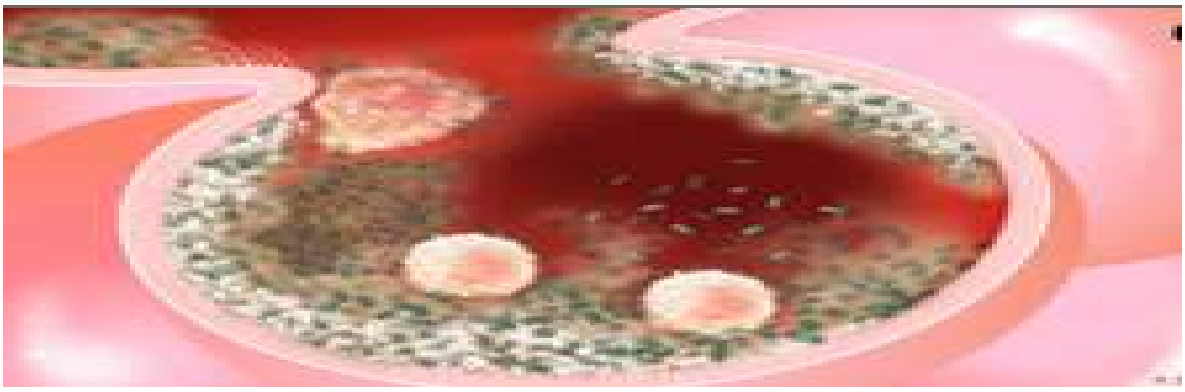

Figure 6: When the immune response is compromised, *Pseudomonas aeruginosa* biofilms are able to colonize the alveoli, and to form biofilms (34).

Antibiotic therapy in patients colonized with *P. aeruginosa* often gives a measure of relief from symptoms but fails to cure the beset ongoing infection. This is because the antibiotic therapy cannot eliminate the antibiotic resistant sessile biofilm communities. In *Pseudomonas aeruginosa* biofilms, the polysaccharide alginate protects biofilm bacteria from macrophage engulfment and killing (figure 7). When macrophages encounter *P. aeruginosa* biofilm bacteria that lack alginate, they can be engulfed and destroyed. However, if the biofilm bacteria transition to a mucoid phenotype, where alginate is present, the macrophages still respond to the presence of the pathogen community, but are no longer able to engulf and kill these organisms (19).

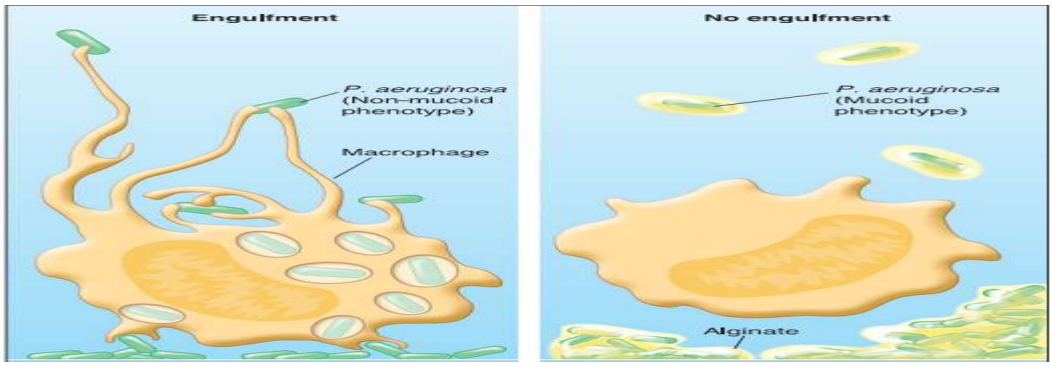

Figure 7: *Pseudomonas aeruginosa* biofilms, the polysaccharide alginate protects biofilm bacteria from macrophage engulfment and killing (19).

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6.6. *Mycobacterium tuberculosis* biofilms

Successful treatment of human tuberculosis requires 6–9 months' therapy with multiple antibiotics. Incomplete clearance of tubercle bacilli frequently results in disease relapse, presumably as a result of reactivation of persistent drug-tolerant *Mycobacterium tuberculosis* cells. Recently, it has been shown that several *mycobacterial* species, have strong propensity to grow *in vitro* as biofilms, which provide a microenvironment that promote development of drug tolerant persisters. While fast growing environmental species such as *M. smegmatis* can readily form biofilms in detergent-free media, the slow growing pathogenic species *M. tuberculosis* require specific environmental and genetic requirements distinct from those for planktonic growth, which contain an extracellular matrix rich in free mycolic acids, and harbour an important drug-tolerant population that persist despite exposure to high levels of antibiotics. *M. tuberculosis* biofilm formation as a potential new target for drugs that facilitate the use of current anti-tuberculosis antibiotics administered in ultra-short regimens (67, 68) and *M. tuberculosis* biofilm development represent an excellent drug target (69).

6.7. *Vibrio cholera* biofilm

Vibrio cholerae is a diarrheal pathogen and a natural inhabitant of fresh- and saltwater environments. Seasonal outbreaks in areas of endemicity that can develop into worldwide pandemics are linked to the persistence of *V. cholerae* in aquatic ecosystems, providing a reservoir for the initiation of new cholera epidemics via human ingestion of contaminated food or water (70).

Biofilm formation is dependent on the production of an exopolysaccharide (EPS) and the previous finding by the Klose laboratory that a flagellum-dependent pathway induces EPS expression has led to their surprising discovery reported in this issue: the sodium-driven flagellar motor is intimately involved in EPS expression, biofilm formation, and the virulence of *V. cholerae*. The EPS signaling cascade appears to operate through a flagellar motor-based mechanism (71) adapted by bacteria to induce appropriate behaviors on solid surfaces and perhaps additional behaviors in the diverse microenvironments encountered during their life cycle (70).

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7. DETECTION OF BIOFILM

The identification of biofilms in persistent infections may assist in deciding suitable therapies. A large number of techniques are being used to study biofilms. The diagnosis starts with establishing the surface-associated biofilms using bright-field microscopy, epifluorescence microscopy, scanning electron microscopy. Confocal laser scanning microscopy (CLSM) has further made it easy to carry out *in situ* examination of biofilms using lower magnification (41).The clinical and laboratory parameters for diagnosing biofilm infections are outlined based on the patient's history, signs and symptoms, microscopic findings, culture-based or culture-independent diagnostic techniques and specific immune responses to identify microorganisms known to cause biofilm infections (72).

With the emergence of biofilm associated diseases, there are considerable diagnostic problems for the clinical laboratory. These problems can be classified into five categories: false negative cultures, visible but non cultivable organisms, underestimated or low colony count, inappropriate specimen and loss of or decreased antimicrobial susceptibility. Biofilms are resilient, adherent and with EPS, quite resistant to culturing by swabs (6). But there are various methods to detect biofilm production like Tissue Culture Plate (TCP), Tube method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination (73, 74). One study (73) found that the TCP method was considered to be superior to TM and CRA. According to this study, from the total of 110 clinical isolates, TCP method detected 22.7 % as high, 41% moderate and 36.3 % as weak or non-biofilm producers.

8. TREATMENT OF MICROBIAL BIOFILM INFECTIONS

In vitro experiment showed that young biofilm could be easily cleared by antibiotic treatment compared to the matured biofilm. Therefore early and aggressive antibiotic treatments are recommended for biofilm infections. However, early diagnosis of biofilm infection is currently difficult and most of the clinical biofilm infections are actually matured biofilms which are usually difficult to eradicate with antibiotic treatment (75). It is therefore important and crucial to legitimately apply currently available antibiotics in the treatment of biofilm infections. Treatment of biofilm infection requires sensitive and well-penetrating antibiotics to ensure a sufficient concentration of effective antibiotic at the site of biofilm infection and combination therapy of antibiotics against biofilm infection was significantly better than antibiotic monotherapy (76).

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9. CONTROL AND PREVENTION OF BIOFILM INFECTION

Biofilms are medically important, accounting for over 80 % of microbial infections in the body. Yet bacterial biofilms remain poorly understood and strategies for their control remain underdeveloped. Standard antimicrobial treatments typically fail to eradicate biofilms, which can result in chronic infection and the need for surgical removal of afflicted areas. The need to create effective therapies to counter biofilm infections presents one of the most pressing challenges in anti-bacterial drug development (77). However biofilms can be prevented by early aggressive antibiotic prophylaxis or therapy and they can be treated by chronic suppressive therapy. A promising strategy may be the use of enzymes that can dissolve the biofilm matrix (e.g. DNase and alginate lyase) as well as quorum-sensing inhibitors that increase biofilm susceptibility to antibiotics (78).

Currently intervention strategies are designed to prevent or control biofilms on medical devices are prevent initial device colonization, minimize microbial cell attachment to the device, penetrate the biofilm matrix and kill the associated cells, or remove the device from the patient. Recent advances focus on bacteriophages as specific and effective therapeutic agents with lytic action against target bacteria. Thus, combination of antibiotics and bacteriophage application has been suggested as a valuable approach for biofilm control (28).

10. CONCLUSION AND RECOMMENDATION

The nature of biofilm structure and the physiological attributes of biofilm organisms confer an inherent resistance to antimicrobial agents, whether these antimicrobial agents are antibiotics or disinfectants. Biofilms are also resistant to phagocytosis, and the phagocytes that attempt an assault on the biofilm may actually do more harm to surrounding tissues than to the biofilm itself (7). But bacterial biofilms remain poorly understood and strategies for their control remain underdeveloped spatially developing countries. So much work is required to devise ways to prevent their occurrence and clear them from the host.

All of the chronic infections described in this review share the fundamental characteristics of all bacterial biofilms. Treatment of biofilm infections is currently a difficult and complicated challenge for microbiologists and clinicians. Therefore, it is recommended to prevent their

formation rather than treatment. Further study on the biofilm also needed that effective control strategies to prevent the formation of biofilm and effective treatment strategies for complete eradication of biofilms.

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