Bacterial biofilms: Medical impact, development, control and threats

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Bacterial biofilm has great medical and industrial impact and is of concern as ultimately it not only affects human health and social life but equally distresses the performance and productivity of industrial organizations. Biofilm facilitates bacterial cell to adopt a provisional lifestyle to survive even under adverse environmental state. The bacterial cells in a biofilm have been reported to possess great deal of genetic energy, supporting them in growth and development. Considerable attention towards the development, control and threat of bacterial biofilm upon human lives have been reviewed and acknowledged. Recent advances in the diagnosis and treatment of biofilm related bacterial infections also highlight importance of bacterial biofilm and the challanges. Accordingly, a number of pathogens responsible for some disease have now been identified and studied to find possible reasons of biofilm formation and its eradication. Some very common examples include; *Pseudomonas aeruginosa* (upper respiratory infections), *Escherichia coli* and *Klebsiella pneumoniae* (urinary tract infections), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (wound infection). Bacterial cells which stick to the surface of various organs and tissues with the formation of biofilm accumulate in an extracellular polymeric substances (EPS) matrix for their own growth and survival. The changeover from acute to chronic bacterial infections is frequently associated with the biofilm formation. Bacteria in biofilms are characteristically more resistant to antibiotics or antibacterial agents. The presence of indwelling medical devices further increases the risk for biofilm formation and subsequent infection, such as *Escherichia coli*, *Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae and Proteus mirabilis* are most commonly responsible for catheter-induced and other device-associated infections. The exterior of medical tools have been indetified as a hub for device related infections exposing the presence of large number of slime encased bacteria and can be seen by electron microscopy. At present, therapy of complicated and multiple bacterial infections using even most potent antibiotics are of limited effectiveness in resolving biofilms infection. A number of bioactive compounds (such as alkaloids and flavonoids) especially from plants and marine sources are also under investigations to get a breakthrough to control biofilm formation. Oral health has also been reported as one of the leading discipline affected by the bacterial biofilm formation with the development of dental caries and periodontitis by certain species of Gram positive organism, such *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus oralis*, *Rothia dentocariosa*, and *Staphylococcus epidermidis*, as well as Gram negative species, such as *Actinomyces viscosus*, *Actinomyces israelis*, *Actinomyces gerencseriae*. With all these aims and objectives, present chapter has been written to highlight its medical impact to review the phases in bacterial biofilm formation, their pathogenic mechanisms, effect of certain new antibiotic or antibacterial agents, detection and eradication as well as the threats associated with biofilms.

Keywords: Bacteria; Biofilms; Gram positive & Gram negative organisms; Medical devices; EPS; Bioactive alkaloids & flavonoids; Antibiofilm enzymes

1. Introduction

Bacterial biofilms are now being regarded as one of the most strenuous discipline in medicine and medical science in view of its prodigious impact and role in the development and treatment of chronic infections. Historically, bacterial infections have been cogitated to be caused by various species occurring either as segregated cells (planktonic form) or present in tiny bunches (sessile aggregates) fastened exclusively by extracellular adhesive appendages. However, the biochemical and physiological statuses of these bacterial species are often thought to match those, grown in the laboratory under ideal conditions using selective or differential growth media. This statement is still quite relevant and appropriate while diagnosing the acute bacterial infections which involve planktonic bacteria and generally respond well with antibiotics and are thus treatable. Yet, many chronic or recurrent infections which are usually unmanageable to single antibiotic therapy or some time even difficult to treat with the combined or multi-antibiotic therapy because bacteria may have succeeded in forming a biofilm within the human host need more in-depth understanding. Intense resistance to most common antibiotics and synthetic antibacterial agents, is one of the leading characteristic feature of these chronic biofilm-based infections which have extreme capacity for shirking the host defences. Biofilms are usually observed as oppressive and inflexible structure of exopolysaccharides and proteins (organic molecules), commonly known as "Extracellular Polymeric Substances" (EPS). The EPS may contain nucleic acid, phospholipids and humic substances as well and constitutes around 50% to 90% of a biofilm's total organic matter [1-3]. During the last two decades, a number of research papers have been published with different aspects to correlate how bacterial biofilms exist under favourable conditions (stable ecological habitats) and how they may survive in unfavourable conditions (unstable ecological habitats), such as infections. The biofilms have now been established to cause or being associated with a number of chronic infections, including nosocomial infections coupled with medical equipment or devices. Even the household equipment, cutting boards, kitchen sinks and serving countertops may act as reservoirs. Despite the fact

that bacteria may have a similar lifestyle in both the habitats, yet the hostility for existence and authority to produce the power of resistance against antibiotics may be different.

Considerable evidence exists in the scientific literature that implicates biofilms as being responsible for a variety of chronic infections associated with medical devices, hospital equipment, and other hard surfaces. There is some strong evidence linking biofilms to diseases such as otitis media (OM) associated with *Haemophilus influenzae*, bacterial endocarditis linked with *Streptococcus mutans* or *Streptococcus gordonii*, and *Legionella pneumophila* related Legionnaire's disease as well [4-6]. Biofilms have also been found in patients with cystic fibrosis caused by *Pseudomonas aeruginosa* [7]. More details on biofilm forming organisms have been depicted in Table – 1. Almost 99.9% of all bacterial species have been reported to live as community and attached to surface as biofilm in their natural habitat. According to The National Institute of Health report published, around 80% of all chronic infections are linked to biofilms [8-9]. Review of literature has indicated that the concept and existence of biofilm was present even before the commercial launching of antibiotic, "Penicillin" [10-11] or perhaps among the first to be noticed throughout the initial progress of microbiology—the "Animalculi" (the lowly or tiny animal) that Anton von Leeuwenhoek perceived with a microscope was plaque biofilm from teeth. However, it was not until the 1970s when biofilms were taken into consideration from academic point of view [12].

It is now well established that the development of biofilms by pathogenic bacterial species, can be of significant health hazard often leading to recurrent infections. Biofilms have now been projected as highly efficient and effective mode for the survival of bacteria, even in hostile immune conditions and presence of potent antibiotics or antibacterial drugs. In view of the significance of biofilm in the development of chronic infections and challenges in their treatment and ever increasing threats, focus has been given to highlight various aspects to understand the concept and overcome challenges.

2. Medical impact

Bacterial biofilms are now being critically evaluated in terms of their nature and role in the development of various diseases and the treatment with antibiotics. It has high impact due to its capability to reduce susceptibility to antibiotics or antibacterial drugs and develop the wide range of antimicrobial resistance. In addtion, biofilm forming organisms are now well documented to participate and hold prominent position in the development of multiple infectious diseases, particularly in their flimsiness and reappearance. Biofilm has impact on society as well. According to a review report, over 99% of the world's bacterial populations have been thought to live in form of biofilms and have the great capacity to infect medical devices, thus contributing around 60% of the hospital-acquired infections (HAIs) associated with the use of orthopaedic prostheses and intravascular catheters, common implantable biomedical devices. Around 6% of the patients with infected orthopaedic implants have been reported to require admission in ICU, thus increasing the mortality rate to about 4.6%. It is further reported that the infected biomedical devices with biofilms are associated with increased mortality in other infectious diseases as well. Cardiovascular medical devices contribute highest mortality percentage, which ranges around 5% for vascular grafts to around 25% in heart assist devices (such as, mechanical assist devices, ventricular assist devices – VAD, left ventricular assist devices – LVAD, total artificial hearts – TAH or simply heart pumps) [13]. Some of the common biofilm forming bacterial species and the respective disease have been depicted in Table – 1. The findings highlighted in the table provide ample evidence to support that the biofilm produced by various bacterial pathogens can significantly contribute in the development of various diseases which are difficult to manage, thus having great medical impact and are required to be addressed.

Table 1 List of some common biofilm forming bacterial species and related disease or infections.

Name of Disease / Infection	Common bacterial species involve in biofilm formation	Classification and Morphology
Muscluskeletal		
Osteomylitis, Cellulitis, Brusitis, Septic arthritis etc.	*Staphylocouus aureus* *Pseudomonas aeruginosa* *Salmonella species* *Streptococcus pyogenes* *Streptococcus pneumoniae*	Gram-positive round cocci Gram-negative motile rods Gram-negative rods Gram-positive spherical or oval cocci Gram-positive spherical or oval cocci
Cardiovascular		
Endocarditis / Native Valve Endocarditis etc.	*Streptococcus viridans* *Streptococcus gordonii* *Staphylocouus aureus* *Staphylococcus epidermidis* *Haemophilus species* *Actinobacillus actinomycetemcomitans,* *Cardiobacterium hominis*	Gram-positive spherical or oval cocci Gram-positive spherical or oval cocci Gram-positive round cocci Gram-positive round cocci Gram-negative coccobacilli Gram-negative rods Gram-negative rods

Respiratory		
Upper & Lower RTI – Otitis media, Tonsillitis, Cystic fibrisis, Legionnaire's disease etc.	*Streptococcus pneumoniae* *Haemophilus influenzae* *Moraxella catarrhalis* *Klebsiella penumoniae* *Pseudomonas aeruginosa* *Burkholderia cepacia* *Legionella pneumonia*	Gram-positive spherical or oval cocci Gram-negative coccobacilli Gram-negative coccobacilli Gram-negative rods Gram-negative motile rods Gram-negative rods Gram-negative thin rods
Gastrointestinal		
H. Pylori infection, Biliary Tract Infection, Peritonitis etc.	*Helicobacter pylori* *Escherichia coli* *Bacteroides fragilis* *Fusobacterium species*	Gram-negative rods Gram-negative rods Gram-negative rods Gram negative fusiform rods
Genito-Urinary		
Prostatitis, Cystitis, Urethritis, pylonephritis etc.	*Escherichia coli* *Proteus mirabilis* *Staphylococcus epidermidis* *Enterococcus faecalis* *Pseudomonas aeruginosa*	Gram negative rods Gram-negative rods Gram-positive round cocci Gram-positive spherical or oval cocci Gram-negative motile rods
Skin & Soft Tissue		
Necrotizing fascitis, wounds etc	*Streptococcus pyogens* *Staphylococcus aureus* *Vibrio vulnificus* *Clostridium pefringens* *Bacteroides fragilis*	Gram-positive spherical or oval cocci Gram-positive round cocci Gram-negative curved motile rods Gram-positive encapsulated rods Gram-negative rods
Public Health		
Meliodiosis	*Burkholderia pseudomallei*	Gram-negative bipolar motile rods
Oral Helath		
Dental caries, Periodonititis etc.	*Streptococcus mutans* *Lactobacilli species* *Porphyromonas gingivalis*. *Actinobacillus actinomycetemcomitans* *Bacteroides forsythus*	Gram-positive spherical or oval cocci Gram-positive rods Gram-negative rods Gram-negative rods Gram-negative rods
Hospital Acquired Infections		
Arteriovenous shunts,	*Staphylocouus aureus* *Staphylococcus epidermidis*	Gram-positive round cocci Gram-positive round cocci
Biliary stent blockage	*Enterococcus species* *Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella species* *Enterobacter species*	Gram-positive spherical or oval cocci Gram-negative rods Gram-negative motile rods Gram-negative rods Gram-negative rods
Central venous catheters	*Staphyloccus aureus* *Staphylococcus epidermidis* *Enterococcus faecalis* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Pseudomonas aeruginosa*	Gram-positive round cocci Gram-positive round cocci Gram-positive spherical or oval cocci Gram-negative motile rods Gram-negative rods Gram-negative motile rods
Contact lens	*Staphylococcus aureus*	Gram-positive round cocci
Endotracheal tubes / Ventilator	*Acinetobacter baumannii* *Pseudomonas aeruginosa* *Staphylocouus aureus* *Streptococcus pneumoniae*	Gram-negative coccobacilli Gram-negative motile rods Gram-positive round cocci Gram-positive spherical or oval cocci
Exit sites	*Staphylocouus aureus* *Staphylococcus epidermidis*	Gram-positive round cocci Gram-positive round cocci
Hickman catheter	*Staphylococcus epidermidis*	Gram-positive round cocci

IUDs	*Staphylococcus aureus*	Gram-positive round cocci
	Staphylococcus aureus	Gram-positive round cocci
	Corynebacterium sp.	Gram-positive rods
	Enterococcus sp.	Gram-positive spherical or oval cocci
	Streptococcus agalactiae	Gram-positive spherical or oval cocci
Mechanical heart valves	*Streptococcus viridans*	Gram-positive spherical or oval cocci
	Streptococcus gordonii	Gram-positive spherical or oval cocci
	Streptococcus mutans	Gram-positive spherical or oval cocci
	Staphylococcus aureus	Gram-positive round cocci
	Enterococcus sp.	Gram-positive spherical or oval cocci
Orthopedic devices	*Streptococcus pyogenes*	Gram-positive spherical or oval cocci
	Enterococcus sp.	Gram-positive spherical or oval cocci
	Proteus mirabilis	Gram-negative rods
	Bacteroides sp.	Gram-negative rods
	Pseudomonas aeruginosa	Gram-negative motile rods
Peritoneal dialysis peritonitis	*Escherichia coli*	Gram-negative rods
	Staphylococcus aureus	Gram-positive round cocci
	Staphylococcus epidermidis	Gram-positive round cocci
	Bacteroides fragilis	Gram-negative rods
Penile prostheses	*Staphylococcus aureus*	Gram-positive round cocci
	Staphylococcus epidermidis	Gram-positive round cocci
Sutures	*Staphylococcus aureus*	Gram-positive round cocci
	Staphylococcus epidermidis	Gram-positive round cocci
Sceleral Buckles	*Staphylococcus aureus*	Gram-positive round cocci
	Staphylococcus epidermidis	Gram-positive round cocci
	Pseudomonas aeruginosa	Gram-negative rods
	Corynebacterium sp.	Gram-positive rods
Urinary catheter cystitis	*Escherichia coli*	Gram negative rods
	Proteus mirabilis	Gram-negative rods
	Staphylococcus epidermidis	Gram-positive round cocci
	Enterococcus faecalis	Gram-positive spherical or oval cocci
	Pseudomonas aeruginosa	Gram-negative motile rods
Vascular grafts	*Staphylococcus aureus*	Gram-positive round cocci
	Staphylococcus epidermidis	Gram-positive round cocci
	Pseudomonas sp.	Gram-negative rods
	Klebsiella sp.	Gram-negative rods

3. Development of biofilms

The biofilm development has been report to happen gradually over a period in stages with characteristic features. The biofilms have been noted to possess substantial adherent property and are quite strong in nature, even resistant to culturing by swabs. For simplicity, most investigators have divided the development into several genetically distict stages as described below [14-16]. The advancement in bacterial physiology and genetics as well as molecular biology has contributed well to understand the mechanism of biofilm formation. The biofilm formation is greatly dependent upon both environmental and genetic constraints. The behaviour of an individual organism differs to a great extent with respect to environmental conditions under which it will produce maximum level of biofilm. This ultimately reflects the biological or ecological properties of the organism and as a result many laboratory bacterial strains have been noted to produce only frail or week biofilm as compared to the wild strains of the same species [16].

3.1 Stage one and two

The formation of biofilm starts with the surface attachment, followed by adhesion or monolayer formation (Fig. 1,2). Significant change in biochemical parameters can be noted due to change in environmental signalling relating to pH, temperature, osmolality and nutritional condition. This extended an opportunity to the bacterial cell to get attach to a living tissue, surface or medical device etc. The stage is reversible and the bacterial cell may repeatedly detaches and then reattaches before entering into next stage. Various long and short forces, such as van der Waal's forces, gravitational forces, hydrogen bonding and hydrophobic interactions etc., have been reported to contribute in this reversible and non-specific association [15].

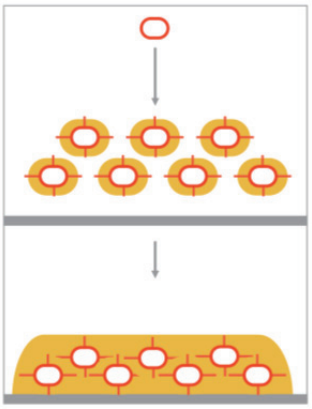

Fig. 1

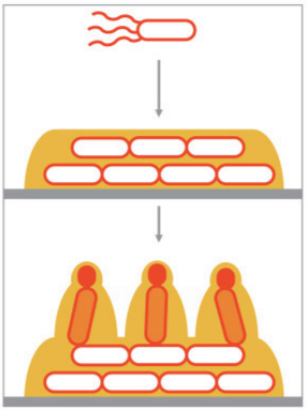

Fig. 2

Fig. 1-2 Stage one and two of biofilm development. Authors would like extend their thanks to Dr. Roberto Kolter, Professor of Microbiology, Harvard Medical School for granting permission to use Fig. 1 & 2 in the chapter. **Adopted from:** Lemon KP, Earl AM, Vlamakis HC, Aguilar C, Kolter R. Biofilm Development with an Emphasis on *Bacillus subtilis*. *Current Top Microbiol Immunology*. 2008; 322: 1–16 [16].

Fig. 1, 2 represent a proposed model to fit two general modes of bacterial lifestyle or lifestyle of unicellular organism, i.e., nonmotile and motile. Fig.1 represents the mechanism of biofilm formation of nonmotile organisms, while Fig. 2, describes that of motile one. In the case of non-motile species (Fig. 1), when conditions are favourable for biofilm formation, organisms appear to increase the expression of adhesions on their outer surface, i.e., they increase their "adhesiveness". This increased adhesiveness promotes both cell-cell adherence and cell-surface adherence when these organisms encounter a surface. Whereas, in case of nonmotile species, the individual organism is supposed to localize to a surface and initiate an amazing lifestyle switch when conditions favour the biofilm formation. The motility of organism is lost and bacteria begin to produce EPS that holds the cells together [16].

3.2 Stage three and four

During stage three and four, cementing the cell and colonization occurs, i.e., migration of organism to form multi-layered micro-colonies and production of extracellular matrix takes place. As colonization starts, the biofilm moves towards maturation through a mechanism in which both the cell division and recruitment are involved. The EPS matrix typically enfolds bacterial biofilms.

3.3 Stage five

The fifth and final stage is designated as fully maturated and functioning biofilm with characteristic three-dimensional architecture. The stage is also called dispersion. Although, the biofilms are fully matured and established, yet may change shape and size. The development of a biofilm, thus may permit for a cumulative cell colony (or colonies) to respond increased resistance against antibiotic. In the whole process, quorum sensing (QS) or cell-cell communication has been reported to play important role in the formation of biofilms in a number of bacterial species. The overall stages of biofilm formation has been depicted in Fig. 3. Once the biofilms reach to a fully mature stage, it starts sheltering planktonic bacteria, microcolonies and fragments of biofilm, which can then diffuse and attach to a new surface or tissue or in a specific parts of an organ or to a wound, forming new biofilm colonies [3, 12].

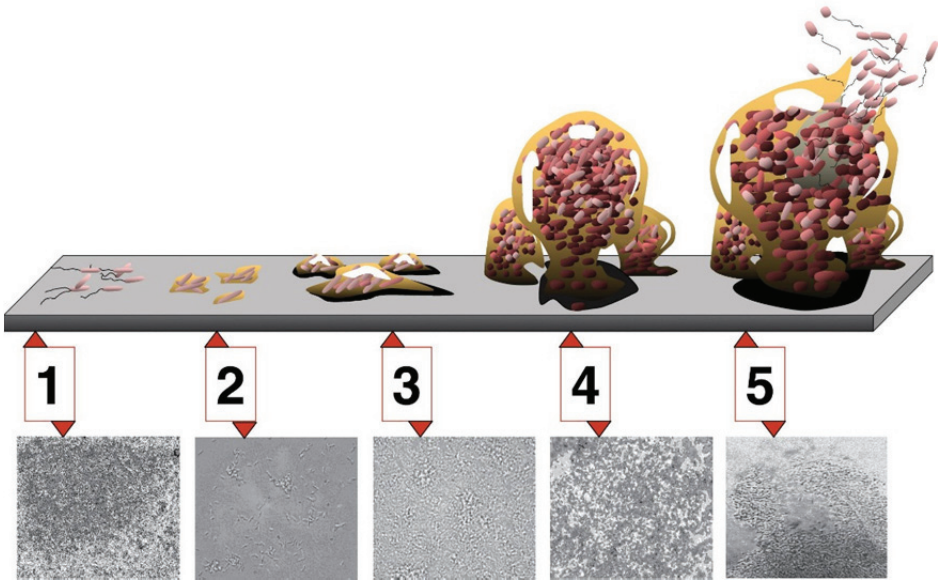

Fig. 3 Five different stages in the development of bacterial biofilm. Stage 1, initial attachment; stage 2, irreversible attachment; stage 3, maturation I; stage 4, maturation II; stage 5, dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing *Pseudomonas aeruginosa* biofilm. **Adopted from:** Monroe D (2007) Looking for Chinks in the Armor of Bacterial Biofilms. PLoS Biol 5(11): e307. doi:10.1371/journal.pbio.0050307 [17].

4. Control

Biofilm related bacterial infections have appeared as a key public health concern in recent years due to highly resistant nature of biofilm-growing cells against drugs (antibiotics) and immune response (host immune defenses mecanaism). Therefore, focus in developing noval antibiofilm drugs is underway on global level through exploring and understanding the mechanisms of biofilm-specific antimicrobial resistance. In most cases, the biofilm related infections are slow in generating apparent symptoms and also rarely fatal. However, the slow and gradual development of disease may affect the quality of life and may persist for months, years and even in some cases for lifetime. Despite this, some antibiotics have been observed to penetrate biofilms in some cliniacl state, yet the EPS matrix, which acts as a physical barrier limits the treatment with a large number of other antibiotics because of their poor diffusion into the matrix.

No approved treatment pattern is currently available for biofilm related bacterial infections. Therefore, bacteria forced into forming stronger biofilms will become more difficult to treat and is expected to cause more severe chronic infections. Review of literature on penetration or diffusion of various antibiotics in the biofilms have indicated some very interesting findings reported elsewhere. Aminoglycosides have been reported to have the most poor penetration when tested against *Pseudomonas aeruginosa* biofilm matrix because of the interaction between the positively charged drug and negatively charged biofilm matrix. Thus, the binding of positively charged drug with negatively charged matrix can significantly delay the penetration and availability of drug into the matrix. In comparison, the diffusion of fluoroquinolones into the EPS matrix have been reported to occur immediately and without delay when tested against *Pseudimonas aeruginosa* and *Klebsiella pneumoniae* biofilms. Similarly, the penetration of β-lactams (such as oxacillin and cefotaxime) and glycopeptides (such as vancomycin and teicoplanin) have been reported to be considerably reduced when tested against *Staphylococcus aureus* biofilms. In comparison, the aminoglycosides (such as amikacin) , ansamycin (such as rifampicin) and fluoroquinolones (such as ciprofloxacin) against the same biofilm remain unaffected. It is further reported that tetracycline and vancomycin can diffuse biofilms of *Escherichia coli* and *Staphylococcus epidermidis* rapidly [18-19].

The high level of resistance offered from biofilm-growing cells has greatly obstructed in delivering a successful treatment for biofilm-associated infections for most antibiotics, thus making very difficult to completely kill the bacterial populations embedded in a biofilm. Scientists are working continuously on various new antibiofilm approaches to explore and developed novel natural products, synthetic or bioengineered molecules which can be used as alternate to classical antibiotic or antibacterial treatment. It is believed that the mechanism of action of these novel antibiofilm agents obtained from natural habitat are less vulnerable to the development of resistance compared to existing antibiotic available for treatment. Natural antibiotics and their semi-synthetic analogues, followed by genetically engineered antibacterial drugs as well as some botanical antibacterial agents have been given significant attention worldwide in view of their role in the management of chronic infectious disease, linked with biofilm former organisms. Since the discovery of penicillin, antibiotics always have had great therapeutic applications in the treatment of infections, however, despite all these new introductions, including that of new semi-synthetic antibiotics and some

of the highly potent synthetic antibacterial drugs, it is still challenging to manage chronic and recurrent bacterial infections in view of the development of biofilms.

In recent years, antibiotics from seaweeds and other marine organisms as well as botanical antibacterials have been investigated very comperhensively to find alternate drugs against existing antibiotics or their semi synthetic analogues. The seaweeds and other marine organisms have been found to have remarkable capability to resist biofilm formation. An endonuclease NucB isolated from marine strain of *Bacillus licheniformis* (Fig. 4, found on the surface of seaweeds - Green Mussel: *Perna viridis*, Fig. 5) has been noted to degrade the external DNA (eDNA) within biofilms more efficiently in comparison to previously known nucleases. The enzyme, NucB in the form of nasal spray has been tested and found quite effective in managing chronic sinusitis. When applied in the form of nasal spray, the enzyme NucB causes biofilm to break resulting in the release of bacteria from the matrix. The growth of these planktonic bacteria are then manageable through common antibiotics [20-21]. An in vitro study conducted on fourteen biofilm forming bacterial strains isolated from chronic rhinosinusitis (CRS) patients, including nine extracellular nuclease-producing bacteria, the enzyme NucB significantly disrupted the biofilm, providing an opportunity to treat CRS patients with NucB [21]. The seaweed-derived *Bacillus licheniformis* are also reported to produce bacteriocins, such as Lichenicidin (small microbial two component peptide - Bliα and Bliβ) classified as Lantibiotics, capable of controlling biofilms produced by several Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) [22].

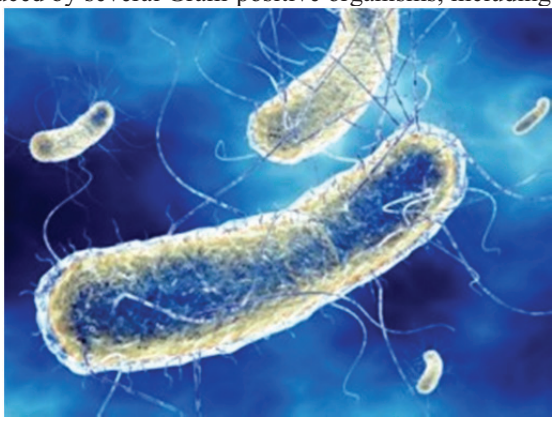

Fig. 4 Bacillus licheniformis

Fig. 5 Green Mussel: *Perna viridis*

Some more biofilm-disrupting enzymes have been studied and reported [23]. These include, Deoxyribonuclease I (DNase I), Lysostaphin (natural staphylococcal endopeptidase), α-Amylases, Lyase and Lactonase. One of the most important phytochemical constituent, Andrographolide (Fig. 6) extracted from *Andrographis paniculata* has shown to act on bacterial surface protein by inhibiting the bacterial quorum sensing (QS) system involved in biofilm formation. The molecule has been noted to be effective in the clinical management of cystic fibrosis, a chronic lung infection due to *Pseudomonas aeruginosa* [24-25].

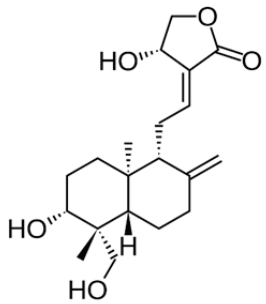

Fig. 6 Andrographolide - $C_{20}H_{30}O_5$

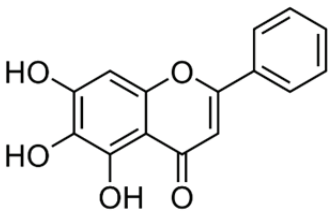

Fig. 7 Baicalein - $C_{15}H_{10}O_5$

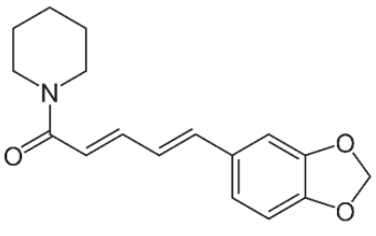

Fig. 8 Piperine - $C_{17}H_{19}NO_3$

Some bioactive flavonoids and alkaloids from natural habitat have also been reported to act against biofilm forming organisms. Flavonoids, especially naringenin, quercetin, sinensetin and apigenin are reported to disrupt *E. coli* biofilms [26]. Baicalein (Fig. 7), a flavone (type of flavonoids) obtained from a Chinese medicinal plant – *Scutellaria baicalensis* has been observed to block the ability of bacteria to adapt to antibiotics by removing the drug through multiple drug resistance pumps and thus prominently increases the efficacy of a number of antibiotics [27]. In addition, a traditional Thai herbal recipe, containing *Curcuma longa* (Turmeric), *Areca catechu* (Areca palm seed), *Oryza sativa*

(Asian rice seed) and *Garcinia mangostana* (Mangosteen) has been reported to inhibit *Staphylococcus epidermidis* biofilm growth by 30% to 40% [28]. Piperine (Fig. 8), a bioactive alkaloid from *Piper nigrum* (black pepper) has been reported to increase the diffusion of certain antibiotics (such as ciprofloxacin and azithromycin) when tested against *E. coli* CFT073 biofilms, enhancing the capability of these antibiotics to dissolve the biofilm and granting availability of the drug to destroy the organisms. Essential oils have also been reported to act as antibiofilm agent [29]. These include, eugenol (inhibiting EPS production and biofilm formation), cinnamaldehyde (interfering with motility, adhesion and biofilm formation) peppermint (act as QS inhibitor), lavender, carvacrol and thymol (Suppressing biofilm). Encouraging results with certain phytochemical (such as 7-hydroxycoumarin, indole-3-carbinol, salicylic acid and saponin) have been reported recently [30]. 7-hydroxycoumarin and indole-3-carinol were observed to affect the motility and quorum-sensing (QS) activity when tested against *E. coli* and *Staphylococcus aureus.* A synergestic effect of these phytochemicals has also been noted when used together with tetracycline, erythromycin and ciprofloxacin.

A large number of plant extracts, such as *Rhodiola crenulata* (arctic root), *Epimedium brevicornum* (rowdy lam herb), *Polygonum cuspidatum* (Japanese knotweed), *Melia dubia* (bead tree), *Capparis spinosa* (caper bush), *Terminalia catappa* (bengal almond), *Allium sativum* (Fresh garlic extract), *Commiphora leptophloeos* (corkwood) *Senna macranthera* (fruit extract) and *Croton nepetaefolius* (bark) have been tested and reported to be quite effective in controlling biofilm formation in various bacterial species through different mechanisms [23]. Natural habitat, especially the plants and marine source are now being considered as the greatest source to obtain new antimicrobials for clinical applications. Few examples cited in text supports our view that these bioactive molecules and many more from natural source will contribute a major role in near future against bacterial biofilm formation through various mechanisms and can potentially be used to fight against bacterial colonization through biofilm formation.

5. Threat

One of the greatest clinical threat is the development of resistant strains of various bacterial species against existing antibiotics in treating chronic infections linked to biofilms formation. The biofilms are difficult to target and control by drugs and thus challenging for researchers and clinician. Antibiotics are most likely to attack the planktonic cells that are released from the biofilm. The bacterial colony surrounded by biofilm has been reported at least 500 times more resistant to antibiotics. Even after the planktonic bacteria dispersed throughout the body are eliminated, the biofilm and the source of the infectious disease persists [12, 31]. Researchers from the University of Southern California and the Oak Crest Institute of Science have discovered the link between antibiotics and bacterial biofilm formation, i.e., antibiotics may promote biofilm formation if taken in sub-inhibitory concentration (Sub-MICs) leading to chronic infection**,** indicating an alarming situation [32]. Some of the examples quoted in the earlier reviews and research studies, indicated that antibiotics (such as vancomycin, tigecycline, linezolid and novobiocin etc.) can stimulate *in vitro* biofilm formation of *Staphylococcus epidermidis* [33]. Similarly, sub-inhibitory concentrations of tobramycin, tetracycline and norfloxacin can stimulate biofilm formation in *Pseudomonas aeruginosa* [34]. It has further been reported that sub-inhibitory concentration of the antibiotics in both *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* is a consequence of altered gene expression which is now a well-documented event, linked to modification of ribosome function [35-37]. The modulation of gene expression in bacteria by antibiotics (such as beta-lactams) is still not clear but believed to be linked with the formation of colanic acid in certain organisms (such as *E. coli*) which is important for maturation of biofilm architecture. Thus any increase in the production of colanic acid is expected to aggravate the foundation or perseverance of biofilms [32,38]. Earlier reports [15,39] on biofilm related disease and their prevalence are of great concern as situation looks quite alarming and of global threat, demanding to address the issue. According to these reports around 40 to 50% of adults had biofilm related gingival infections, out of 4000 infants with cerebrospinal- fluid shunts around 15 to 20% had biofilm related infections. Similarly, 95% of the urinary tract infections were associated with urinary catheters and around 86% pneumonias are coupled with mechanical ventilation, while 85% of the blood related infections are closely linked to intravascular devices infected with biofilm. Therefore more understanding and in-depth studies are required on subject to address the issues relating to ever increasing resistance of antibiotics with the use of Sub-MICs of antibiotics and antibacterial drugs. It is believed that this will also help in minimizing the prevalence of chronic and recurrent infections as well.

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