Chapter

Combating Biofilm and Quorum Sensing: A New Strategy to Fight Infections

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Abstract

Biofilms are structured aggregates of bacterial cells that are embedded in self-produced extracellular polymeric substances. Various pathogens initiate a disease process by creating organized biofilms that enhance their ability to adhere, replicate to accumulate, and express their virulence potential. Quorum sensing, which refers to the bacterial cell-to-cell communication resulting from production and response to *N*-acyl homoserine lactone signal molecules, also plays an important role in virulence and biofilm formation. Attenuation of microorganisms' virulence such that they fail to adapt to the hosts' environment could be a new strategic fight against pathogens. Thus, agents or products that possess anti-biofilm formation and/or anti-quorum sensing activities could go a long way to manage microbial infections. The incidence of microbial resistance can be reduced by the use of anti-biofilm formation and anti-quorum sensing agents.

Keywords: biofilm, quorum sensing, bacteria, acyl homoserine lactone

1. Introduction

Biofilm is a population of cells growing on a surface and enclosed in an exopolysaccharide matrix [1]. The physiology, structure and chemistry of the biofilm vary with the nature of its resident microbes and local environment [2].

Most important feature among biofilms is that their structural integrity critically depends upon the extracellular matrix produced by their constituent cells. They are notoriously difficult to eradicate and are a source of many recalcitrant infections [2]. Biofilms are associated with serious health issues stemming from persistent infections due to the contamination of medical devices (intravenous and urinary catheters), artificial implants and drinking water pollution among others [3].

Intercellular signaling, often referred to as quorum sensing (QS), has been shown to be involved in biofilm development [4]. Quorum sensing relies on small, secreted signaling molecules; much like hormones in higher organisms, to initiate coordinated responses across a population and it contributes to behaviors that enable microbes to resist antimicrobial compounds [5]. Quorum sensing signaling activation can lead to antimicrobial resistance of the pathogens, thus increasing the therapy difficulty of diseases [4].

The key concern about biofilms is their contribution to the development of resistance against antimicrobial agents, and with the on-going emergence of antibiotic-resistant pathogens, there is a current need for development of alternative therapeutic strategies [6].

An anti-virulence approach by which quorum sensing is impeded could be a viable means to manipulate bacterial processes, especially pathogenic traits that are harmful to human and animal health and agricultural productivity [7]. Further research into the identification and development of chemical compounds and enzymes that facilitate quorum-sensing inhibition (QSI) by targeting signaling molecules, signal biogenesis, or signal detection are required [7]. Anti-QS agents can abolish the QS signaling and prevent the biofilm formation, therefore reducing bacterial virulence without causing drug-resistant to the pathogens, suggesting that anti-QS agents could be potential alternatives for antibiotics [8]. An effective clinical strategy for treating bacterial diseases in the near future will be to combine anti-QS agents with conventional antibiotics since this can significantly improve the efficacy of therapeutic drugs and decrease the cost of human healthcare [9].

2. Microbial biodiversity in biofilm systems

Biofilms are mixed microbial cultures normally consisting predominantly of prokaryotes with some eukaryotes. Thus, in addition to microbial cells, the surrounding environment contains a range of macromolecular products in which exopolysaccharide secreted by the cells is the dominant macromolecular component, while the water content is probably about 90–97% [10, 11]. Secreted products also include enzymes and other proteins, bacteriocins, and low mass solutes and nucleic acid released through cell lysis. The lysis may occur either naturally with cell aging or through the action of phage and bacteriocins.

Opportunistic pathogens, viruses, parasitic protozoa, toxin releasing algae and fungi and enteric bacteria e.g. *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Enterobacter agglomerans, Helicobacter pylori, Shigella spp., Campylobacter spp., Salmonella spp., Clostridium perfringens, Enterococcus faecium, Enterococcus faecalis* and environmental pathogenic bacteria like *Legionella pneumophila, Pseudomonas aeruginosa, Pseudomonas fluorescens, Aeromonas hydrophila, Aeromonas caviae, Mycobacterium avium, Mycobacterium xenopi* etc. are associated with biofilms present in drinking water [12, 13].

Biofilms present complex assemblies of microorganisms attached to surfaces. They are dynamic structures in which various metabolic activities and interactions between the component cells occur [10]. Studies on microorganisms and biofilm formation have revealed diverse complex social behavior including cooperation in foraging, building, reproduction, dispersion and communication among microorganisms [14]. The organisms within a biofilm setup may include a single or diverse species of microorganisms. In the biofilm, bacteria can share nutrients and are sheltered from harmful factors in the environment, such as desiccation, antibiotics, and a host body's immune system.

Bacteria, fungi, viruses, protozoa and cyanobacteria that are common pathogens are all involved in biofilm formation [15].

2.1 Bacterial biofilms

About 99.9% of all bacteria live in biofilm communities [16]. A biofilm usually begins to form when a free-swimming bacterium attaches to a surface. Pathogenic organisms are found on most food items including seafoods and biofilm forming

pathogens are found on such seafoods as crabs [17], pacific oysters [18], shrimps [19] etc. Public health and clinical microbiologists recognize that biofilms are present everywhere in nature and are responsible for a number of human infections. Infectious caused by microbial communities include urinary tract infections, middle-ear infections, dental plaque, gingivitis, endocarditis, cystic fibrosis. Biofilms on persistent indwelling devices such as catheter, contact lenses, heart valves and joint prostheses are also responsible for many recurrent infections [20, 21]. Biofilms on indwelling medical devices may be composed of Gram-positive or Gram-negative bacteria. Bacteria commonly isolated from these devices include the Gram-positive *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus viridans;* and the Gram-negative *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa* [22]. It has been shown that virtually all indwelling central venous catheters are colonized by microorganisms embedded in a biofilm matrix. Among these *S. epidermidis* and *S. aureus* are commonly present on cardiovascular devices [23], causing about 40–50% of infections related to heart valve [14].

The organisms that form biofilms on medical devices originate from patient's skin microflora, exogenous microflora from health-care personnel, or contaminated infusates. Biofilms associated with catheters may initially be composed of single species, but with the passage of time they become multi-specie communities. Some urinary tract and bloodstream infections are also caused by biofilm-associated indwelling medical devices with 50–70% of infections related to catheter [12]. Chronic infections, inflammation and tissue damage caused by many strains of single species are often found in polymicrobial communities [24].

Bacteria that reside in a biofilm community usually will not grow when cultured, a situation normally referred to as "viable, but not culturable". The reason is that to change to the planktonic state from a biofilm-producing phenotype, bacteria require complex and specific environmental and signaling factors that are not available in a culture plate [25]. This therefore suggests that analyzing biofilm samples for bacterial infective agents during infections may show negative results and the real cause of the infections may not be detected if culturing is the only investigative procedure.

2.2 Fungal biofilms

Many medically important fungi produce biofilms and they include *Candida*, *Aspergillus*, *Cryptococcus*, *Trichosporon*, *Coccidioides*, and *Pneumocystis*. *Candida albicans* biofilms are primarily made up of yeast-form and hyphal cells, both of which are required for biofilm formation [26]. The formation of *Candida albicans* biofilm follows a sequential process that involves adherence to a substrate (either abiotic or mucosal surface), proliferation of yeast cells over the surface, and induction of hyphal formation [27]. As the biofilm matures extracellular matrix (ECM) accumulates. Many other Candida spp. form ECM-containing biofilms but do not produce true hyphae and they include *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* [28]. Aspergillus biofilms can form both on abiotic and biotic surfaces and the initial colonizing cells that adhere to the substrate are conidia. Mycelia (the hyphal form) develop as the biofilm matures [29]. *Aspergillus fumigatus* produces two forms of biofilm infections: Aspergilloma and Aspergillosis. Aspergilloma infections present an intertwined ball of hyphae while aspergillosis infections present individual separated hyphae [30].

Trichosporon asahii forms biofilms comprised of yeast and hyphal cells embedded in matrix, as do those of *Coccidioides immitis*. *Cryptococcus neoformans* forms biofilms consisting of yeast cells on many abiotic substrates [31]. Although *Cryptococcus neoformans* forms hyphae in the course of mating, no hyphae have

been observed in *Cryptococcus neoformans* biofilms. Similarly, *Pneumocystis species* do not produce hyphal structures as part of their biofilms [32]. Hyphal formation is therefore, not a uniform feature of fungal biofilms.

2.3 Protozoan biofilms

Free-living protozoans are single celled eukaryotic organisms and are divided into amoebae, flagellates and ciliates. All the three protozoan groups have been found in fresh water biofilms. Although many different species are found in association with biofilms, their level of association differs. The protozoans *Cyclospora cayetanensis, Cryptosporidium spp., and Toxoplasma gondii* have all been found in biofilm communities [22].

2.4 Virus involvement in biofilms

Viruses are obligatory intracellular parasites and are found in communities where cells in which they live are found. Viruses are, thus, found in biofilms communities associated with the bacteria, fungi and protozoa they infect.

Many phages may produce polysaccharases or polysaccharide lyases. Some phages are also known to produce enzymes that degrade the poly-Q-glutamic acid capsule of *Bacillus* spp. [33]. Various structures including extracellular polymers and heterologous microbial cells may impede viral access to the bacterial cell surface. Phage may carry on their surfaces enzymes that degrade bacterial polysaccharides including those of biofilm structures. These enzymes are very specific and seldom act on more than a few closely related polysaccharide structures [34]. Numerous phages have been isolated which induce enzymes capable of degrading the exopolysaccharide of various Gram-negative bacterial genera. These include phage for biofilm-forming bacteria. It has been observed that the extracellular matrix of the biofilms does not protect the bacterial cells from infection with phage T4 [35].

Many biofilms possess an open architecture with water-filled channels, which would allow the phage access to the biofilm interior [36]. As biofilms age and cells die and slough off, potential new viral receptor sites may become available. As bacteria excel at adapting to differing nutrient conditions, changes to the host cell surface could be expected with either loss or gain of possible phage receptors. A further factor which might influence phage retention within biofilms lies in the role of hydrophobic and electrostatic interactions. In the interaction of a coliphage with both hydrophobic and hydrophilic membranes, a critical factor in the retention of the phage was its iso-electric point [37].

In complex biofilms in natural environments, eukaryotic algae may also be present [38]. Under these circumstances algal cell lysis through viral action is also possible as many viruses for algal species have now been isolated and identified [39].

3. Biofilms in respiratory tract infections

It is becoming progressively more accepted that biofilm formation is an important cause of morbidity in respiratory tract infections [40]. Biofilms may be involved in some respiratory infections, including ventilator-associated pneumonia, bronchiectasis, bronchitis, cystic fibrosis and upper respiratory airway infections [41].

3.1 Upper respiratory tract infections

Infectious diseases that affect the upper respiratory tract include otitis media, sinusitis, tonsillitis, adenoiditis, pharyngotonsillitis, adenoiditis and chronic

rhinosinusitis [42]. In otitis media, infections may be as a result of both respiratory viruses and bacteria such as non-capsulated *Haemophilus influenza, Streptococcus pneumonia*, *Streptococcus pyogenes, Moraxella catarrhalis* and *Staphylococcus aureus*, triggering the appearance of polymicrobial biofilms [43].

The most cited reason for childhood visits to physicians is otitis media with effusion (OME) and is again one of the most reasons for antibiotic therapy in children. Even though OME is regarded as a sterile inflammatory process, current data using a chinchilla model suggest that viable bacteria are present in intricate communities referred to as mucosal biofilms [44]. It is interesting to know that intracellular *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Moraxella catarrhalis in situ* are found in adenoids from children going through adenoidectomy for the treatment of hypertrophic adenoids or chronic otitis media using Fluorescence in situ hybridization [45]. *Haemophilus influenzae* and intracellular *S. pneumoniae* have also been in middle ear mucosal biopsies in children with chronic otitis media [46].

Biofilms were seen in the sinus tissues of 72% of patients affected by chronic rhinosinusitis and the cultured organisms identified included *H. influenzae* (28%), *P. aeruginosa* (22%), *S. aureus* (50%), and fungi (22%). The presence of bacterial biofilms was linked to persistent mucosal inflammation after endoscopic sinus surgery [47]. Assessment of some chronic infections in the upper respiratory tract including recurrent tonsillitis and chronic rhinosinusitis in human clinical specimens suggests that both attachment and aggregated bacteria are present [48]. For instance, electron microscopy and culture were used to show that biofilms were associated with the mucosal epithelium of tonsils in 73% of tonsils removed for tonsillitis and 75% of those tonsils removed due to hypertrophic tonsils alone [49]. Calo *et al*. [42] found bacterial biofilms in recurrent and chronic infectious diseases of the upper respiratory tract (adenoiditis, tonsillitis, and chronic rhinosinusitis) and concluded that biofilms formation plays a role in upper airway infections.

3.2 Tissue-related infections

3.2.1 Cystic fibrosis (CF)

Cystic fibrosis (CF) is a protracted disease of the lower respiratory tract. The most frequent serious clinical complication in CF today is chronic endobronchial infection with *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a microorganism characterized by the capacity to produce large amounts of alginate and developed as a biofilm where micro-colonies of bacteria embedded in a matrix of alginate attack the lower respiratory tract [42]. Cystic fibrosis occurs as a result of a mutation in the CF transmembrane conductance regulator gene that encodes a cyclic AMP-regulated chloride ion channel. The mutation causes defective ion transport across epithelial cell surfaces in the upper airways, interfering with the removal of particles and microbial cells trapped in the overlying mucus and causing increased susceptibility to bacterial infection. Therefore, the airways of patients with CF are almost always infected with different bacterial species, but *P. aeruginosa* infection causes the greatest problem of morbidity and mortality [43]. *Pseudomonas aeruginosa* is the most common bacterial species that causes respiratory tract infection in CF patients and can be seen in about half of all cases and in up to 70% of adults [44]. Other pathogens such as *Staphylococcus aureus*, *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* have also been found to cause CF and are linked to biofilm formation [45].

3.2.2 Cystic fibrosis with chronic lung infections

A major difficulty in this type of infection is contamination of lower respiratory secretions with the normal oropharyngeal flora, particularly as members of the normal flora (e.g. *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Moraxella catarrhalis*) are common lung pathogens in CF [46, 47]. The incidence of bacterial lung infections in CF is high because the mucoid polysaccharidic material that accumulates on the respiratory epithelium due to the fact that impaired mucociliary removal in the bronchi of such patients favors biofilm formation. The capacity of *Pseudomonas aeruginosa* to form biofilms is believed to be the primary reason for its survival in the CF lung, despite a high inflammatory response and intensive antibiotic treatment [48]. Chronic airway infections cause an increase deterioration of lung tissue, a decline in pulmonary function and, finally, respiratory failure and death in cystic fibrosis (CF) patients [49].

3.2.3 Chronic obstructive pulmonary disease (COPD)

The role of biofilms in patients with COPD has not been directly validated but has been hypothesized considering the evidence showing that the respiratory tracts of these patients are frequently colonized by pathogens. Murphy and Kirkham [50] have recently confirmed that biofilms do play a role in COPD where they identified major outer membrane proteins of Non-typeable *H. influenzae* during its growth as a biofilm. Even if direct proof of biofilm formation *in vivo* is lacking, biofilms may reasonably be considered to be involved in the vicious cycle of infection/inflammation leading to disease development in patients with COPD [51].

3.2.4 Non-cystic fibrosis bronchiectasis

In bronchiectasis not due to cystic fibrosis, infections result in changes in the muscular and elastic components of the bronchial wall, which become distorted and expanded. Airways gradually become unable to clear mucus, leading to serious lung infections, which in turn cause more damage to the bronchi [52]. Recently biofilm formation has been demonstrated *in vivo* and is assumed to play a significant role in the pathophysiological cascade of the disease [53]. Bacterial biofilm formation by *Pseudomonas aeruginosa* or *Klebsiella pneumoniae* is common in bronchiectasis and could be an essential factor that makes infections in bronchiectasis obstinate. Other pathogens such as *Prevotella sp*., *Veillonella sp*. and *Neisseria sp*. have also been identified recently in patients with bronchiectasis to form biofilms [54].

3.2.5 Bronchitis

Prolonged bacterial bronchitis may be caused by chronic infections of the respiratory tract. In children especially, the condition appears to be secondary to impaired mucociliary removal that produces an environment favorable for bacteria to become established, usually in the form of biofilms. The most commonly involved bacteria include *Haemophilus influenzae* (30–70%), *Moraxella catarrhalis and Streptococcus pneumonia* [55].

3.2.6 Diffuse pan-bronchiolitis

Diffuse pan-bronchiolitis (DPB) is an unusual inflammatory lung disease of unknown etiology found in adult Japanese patients. With this disease, chronic

endobronchial infection with *Pseudomonas aeruginosa* biofilms leading to respiratory failure is common. It is a severe, progressive form of bronchiolitis (Inflammation and congestion in the bronchioles of the lung) [56].

3.3 Device-related infections

In device-related infections such as ventilator-associated pneumonia (VAP), biofilms result in microbial persistence and reduced response to treatment. Biofilm formation within the first 24 h after intubation has been reported in 95% of endotracheal tubes [57]. Pathogens in both endotracheal tube biofilm and secretions accrued within the airways/endotracheal tubes in 56 to 70% of patients with VAP have been reported. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most common bacteria that colonize these devices [57].

3.4 Biofilm forming organisms associated with respiratory tract infections

This section presents the role of biofilms in respiratory tract infections, with specific emphasis on the biofilms formed by *Pseudomonas*, *Staphylococcus*, and *Haemophilus*, the primary pathogens associated with respiratory tract infections [58] although additional important pathogens, including *Streptococcus pneumoniae*, *Bordetella* and *Mycobacterium* species do play a role [59].

3.4.1 Biofilms formed by Pseudomonas aeruginosa

Pseudomonas aeruginosa is a recognized common pathogen in respiratory tract infections although other members of the genus *Pseudomonas* are able to form biofilms [7]. Respiratory infections caused by *P. aeruginosa* are a major globally clinical issue, especially for patients with chronic pulmonary disorders, such as those with cystic fibrosis (CF), non-CF bronchiectasis, severe chronic obstructive pulmonary disease (COPD) and ventilator-associated pneumonia [60]. This bacterium is a difficult opportunistic pathogen that readily forms biofilms on most surfaces [5]. The intricate steps of biofilm formation by *P. aeruginosa* are considered to be a developmental process. The stages of *P. aeruginosa* biofilm formation can be seen by several strategies. One easy technique is the scanning electron microscope (SEM) of *P. aeruginosa* grown on glass surfaces or tracheal explants. Biofilms form when planktonic *P. aeruginosa* bacteria get attached to a surface using adhesins such as type IV fimbriae and flagella, and begin to colonize. In this regard type IV fimbriae and flagella *P. aeruginosa* mutants are severely compromised in initiation of biofilm formation [58, 61]. Additionally, the process of surface translocation mediated by type IV fimbriae (twitching motility) is essential for initiation of biofilm formation by *P. aeruginosa* [58]. Most probable, twitching motility confers synchronized cell movement along the surface as well as cell–cell communications that lead to the formation of micro-colonies. The coordination of events for the initiation and formation of biofilms requires cell–cell interactions that are mediated by quorum sensing [62]. Following this, the micro-colonies mature into distinctive three-dimensional structures that pose the most severe scenario for clinical treatment. This structure is typically trapped in a matrix material that may be composed of protein, polysaccharide, or nucleic acid. Nonetheless, it has been proposed guluronic and mannuronic acids [63] are the major constituents of the biofilm matrix [64]. Recent data also suggest that DNA also contributes to this matrix [60].

3.4.2 Biofilms formed by Staphylococcus species

The adherence of *Staphylococcus* directly to an implanted device (intravascular catheters, prosthetic devices, and other indwelling medical devices) or indirectly via host proteins is the first step in the development of a biofilm. This is followed by a buildup of multilayered cell clusters on the polymer surface [65]. When *Staphylococcus* bacteria get within 50 nm of a surface, they adhere through hydrophobic interactions, van der Waal's forces, and when present, fimbriae and pili also contribute to its adhesion [66]. A biofilm-associated protein (Bap) is reported to contribute to *S. aureus* biofilm formation. The second phase of *Staphylococcus* biofilm formation is the accumulation of complex cell clusters mediated by intercellular adhesion. A 140 kDa extracellular protein, known as the accumulation associated protein (AAP), appears responsible for accumulative growth on polymer substances [67]. It has been hypothesized that AAP is involved in anchoring the polysaccharide adhesion PIA (polysaccharide intercellular adhesion) to the cell surface [63]. The extracellular polysaccharide adhesion antigen PIA is a well-described polysaccharide antigen that is linked to cellular aggregation or clustering. Lastly, the generation of a slime glycocalyx is believed to be the climaxing event in the staphylococcal biofilm developmental process. This slime layer is not essential for surface colonization and appears variable between strains. However, when present, the slime layer protects the bacteria from host defenses and some antibiotics. As in *P. aeruginosa*, organization of complex communities within *Staphylococcus* biofilms is a coordinated effort and requires cell–cell communication [68].

3.4.3 Biofilms formed by Haemophilus influenzae

Non-typeable *H. influenzae* (NTHI) strains are members of the normal human nasopharyngeal flora, as well as frequent opportunistic pathogens of both the upper and lower respiratory tracts. It is an important cause of otitis media in children and lower respiratory tract infection in adults with chronic obstructive pulmonary disease (COPD). Recently, it has been shown that NTHI can form biofilms both *in vitro* and *in vivo* [69]. Considerable diversity in the ability of NTHi isolates to form biofilms has also been reported. A NTHi pilus defective strain was reduced three-to four fold in biofilm formation compared with its isogenic parental NTHi isolate, signifying a role of the pilus in biofilm development. Although this is the case for other gram-negative bacteria [70], nonetheless, it is quite clear that NTHi strains have the ability to form biofilms both *in vitro* and *in vivo* [69]. Earlier studies of cell envelopes during growth of *H. influenzae* as a biofilm established an increased abundance of a ~30 kDa protein [58], peroxiredoxin-glutaredoxin (PGdx) [71], that is expressed by *H. influenzae* during biofilm growth and this probably contributes to its persistence in the upper respiratory tract infections.

3.4.4 Biofilms formed by other microorganisms

Streptococcus pneumoniae: *Streptococcus pneumoniae* is a frequent colonizer of the human nasopharynx and a significant human respiratory pathogen that causes a variety of diseases such as community-acquired pneumonia and otitis media in children [72]. Colonizing pneumococci form well-ordered biofilm communities in the nasopharyngeal environment, but the exact role of biofilms and their interaction with the host during colonization and disease is not yet explicit [73]. However, investigators have speculated that pneumococci form biofilms in the nasopharynx *in vivo* [74]. Recently, pneumococci have been reported for the first time to form

highly structured biofilms during colonization of the murine nasopharynx [75]. Mice were also inoculated intranasally with the pneumococcal strain EF3030, a clinical isolate known to be non-invasive and an efficient colonizer in murine models, and found to form biofilms [76].

Bordetella species: *Bordetellae* are respiratory pathogens that infect both humans and animals. *Bordetella bronchiseptica* causes asymptomatic and long-term to life-long infections in animal nasopharynges while the human pathogen, *B. pertussis* is the etiological agent of the acute disease whooping cough in infants and young children. One proposed hypothesis to explain the survival and continued persistence of *Bordetella spp*. in the mammalian nasopharynx is that these organisms produce surface-adherent communities known as biofilms [77]. Researchers have recently established the ability of the three classical *Bordetella species* (*Bordetella pertussis, Bordetella bronchiseptica,* and *Bordetella parapertussis*) to form biofilms on abiotic surfaces [78]. It is assumed that *Bordetella* biofilm formation may play a role in the pathogenic cycle, precisely in persistence within the nasopharynx [79]. The capacity to form biofilms in mice suggests a role for *Bordetella* mode of existence during human infections. Clusters and tangles (reminiscent of biofilms) of *Bordetella pertussis* adherent to ciliated cells in explant cultures and tissue biopsies of pertussis patients have been documented [79]. As reported for other biofilm-forming organisms, extracellular DNA and exopolysaccharide are vital for biofilm formation by *Bordetella bronchiseptica*. The observation of biofilm-like structures *in vivo* in the nasal epithelium of *Bordetella bronchiseptica* infected mice showed that these communities expressed a polysaccharide essential for *in vivo* biofilm development [75, 76]. In *Bordetella*, BvgAS-regulated factors, including the filamentous hemagglutinin and adenylate cyclase, may also contribute to biofilm formation [79].

Mycobacterium species: Mycobacterial infections have been shown to form biofilms, most notably *Mycobacterium tuberculosis*, which under the conducive environments, can self-assemble. Among the non-tuberculous mycobacteria, *Mycobacterium avium* complex (MAC) and the rapidly growing mycobacteria, including *Mycobacterium abscessus* complex, have been reported to produce biofilms either *in vitro* or in environmental reservoirs [80], but *in vivo* conditions have not been investigated. *Mycobacterium abscessus* complex is an evolving threat to patients with cystic fibrosis [81], that become infected at an early stage and worsens clinically as the persistent infection results in inflammation and tissue damage.

4. Quorum sensing

In the control of microbial infections, two strategies are normally envisaged; killing the organisms or attenuation of the organisms' virulence such that they fail to adapt to the host environment. The former approach is what is generally favored; the latter lacks specific targets for rational drug design. It has, however, been realized that Gram-negative bacteria use small molecules known as acyl homoserine lactones to regulate the production of secondary metabolites and virulence factors, and this could offer a novel target to address the strategy of attenuating the organisms' virulence thereby impairing their adaptation to the host system. Recent research has highlighted the importance of cell-to-cell interactions or communications, referred to as Quorum Sensing (QS), in microorganisms. Many bacterial species employ a complex mechanistic communication system to transmit information among themselves. Bacteria can act in response to a variety of chemical signals produced by the same species along with others produced by other species, and this provides a way for intraspecies and interspecies cross-communication

and interruption of signals. The ability of bacteria to dispatch, pull together, and process information allow them to act as "multicellular" organisms and enhance their survival in complex environments [82].

Any mechanism capable of disrupting QS signals can be used to reduce survival of the microorganism thereby preventing or reducing virulence in the host environment. Such methods of interruption of the QS include:

- Disruption of biosynthesis of signal molecules,
- Application of QS antagonists (e.g. use of extracts from higher plants and algae and other chemical compounds),
- Chemical inactivation of quorum sensing signals,
- Biodegradation of signal molecule.

Agents capable of inhibiting the growth of microorganisms or disrupting the quorum sensing mechanisms of the microorganisms or interrupting the biofilm formation may be useful in the fight against microbial pathogenicity.

4.1 Anti-quorum sensing activity

It has now become apparent that different types of microorganisms have evolved the ability to recognize and act in response to the presence of other microorganisms in their neighborhood. Most Gram-negative bacteria produce and respond to *N*-acyl homoserine lactone (AHLs) signal molecules to regulate production of secondary metabolites in order to monitor their own population density. These molecules, at a threshold population density, act together with cellular receptors and elicit the expression of target genes such as those involved in virulence, antimicrobial production, motility and swarming, sporulation, bioluminescence and biofilm formation. The concept of quorum sensing (QS) was initially described in *Vibrio fischeri*, a luminescent marine bacterium. It was observed that the organisms express genes controlling light emission (the luciferase enzyme) when in symbiotic association with its hosts, the squid [83]. At low population densities (i.e. free-living in seawater) *Vibrio fischeri* does not express luciferase and so is non-luminescent. However, when cultured in the laboratory to high cell densities, they express bioluminescence with a blue-green light. They do not emit light unless they detect a concentration high enough of their own AHL. These organisms usually form symbiotic relationships with some fish and squid species such as *Euprymna scolopes*. *Euprymna scolopes* appears bioluminescent in dark surroundings because of high-population of the cells (*Vibrio fischeri*) in a specialized light organ. *Euprymna scolopes,* in return, offers nutrients to the *Vibrio fischeri* population. The QS system originally identified in *Vibrios* involved two genes, luxl and luxR. The Luxl codes for an enzyme, which synthesizes 3-oxo-C6-homoserine lactone (an auto-inducer as they are produced by the same cells whose metabolism they regulate) [82].

The unpleasant side effects of antibiotics (such as ototoxicity and nephrotoxicity associated with the aminoglycosides) have led to preference for preventive rather than curative approach towards fighting infectious diseases. Inhibition of quorum sensing activity has been hypothesized as one approach that can be useful in preventing bacterial infection. It could provide an additional approach to antibiotic mediated bactericidal or bacteriostatic activity thereby reducing the risk of successful establishment of infections or resistance development in the bacteria. This is supported by the protective effect of QS inhibition demonstrated

in animal infection models. A simple animal infection model on QS was launched in *Caenorhabditis elegans*, a nematode that feeds on bacteria. When fed on opportunistic pathogens such as *P. aeruginosa*, the worm was mostly destroyed within a short time after taking in the bacteria; presumably annihilated by the actions of cyanide and phenazines produced by the bacteria [84]. However, in instances where the worms ingested *P. aeruginosa* with mutations in the QS-controlling systems, they were not killed but were rather sustained on the bacteria. This model highlights the involvement of QS-regulated virulence factors in pathogenicity of *Pseudomonas aeruginosa*. It is obvious from such models that interruption of the QS apparatus of bacteria by plant extracts or other chemical compounds may offer a novel and an exciting approach to fight the existing problems associated with antimicrobial chemotherapy.

Many bacteria produce AHL molecules in response to QS and so could be used as biomonitor organisms in screening of compounds for anti-QS activity. Such bacteria include *Chromobacterium violaceum, Erwinia carotovora* and *Pseudomonas aeruginosa*.

5. Medicinal plants with biofilm inhibition activity

Natural products have been identified to inhibit biofilm formation in microorganisms. The exact mechanism for most of the agents is yet to be elucidated. Medicinal plants have been identified as rich source of bioactive compounds that have the capability of interfering with biofilm formation but most of these studies are still in the early stages of drug development. The anti-biofilm effects of medicinal plants have been proposed to be due to the inhibition of formation of polymer matrix, suppression of cell adhesion and attachment, interruption of extracellular matrix formation and reduction in virulence factors production and activation, thereby blocking QS network and biofilm development [85].

Medicinal plants belonging to various plant families reported to have biofilm inhibitory activity are listed in **Table 1**; the part of the plant (leaves, fruits, stem

Plant name	Family	Part used	Solvent	Biofilm inhibition activity	Reference
Punica granatum L	Lythraceae	Fruit	Methanol	Inhibit biofilm formation in *E. coli* by 70% at 150 μg/mL	[86]
Salvia fruticosa Mill.	Lamiaceae	Aerial parts	Ethanol	Inhibit biofilm formation by 60.9% at 0.78 mg/mL	[87]
Vaccinium corymbosum L	Ericaceae	Fruit	Decoction	Reducing 47% MRSA biofilm viable counts. 12.5 mg/mL	[88]
Commelina benghalensis L.	Commelinaceae	Whole plant	Distilled water	Inhibited the biofilm formation at 250 μg/mL	[89]
Curcuma longa L.	Zingiberaceae	Rhizome	Aqueous	Removed 30 to 40% of biofilm at 5–0.63 μg/mL	

Plant name	Family	Part used	Solvent	Biofilm inhibition activity	Reference
Euphorbia hirta L.	Euphorbiaceae	Aerial parts	Methanol	Biofilm inhibition and eradication activity against *P. aeruginosa* observed at 0.25 and 0.5 mg/ml, respectively	[90]
Terminalia bellirica (Gaertn.) Roxb	Combretaceae	Dried fruit	Ethanol	Inhibition biofilm formation by 89.8 and 92.2% at 125 and 250 μg/mL, respectively	[91]
Azadirachta indica A. Juss	Meliaceae	Leaf	Distilled water	Reduced biofilm completely by 35% at 5% w/v	[92]
Commiphora leptophloeos (Mart.) J.B. Gillet	Burseraceae	Stem bark	Distilled water	Inhibition of cell adhesion above 80% at 4.0 mg/mL	[93]
Bauhinia acuruana (Moric)	Fabaceae	Fruit	Distilled water	Inhibition of biofilm formation was determined to be 77.8 ± 5.0% at 4.0 mg/mL	
Camellia sinensis (L.) Kuntze	Theaceae	Leaves	Ethanol	Inhibited the cell adhesion by 78.7% 0.5%w/v	[94]

Table 1.
Medicinal plants with anti-biofilm activity.

bark, rhizome) used, the various solvents used for extraction and their ability to inhibit cell adhesion or to eradicate biofilm formed by different pathogens have been mentioned.

6. Conclusion

Combatting biofilm and quorum sensing is a good strategy to reduce microbial pathogenicity and thus fight infections. This can be achieved by finding effective agents that can inhibit biofilm formation and disrupt quorum sensing mechanisms. Natural products particularly medicinal plants are a rich source of bioactive compounds that have served as useful leads in the development of drugs. Rigorous evaluation of medicinal plants can therefore lead to novel anti-biofilm and anti-quorum sensing agents.

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