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Chapter

Innovative Strategies for the Control of Biofilm Formation in Clinical Settings

Aqsa Shahid, Maria Rasool, Naheed Akhter, Bilal Aslam, Ali Hassan, Sadia Sana, Muhammad Hidayat Rasool and Mohsin Khurshid

Abstract

Biofilm formation in clinical settings is an increasingly important issue particularly due to the emergence of multidrug-resistant strains, as it resulted in increased mortality, which poses a considerable financial burden on healthcare systems. The bacterial biofilms are quite resistant to the routine antimicrobial-based therapies; therefore, the novel strategies are desired in addition to the conventional antibiotics for the effective control of infections caused by biofilm-forming microbes. So far, the approaches being proposed to control the biofilm formation in clinical practice settings include the use of biofilm inhibitors and the use of modified biomaterials for the development of medical devices to thwart the formation of biofilms. In this chapter, we have focused on the latest developments in the anti-biofilm strategies through the interruption of the quorum-sensing system, which is crucial for biofilm formation and have summarized the various classes of antibacterial compounds for the control of biofilm formation. This agrees with the recent approaches suggested by the National Institute of Health (NIH) that advocates the use of combinational therapies based on the conventional methods and complementary treatment to explore the potential utility and safety concerns of the natural products. The studies regarding these emerging strategies could possibly lead to the establishment of better therapeutic alternates compared to conventional treatments.

Keywords: biofilms, infections, catheters, antimicrobials, quorum sensing, implants

1. Introduction

Group of microbial cells that are surface-attached and embedded within the extracellular matrix (self-produced), and are strikingly resistant to antimicrobials are called biofilms [1]. Biofilms can adhere to almost different types of surfaces like body tissue and plant, plastics, metals, implant objects as well as medical devices [2]. Formation of biofilm on implants and medical equipment and implants, for example, vascular grafts, prosthetic joints, heart valves, catheters, intrauterine devices, pacemakers, and contact lenses can cause infection. Central line-associated bloodstream infection (CLABSI) can occur due to use of intravascular catheters, furthermore,

CLABSI can cause an increased rate of mortality and morbidity, and every year in the USA almost 250,000 cases of bloodstream infections are reported [3].

When cells adhere and attach to surfaces biofilm formation begins. Several factors can promote the attachment of microorganisms to biomaterials including increased shear forces, bacterial motility, and electrostatic as well as hydrodynamic interactions between the surface and microbial cells [4]. It has been observed that adherence of biomaterials to bacteria via biomaterial-surface interactions and cell-surface is facilitated by numerous factors, such as protein autolysin, surface, and adhesion proteins and capsular polysaccharides, etc. For example, '*Staphylococcus* species' show cell-surface proteins that are vital for adherence of '*Staphylococcus epidermidis*' to polystyrene which is named as staphylococcal surface protein-1 and -2 (SSP-1 and SSP-2). After attachment to the extracellular surfaces, microbial cells will start aggregate, multiply, and eventually differentiate into the biofilm network [5]. Such microbial cells can then be separated from mature biofilms, can cause chronic infections and can spread to other organs also [6].

Another worrying characteristic of infections associated with biofilm formation is increased biofilm cell tolerance to biocides. As biofilms provide an excellent niche for exchange of plasmid, so increased resistance to the drug can affect genes containing plasmids which results in multidrug resistance (MDR) phenotypes. Enhanced drug resistance mechanisms include incomplete or slow infiltration of antimicrobials within the extracellular matrix, the formation of dormant cells during the non-dividing phase, reduced cell's growth rate within the biofilm, hence ultimately decreasing total targets for antimicrobial molecules [7]. Furthermore, it is difficult to treat biofilm formation with the traditional antimicrobial approach and the therapy is further inhibited by increased resistance to the antibiotic because under antibiotic selective pressure microbial cells develop resistance. For instance, it has been observed that almost above 70% of hospital isolate of *'Staphylococcus epidermidis'* show resistance to methicillin and surprisingly there are many strategies to prevent infections associated with biofilm formation other than antibiotic treatment [8]. In this chapter, we have focused on anti-biofilm approaches and some promising efforts for controlling these biofilm-based infections.

2. The process of biofilm development

The production and maturation of biofilm are complex, subsequent and dynamic processes, depending upon several factors i.e. cellular metabolism, intrinsic properties of the cells, genetic control, the substratum, and the medium signaling molecules. Biofilm formation is introduced with a conditioning film of inorganic or organic material on the cell surface; furthermore, this layer modifies the surface feature of substratum which ultimately favors microbes for colonization on the cell surface. The formation of biofilm consist of several different steps: (i) initially the reversible attachment of microbial cells with biotic or abiotic surfaces through weak forces for example van der Waals forces, (ii) irreversible attachment to the cell surface with the help of different attachment structure i.e. lipopolysaccharides, flagella, adhesive proteins or fimbriae by hydrophobic or hydrophilic interactions, (iii) and then eventually biofilm architecture development due to the production and proliferation of extracellular polysaccharide (EPS) matrix which is self-produced and is made up of proteins, extracellular deoxyribonucleic acid (DNA) and polysaccharides [9] (iv) in the

next step mature biofilm is formed which has water channels that are responsible for distribution of nutrients as well as signaling molecules within the biofilm [10], (v) and then due to extrinsic or intrinsic factors separation of biofilm cells occurs individually or in clumps and finally colonization of other niches and dispersal of the cells [11].

3. Inhibition of initial attachment

3.1 Altering physical properties of biomaterials

Biofilm development starts with a reversible weak adhesion of microbial cells to the exterior surface of medical equipment, however, if they are not removed from the exterior of devices, they adhered permanently through their adhesion structures i.e. fimbriae, pili and thereby forming biofilm matrix [12]. Surface charge and hydrophobicity of implant constituents play a significant role in controlling the ability of microbes to anchor to cell surfaces. Therefore, alteration in the hydrophobicity and surface charge of polymeric constituents are proved as efficient for controlling biofilm formation by using numerous antimicrobial agents and backbone compounds [13]. Poly N-vinylpyrrolidone and Hydrophilic polymers i.e. hyaluronic acid [14] on silicone shunt and polyurethane catheters have been widely used to decrease the adherence of '*Staphylococcus epidermidis*'. Furthermore, several hydrogel membranes have been introduced particularly for ureteral stents that decrease bacterial adherence because of their hydrophilic characteristics. It has been observed that due to very low wettability superhydrophobic coatings play a significant role to reduce the biofilm matrix formation and adhesion of bacteria [15]. Later, it has been suggested that *S. aureus* and *Pseudomonas aeruginosa* poorly attached on superhydrophobic fluorinated silica coating as well as on titanium coatings. However, it was demonstrated that *Escherichia coli* and *Staphylococcus aureus* poorly adhered on other superhydrophobic surfaces i.e. (AACVD) aerosol assisted chemical vapor deposition-coated [16]. In some cases, it was observed that hairpin coating affects colonization and adhesion of bacteria because it forms vascular catheter negatively charged, so contribute to reducing the catheter-related infections, inhibiting microbial colonization and thrombosis [17]. It has been described that the surface roughness can modulate hydrophobicity, which ultimately influences the bacterial adhesion [18].

3.2 Altering the chemical properties of biomaterials

There are several chemical approaches used to alter the exterior of biomedical equipment to inhibit the biofilm formation comprising ion coatings, biocides and also antibiotics [19]. Catheters that are impregnated with antibiotics, for example, rifampin and minocycline have been revealed to reduce the occurrence of biofilm-based infections by *Staphylococcus aureus*. Furthermore, catheters are coated with several antibiotics that play a significant role in biofilm production during urinary tract infections (UTI) like norfloxacin, nitrofurazone, and gentamicin [20]. Several chemical molecules are identified through screening of chemical libraries, these molecules are used as potential drugs to control infection and biofilm development. Furthermore, such molecules do not provoke antimicrobial action, and hence reduces the development of resistance due to no selective pressure against biofilm matrix formation. In *Staphylococcus aureus* and *Streptococcus pyogenes* a series of

small chemical molecules have an inhibitory effect on the expression of different important virulent factors during infection and biofilm formation [21]. Several aryl rhodamines showed inhibitory effect on early stages of biofilm development in *Enterococcus faecalis, S. epidermidis,* and *S. aureus*. Moreover, it was reported that a mucolytic mediator N-acetylcysteine has inhibited the formation of exopolysaccharides in the biofilm layer in case of *S. epidermidis* [22]. In another microorganism *Vibrio cholerae*, small substances suppressed the initiation of cyclic di-GMP that acts as the second messenger to control switch in-between the aquatic and sessile way of living of microbes [23].

It has been observed that numerous antibacterial peptides also inhibit biofilm formation in several microbes. For instance, it is considered that peptide 1018 has inhibitory effects in different microbes such as in *Acinetobacter baumannii, Burkholderia cenocepacia, Klebsiella pneumoniae, P. aeruginosa, E. coli, Salmonella typhimurium* and *S. aureus* [24]. Furthermore, class of peptide antibiotics called lantibiotics i.e. gallidermin, epidermin, subtilin, and nisin has been reported and control the biofilm production in *S. aureus, S. epidermidis* and also in *Lactococcus lactis*.

Chelators hindering the role of metal ions in the production of biofilm are considered as biofilm inhibitors, for example, silver salts, metallic silver and also silver nanoparticles are commonly employed as antibacterial agents in clinical implants against *P. aeruginosa, Salmonella typhimurium, Klebsiella species, E. coli,* and *S. aureus* [25]. It is observed that antibiotics i.e. amoxicillin, clindamycin, vancomycin, penicillin G and erythromycin show increased antimicrobial activity against *Staphylococcus aureus* in the presence of nanoparticles [26]. Treatment with silver substances prevents DNA replication, expression of cellular as well as ribosomal proteins, and also respiration process that leads to death of the cell [27]. In addition, It is also suggested that silver-coated implants inhibit *Staphylococcus aureus* biofilm production without aggregating silver inside the host tissue [28].

4. Quorum quenching

In the majority of Gram-negative and Gram-positive bacteria, an essential cellular communicating system is presently called as Quorum sensing, which regulates a variety of genes in accordance with the density of signaling molecules furthermore, signaling molecules are called autoinducers [29]. On the bases of signaling molecules QS is classified into three i.e. autoinducing peptide (AIP-based) for Gram-positive bacteria, N-acyl homoserine lactones (AHLs-based) for Gram-negative bacteria and autoinducer-2 (AI-2-based) for both Gram-negative and Gram-positive bacteria [30]. When the biofilm is formed, after the initial attachment, cells secrete QS molecules that alter the expression of the microbial gene, thus changing planktonic form into a sessile form. Furthermore, QS plays a significant role in biofilm development, so It has been observed that QS inhibition i.e. quorum quenching (QQ) would be a striking approach to control biofilm formation [31]. QS system is thought to be a target for developing new antimicrobial agents, moreover, QS system plays a crucial role in regulating pathogenetic factors and also virulence factors production in several pathogens [32]. The most important benefit of preventing biofilm formation by QQ is that this approach decreases the risk of multidrug resistance (MDR) and thus creating this approach noticeable to prevent biofilm-based infections in clinical settings. The different approaches for the inhibition or removal of biofilms are summarized in **Table 1** and **Figure 1**.

Bacteria	Compound	Mechanism	Antibiofilm activity	References
P. aeruginosa	N-Acyl homoserine lactones	Transcriptional regulators (LuxR and LasR)	Decreased the production of QS signals and virulence factors	[49]
	Patriniae	Biofilm related genes	Reduced the production of exopolysaccharide	[50]
	Hordenine	Quorum sensing related genes	Blocked QS-controlled phenotypes like biofilm formation	[51]
	Quercetin	Transcriptional regulators of quorum sensing related genes	Inhibition of biofilm formation	[52, 53]
	'*Piper betle*' Leaves (Ethanolic Extract)	Pyocyanin	Inhibited Pyocyanin production and reduced twitching ability	[53]
	Parthenolide	Extracellular polymeric substance and transcriptional regulators of quorum sensing related genes	Inhibition of the expression of QS related genes expression and downregulation of extracellular polymeric substance production	[54]
E. coli O157:H7	Ginkgolic acids (GAs)	Curli gene expression, prophage genes	Biofilm formation was inhibited on the polystyrene, glass and nylon membrane	[55]
	Phloretin	Toxin genes, autoinducer-2 importer genes curli genes, prophage genes	Decreased biofilm formation and production of fimbria	[55]
	Cinnamaldehyde	LuxR-DNA-binding	Affected the biofilm formation and virulence	[56]
S. mutans	'*Zingiber officinale*' (Methanolic fraction)	F-ATPase activity, virulence genes, surface protein antigen (SpaP)	Affected the cell-surface hydrophobicity index, Inhibited surface protein antigen (SpaP)	[57]
	Leaf extract of '*Bergenia crassifolia*' (L.)	Exopolysaccharides (EPSs), glucosyltransferases (Gtfs)	Decreased adherence properties of bacterial cells	[58]
	Quercetin	pH	Disrupted the pH in biofilm	[59]
Staphylococcus aureus and *Staphylococcus epidermidis*	'*Rhodomyrtus tomentosa*' (Ethanol extract)	Not mentioned	Inhibition of biofilm formation and disruption of mature biofilm	[60]
S. aureus strains	Phloretin	Efflux protein genes	Anti-biofilm formation at low	[61]

Bacteria	Compound	Mechanism	Antibiofilm activity	References
S. aureus and *C. albicans*	'*Hymenocallis littoralis*' leaf extract	Adhesin proteins	Antimicrobial and anti-biofilm activity	[62]
Streptococcus pneumoniae	Quercetin	SrtA gene	The blockage of SrtA gene function, impairment of biofilm formation	[63]
Enterococcus faecalis	Quercetin	Protein translation and folding pathways	Blocked the protein translation and folding pathways	[64]

Table 1.
Anti-biofilm compounds for various clinically important bacteria.

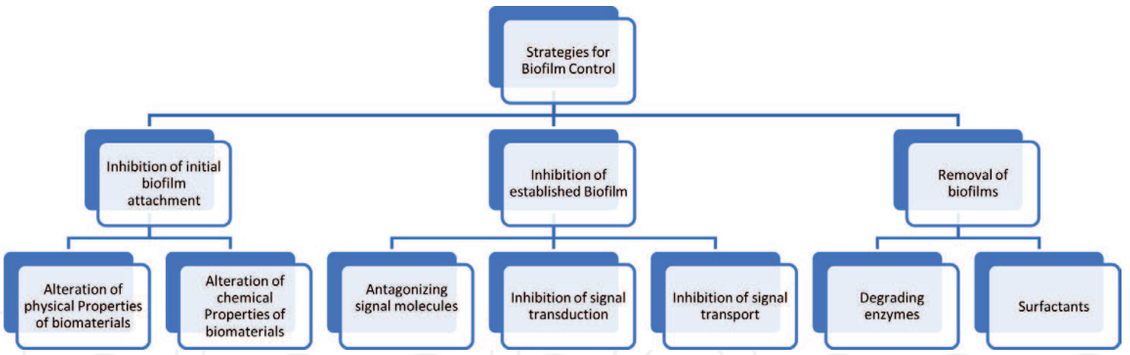

Figure 1.
An overview of the different anti-biofilm strategies.

5. Removal of biofilms

Another anti-biofilm approach is the dissociation of the biofilm matrix which accounts for around 90% of biofilm dry mass. This dissociation will ultimately expose the sessile bacteria to the antibiotics as well as host immune defense. The enzymes majorly employed for biofilm matrix-degradation can be divided into three categories Proteases, nucleases and polysaccharide degrading enzymes [33].

Moreover, the surfactants also possess the antibiofilm activities as the cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Tween 20 have been found to promote the detachment or dispersal of biofilms. Surfactin; a biosurfactant produced by the *Bacillus subtilis* was shown to inhibit the biofilm dispersal in *E. coli*, *Proteus mirabilis,* and *S. typhimurium* [33].

6. Bacteriophages as antibiofilm agents

Bacteriophages are considered as the largest creature in the biosphere, because of antibiotic resistance development, bacteriophages play an important role in the destruction of microbes. Use of bacteriophages is now considered as an alternative strategy to antibiotics, particularly for disruption or biofilm inhibition. Bacteriophages are beneficial than chemical agents and antibiotics. The isolation of bacteriophage is simple and fast, furthermore, its production is also cheap, and these are very distinct against a host or either host range, therefore, do not disrupt the normal flora. Bacteriophages are ecologically friendly, so with the persistence of host bacteria, they can replicate at the target site and have no adverse effects.

Bacteriophages also considered as potent antibiofilm mediators, e.g., phage T4 can cause infection and replicates within *E. coli* biofilms and by destroying microbial cells it can disturb the biofilm matrix. Doolittle and colleagues reported a study and demonstrated the interaction of phages with biofilms. The interaction among biofilm and phage is a dynamic as well as a sequential process. Phage adsorption with the target bacterial receptors is the significant phase in phage infection. The EPS matrix suggests a potent challenge for bacteriophages as EPS must be enough penetrated so that bacteriophages can attach with and reach to the particular host receptors. Furthermore, the EPS matrix also helps in the protection of bacteria in the biofilm. Moreover, by diffusion or through phage derived enzymes, for example, polysaccharide depolymerase can easily penetrate the EPS layer because these enzymes have the ability to destroy the structure of biofilm so that these phages can readily anchor to outer membrane receptors, lipopolysaccharides, or other proteins that are essential for replication process [34]. It is surely suggested that these phages induced depolymerizes can easily disrupt biofilms. Now genetically engineering

for phages have been introduced that explicit biofilm degrading enzymes during infection. The scientist has engineered a gene namely "dispersion" (dspB) into an *E. coli* specific T7 phage to yield an engineered enzymatic phage, which shows more efficacy for the removal of biofilms as compared to non-cloned phages.

Despite the several benefits of phage use, there are some disadvantages also, for example, the release of a considerable amount of bacterial membrane-bound endotoxins, decreased number of phages encoding toxins, insufficient pharmacokinetic data and conversion of lytic phages to prophages is also a big concern. Some of the above-mentioned problems have been well determined through different processes like designing a recombinant phage from *Pseudomonas aeruginosa* filamentous phage to minimize the mortality rate in experimental animals and release of membrane-bound endotoxins to report the endotoxin release issue is major advances to overcome the above-mentioned concerns [35]. It has been observed that bacteriophages and antibiotics have a big potential to control biofilms such as phage PhilBB-PF 7A plays role in the removal of *Pseudomonas fluorescens* biomass and has shown almost 63–91% activity.

Different studies show some of the strongest inhibitions, for example, the existence of biofilm EPS matrix hindering the control of biofilm via antibacterial agents and higher antibiotic resistance can be controlled through phage use. Furthermore, there are many limitations of phage use such as microbial resistance to phages, virulence genes that are phage-encoded can incorporate inside the host bacterial genome and the narrow host range. Phage efficacy can also be reduced by the immune system, and phage preparations that are improperly obtained can also contain endotoxin. To control these obstacles engineered phages or phage mixtures can be an effective alternative. Moreover, after proper selection and several studies phages has become one of the most useful anti-biofilm agents.

7. Natural anti-biofilm strategies

7.1 Plant extracts

Many extracts of plants and their derivatives were widely studied to eliminate the '*Propionibacterium acne*' biofilm [36]. It has been reported that out of 119 plant extracts, five showed strong antibiofilm activity i.e. *Rhodiola crenulata, Dolichos lablab*, *Malus pumila, Epimedium brevicornum,* and *Polygonum cuspidatum*. These scientists also suggested that extracts of *P. cuspidatum* and *E. brevicornum* and their active derivatives i.e. resveratrol and icartin show a potential antibiofilm activity even when used at lowest MIC. Bark extracts of *Melia dubia* were evaluated with 30 mg/mL concentration [37]. Furthermore, these extracts exhibit potential suppression of hydrophobicity, swarming motility, hemolysis, and biofilm production in *E. coli*. Other colleagues also reported similar results about *Capparis spinosa* (caper bush) extract, this extract shows inhibitory effect on the EPS production and biofilm production in *Serratia marcescens, Pseudomonas aeruginosa, Proteus mirabilis,* and *Escherichia coli* at 2 mg/mL concentration [38]. In addition, well-known biofilm formation of 3 microbes was dispersed. A medically important plant '*Lagerstroemia speciosa*' usually present in Southeast Asia, fruit extract from this plant is capable of inhibiting biofilm formation by '*P. aeruginosa*' PAO1 at 10 mg/mL concentration [39].

Other two plant extracts Dandasa (*Juglans regia* Tree Bark) and green tea (*Camellia sinensis*) show a potential antibiofilm activity individually. Recently, researchers observed that both Green tea and Dandasa exhibit potential antibiofilm activity of *Streptococcus mutans* at 12.5 and 6.2 mg/mL concentration, respectively, and on *E. coli* at 3.1 and 12.5 mg/mL concentration, respectively.

Allium sativum extract i.e. fresh garlic extract (FGE) has a potential inhibitory effect against biofilm formation, it has been observed that FGE decreased '*P. aeruginosa*' biofilm formation [40]. In-vitro screening of antibiofilm activity of '*Staphylococcus epidermidis*' of different 45 aqueous extracts from twenty-four Caatinga (Brazilian xeric shrubland) medicinal species was published. Extremely favorable extracts were taken from *Chamaecrista desvauxii* fruits, *Pityrocarpa moniliformis* leaves, *Bauhinia acuruana* fruits and *B. acuruana* branches, which show decreased the formation of biofilm even when they were tested at the lowest concentration. In addition, it was also suggested that *Senna macranthera* and *Commiphora leptophloes* fruit extracts decreased biofilms by 66.7% and 67.3% respectively. *Mycobacterium smegmatis* which plays a significant role in biofilm development was observed by using many quantitative and qualitative techniques. Other scientists examined different plants i.e. *Vaccinium oxycoccos*, *Hippophae rhamnoides, Azadirachta indica* and *Juglans regia* and spices to look for useful antibiofilm natural substitutes. When the efficiency of plant extracts as an antibiofilm agent was checked it showed that the extract of *Azadirachta indica* usually named as "Neem" was surprisingly helpful at removing and lowering *M. smegmatis* biofilms [41].

Another plant extract 'casbane diterpene' isolated from "*Croton nepetaefolius*" extract, is used to suppress the biofilm production of five Gram-negative bacterial species (*Klebsiella oxytoca, Klebsiella pneumoniae, Escherichia coli, Pseudomonas fluorescens* and *Pseudomonas aeruginosa*), two Gram-positive bacterial species (*S. epidermidis* and *S. aureus*), and three yeast species (*Candida glabrata, Candida tropicalis* and *Candida albicans*) [42]. Furthermore, another study demonstrated that *Candida* biofilm formation was remarkably decreased by *Boesenbergia pandurata* also known as "finger root oil" almost by 63–98% when MIC levels were used from 4 to 32 μL/mL [43]. Later studies showed that different plant extracts were isolated against *Enterohemorrhagic E. coli* (EHEC) O157:H7 biofilm. Furthermore, this study displayed that out of 498 plant extracts, almost 16 of them showed an inhibitory effect on biofilm formation of EHEC above 85% with no-growth of planktonic cells [44]. Certainly, these results specify that these different plant extracts show maximum inhibitory effect on biofilm formation of several microbes. Hence, it is suggested that further efforts are required to study the potential of these plant extracts as antibiofilm agents in detail.

7.2 'Honey'

A natural product extracted by 'honey' bee from floral nectar is called as 'honey' however, 'honey' is generally common and is usually used for its remarkable activity in wound-healing, anti-inflammatory, and antibacterial activity and used as an antioxidant. It has antimicrobial activities against 60 species of fungi and bacteria. 'Honey' was reported as a useful agent to control the biofilm formation. Furthermore, it was described that 'honey' is effective in the prevention of *Enterococcus spp.* biofilm production and can also use as a therapeutic agent against many *Enterococcal* infections that are biofilm-related. It can also decrease the biofilm production of EHEC O157:H7. Recent studies show that very low quantity of 'honey' can significantly decrease the formation of biofilm, the virulence of *E. coli* O157:H7 and Quorum sensing. So, a very low 'honey' concentration can decrease the formation of biofilm by preventing the virulence genes transfer in microbes and the expression of biofilm-associated curling QS, without inhibiting the cell growth. Due to its antimicrobial properties, high concentration of 'honey' can also prevent biofilm formation as well as adhesion of bacteria. Despite its antibacterial activity, it is also observed that 'honey' inhibits biofilm formation by antibacterial peptide which is bee defensin 1 that prevents microbial viability as well as biofilm formation indirectly [41].

7.3 Essential oils

Naturally plant-derived volatile substances are called as essential oils (EOs). Because of their antibacterial and preservative properties, these are effective and favorable natural products for the food industry. These essential oils are commonly used against a wide diversity of microorganisms since ancient time. These oils exhibit antimicrobial impact on the cell wall of microbes, leading to the destruction of microbes. Furthermore, it is suggested that these oils are very effective in inactivating many microbes without producing antimicrobial resistance [45]. Because of little mammalian toxicity, rapid degradation in the environment and availability of many essential oils make them beneficial antibiofilm agent [46].

Cumin oil scientifically named *Cuminum cyminum,* a derivative of an aromatic, therapeutic plant of "Apiaceae" family, has various medicinal properties and in the digestive system, it acts as an astringent. It has been widely used for acute gastric diseases as a carminative and eupeptic, and as an analgesic. It is also widely used to flavor foods, for example, added in food for fragrance. Cumin seeds have been used since ancient time. The efficiency of cumin seed against biofilm development on *Klebsiella pneumoniae* strains was observed, which showed that cumin seeds has decreased biofilm activity with improved ciprofloxacin efficiency [47].

Cinnamon oil is derived from the inner bark of the "*Cinnamomum zeylanicum*' as well as "*Cinnamomum cassia*" and is mostly used in the food industry due to its specific fragrance [48]. It is suggested that this oil is efficient against biofilm cultures *Lactobacillus plantarum, S. mutans*, and *S. epidermidis*. *Oregano* also is known as *Origanum vulgare* has inhibitory activity on biofilm production in case of *E. coli* and *Staphylococci*. A study revealed that Oregano essential oil exerts antimicrobial action on *E. coli, S. haemolyticus, S. sciuri, S. aureus,* and *S. lugdunensis* and could prevent biofilm formation. Moreover, it also able to detach active biofilm even at very low MIC. Inhibitory activity of *"Brazil nut oil"* named as *Bertholletia excelsa* (a vegetable oil) on commercially available dentifrice to prevent dental biofilm was also assessed. Scientists showed that by adding this vegetable oil to commercially available dentifrice, dental biofilm formation can be inhibited. Furthermore, this oil helps in preventing and controlling periodontal diseases [41].

The antimicrobial activity of "tea tree" essential oils scientifically named *Melaleuca alternifolia*, synergistically with ciprofloxacin was also evaluated against '*P. aeruginosa*' biofilms. The consequences showed that the combined effect of TTO with ciprofloxacin has decreased biofilm biomass significantly by more than 70% and lowered the number of cells at the lowest (1.25 μg/mL) ciprofloxacin concentration. The efficacy of essential oils from *cinnamon (Cinnamomum verum),* namely *thymol*, and *oregano* at sub-lethal concentrations on biofilm formation of 3 biofilm-forming bacterial strains i.e. *Stenotrophomonas*, *Acinetobacter* and *Sphingomonas* were assessed. Researchers showed that at the MIC, two out of three strains revealed resistance on microbial biofilm formation. Furthermore, among the three tested oils, "*thyme oil*" was considered as more efficient and showed inhibitory effect even on sub-lethal concentrations of 0.001% (w/v) [41].

8. Conclusion

Since biofilms are abundant in nature, the importance of biofilms in hospitals especially regarding their role in infections is often undervalued. Future studies should attempt to comprehend the biological forces controlling the colonization to develop innovative strategies for controlling biofilm biomass within a clinical context. Additionally, comprehensive research is required to recognize the potential

of various synthetic and natural quorum sensing inhibitors (QSIs) for their applicability for humans. As these QSIs do not encourage the antibiotic resistance, therefore they can surely be the future therapeutic agents for the management of biofilm-based bacterial infections in clinical settings.

Conflict of interest

The authors declare no conflict of interest.

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