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# Biofilm Formation by Quorum Sensing and Manners to Deal It

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**ABSTRACT**

***Background:*** Quorum sensing (QS) is one of the main regulatory systems which have various effects on populations of microorganisms. This process has been found in a diverse array of microorganisms (various bacterial taxa, microalgae and fungi). QS is required for different activities of microorganisms such as, virulence factor secretion, motility, competence, biofilm and sporulation. There are different molecules as signals in disparate microorganisms. Biofilm formation is one of the significant functions of QS. Biofilms are groups of microorganisms that are tied to a surface (biotic or abiotic). One of the remarkable effects of biofilm formation seems to be the persistence against hostile environmental condistions. Biofilm formation have been widely reported as a pathogenesis strategy in microorganisms. Here we describe QS and biofilm formation in some important microorganisms and describe some of the suggested strategies for eradication of microbial biofilms.

***Conclusion***: Inhibition of biofilms formation can have detectable effects on the treatment of infectous diseases. In this line, multiple approaches have been suggested to inhibit the biofilm formation by microorganisms.

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## Introduction

Communications among the bacterial cells happens via small molecules which are known as the regulatory signals of QS (1). This regulatory system was first described in *Vibrio fischeri* by Nealson et al. in 1970 (2). Essentially, three elements are used during this phenomenon; the signal synthase, signal receptor, and signal molecules (3). QS is dependent on population density and environment (4). When bacterial cells density increased, signal molecules are secreted. Microbial populations are sensitive to defined concentions of such molecules and physiochemical changes will occur when the signal molecule concentration pass the threshold level of receptor sensitivity (5). The regulatory effects of the QS have been widely described in gram-positive and gram-negative bacteria, and fungi as demonstrated in Table 1. Among those microorganisms; *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Candida albicans* have been well studied.

Formation of biofilm which is controlled via the regulatory effects of QS is indeed a cellular response to environmental stresses. One of such stresses is the antimicrobial compounds which have inhibitiory impacts on the microbial populations (6). In fact, QS help microorganisms to have communication with others and this mechanism is necessary to resist against various stresses (7, 8). So, QS needs to achieve energy by energy mechanisms (7-9). Genetic mechanisms and environmental signals are used to control microorganism's biofilm formation. Among the main regulatory molecules in bacterial biofilms, bis-(3'-5')-cyclic diguanosine monophosphate (c-di-GMP), and small RNAs (sRNAs) have been well studied (10). Also, horizontal gene transfer, alternative sigma factors and toxin–antitoxin systems are other QS factors (11).

Hence, the main aim of this review was the description of QS, biofilm formation and available approaches to inhibit the biofilm formation in several important microorganisms.

### *QS Molecules*

Small diffusible molecules are used by bacteria in QS prosses (12). These molecules have a key role to coordinate the gene expression of the individual cells which will direct the formation of biofilms in various microbial taxa. Bacteria produce one or more molecular compounds and transfer them out of the cell. From these molecules (several more classes of auto inducer) can mention autoinducer-2, boron-bearing compound, bradyoxetin, several diketopiperazines, farnesol, cis-2-alkenoic acids and a variety of peptides (1). Autoinducers due to coordinate individual cells to initiate biofilm formation and in terms of maintaining established biofilms are really important (13). Although specially auto inducers are found in close bacterial species, some of them may be also found in different species (14). Furthermore, gram-negative bacteria have both central protein compounds the LuxR-type (the signal receptor) and LuxI-type (signal synthase). First, LuxI catalyzes the synthesis of signaling molecules called N-acyl homoserine lactones (AHLs). AHLs and LuxR form a complex which enhances the expression of target genes, luxICDABE, (for bioluminescence production and also the LuxI production) (15).

Diverse bacteria maybe have different autoinducers (bacterial pheromone). Generally, three types of autoinducers are used by bacteria: the gram-negative bacteria use first class, the gram-positive bacteria use molecules in second class and the third class is biomolecules that used by both gram-negative and gram-positive bacteria. In contrast to gram-negative bacteria, gram-positive bacteria hire secreted peptides as HSL autoinducer in QS prosses. Autoinducer signals in gram-positive bacteria are transferred out of cell by ABC transporter. Also, different bacteria can use multiple autoinducers and sensors (16).

*QS Applications*

QS has a key role in different metabolic pathways in various microorganisms. Maybe can say that, the important function of QS is biofilm formation in bacteria (2). Furthermore, bacteria utilize QS to control biofilm development, bioluminescence, sporulation, motility, conjugation, genetic competence and bacteriocin production. Surprisingly, studies have shown that QS has critical role in formation of persister cells. QS molecules, phenazine pyocyanin and acyl-homoserine lactone in *Pseudomonas aeruginosa*, indole in *E. coli* and *Salmonella typhimurium*, and CSP pheromone in *Streptococcus mutans* can induce formation of persister cells (5). Biofilm is the close communication of bacterial population. This structure has been made from polysaccharides, nucleic acids, proteins, lipids, and humic substances.

It is recognized that bacteria in biofilm have more resistant to environmental stresses (such as antibiotics) compared with planktonic bacteria. So, easily could be found the importance of QS in bacteria (17). Persister cells are species of bacteria that are resistant to special antibiotics without expressing antibiotic resistance genes. Accurate mechanism to persister cells still is not found but in recent years have been shown that QS has a major role in persister cells in some bacteria for example *Streptococcus mutans* (18), *Acinetobacter baumannii* (19) and *Pseudomonas aeruginosa* (20).

*QS and Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is gram-negative bacteria that leads to critical infections. This organism is resistant to most conventional antibiotics. Also, it is the main reason for nosocomial infections especially in patients with cystic fibrosis, pneumonia, sepsis and urinary tract infections. More important than all these sayings, *Pseudomonas aeruginosa* uses QS to survive on surfaces, formation of biofilm and regulatory gene expression. Here, QS has been connected to the systems, including las, iqs, pqs and rhl (21). Many researches have been carried out about QS in *Pseudomonas aeruginosa* and most of them have shown that there are three connected mechanisms in this bacterium. The first two use acyl homoserine lactones and the third uses a quinolone signaling molecule. N-(3-oxododecanoyl)-L-homoserine lactone is synthesized by AHL synthase, LasI and RhlI AHL synthase synthesizes N-butyryl-L-homoserine lactone. Also, expressing genes of virulence factors, proteases, elastase, siderophores, rhamnolipids, iron metabolism and swarming motility in *Pseudomonas aeruginosa* are controlled by QS (22, 23). Cyclic di-GMP, second messenger, is controller biofilm formation in *Pseudomonas aeruginosa*. When intracellular level of cyclic di-GMP is high, production of biofilm matrix is increased and contrarily. In addition, cyclic di-GMP causes synthesis of *Pseudomonas aeruginosa* adhesin CdrA (could act as a cross-linker of Psl strands within the matrix ) (24). 3-oxo-C12-HSL acts as virulence modulating the responses of the host's defense. It is down-regulating the host defense by restraining activation immune system cells such as dendritic cells and T-cells and promotes apoptosis of neutrophils and macrophages. RsaL acts as the antagonist to the 3-oxo-C12-HSL-LasR complex and binds to lasI promoter, thus repressing the expression of LasI. There is the transcriptional activator RhlR in Rhl system and RhlI synthase, synthesis of the N-butanoyl-homoserine lactone (C4-HSL) signal molecule. Also, Rhl system control production of rhamnolipids, elastase, LasA protease, hydrogen cyanide, pyocyanin, the stationary-phase sigma factor RpoS, siderophores, LecA and LecB lectins. *Pseudomonas aeruginosa* quinolone signal 2-heptyl-3-hydroxy-4-quinolone (PQS) increases the level of complexity to the QS network and is controlled by Las and Rhl systems. Interestingly, PQS itself controls the expression of RhlR and RhlI (22, 25). So, the pathogenicity can

be reduced by preventing the formation of biofilm in *Pseudomonas aeruginosa*. Many studies have been done about inhibition of formation biofilms in bacteria. Kazemian et al. showed that *Chamaemelum nobile* extracts (anti-inflammatory, deodorant, bacteriostatic, antimicrobial, carminative, soothing, anti-infection, anti-catarrhal, and spasmolytic properties) has inhibitory activity which affect the biofilm formation in some bacterial taxa (26). Also, Soković et al. has shown the edible mushroom *Agaricus blazei* has anti- quorum sensing activity. They exhibited the impact of the sub-MICs of *Agaricus blazei* on QS regulated virulence factors and biofilm formation in *Pseudomonas aeruginosa* (27).

More, another way to the inhibition of QS in *Pseudomonas aeruginosa* decreases the expression of QS genes and production of autoinducers by catechin, naringenin, and taxifolin of *Combretum albiflorum* (28, 29). it has been shown that, extraction of *Moringa oleifera* and *Hibiscus sabdariffa* have the effect on the prevention of biofilm formation in *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* (30). As a result of one study, the biofilm formation in *Pseudomonas aeruginosa* is affected in response to defined concentrations of cladodionen (a biochemical compound extracted from a filamentous fungi *Cladosporium* sp. Z148) (31).

As well as, extraction of extremophilic *Natrinema versiforme* has inhibitory potential on both QS (las and rhl) and biofilm formation of *Pseudomonas aeruginosa* (32). Based on the results of Ahmed et al. *Mycoleptodiscus indicus* PUTY1 has the maximum quorum quenching potential against *Pseudomonas aeruginosa* with 20-mm inhibitory zone at a dose of 250 µg/mL (33).

*QS and Burkholderia cepacia*

*Clinical and Necropsy Findings*

*Burkholderia cepacia* complex (Bcc) is a group of at least 20 closely related species that resulted in threatening infections in cystic fibrosis (CF) patients and chronic granulomatous (CGD) disease. Virulence factors including toxins, proteases, lipases and siderophores as well as swarming motility and biofilm formation are related to QS in *Burkholderia cepacia* (34).

Biofilm formation in Bcc strains has an important role in the resistance of antibiotics and persistence to infections. At least one QS system is encoded by all Bcc members. QS in these members has homologs of the LuxR (is an AHL receptor protein that activates or represses gene expression) and LuxI (synthesizes an AHL signal) proteins of Vibrio fischeri. LuxR bind to a consensus sequence lux box in the promoter regions of target genes. LuxR/AHL complex activates transcription of luxI. CepIR QS system is conserved in Bcc. N-octanoyl-homoserine lactone (C8-HSL) and less amounts of N-hexanoyl-homoserine lactone (C6-HSL) are synthesized by CepI (35, 36). In CF patients, *Burkholderia cepacia* and *Pseudomonas aeruginosa* co-infections have been also described. There is hypothesis that suggests *Pseudomonas aeruginosa* facilitates *Burkholderia cepacia* binding to the epithelial cell surface of the lungs in CF patients. Also, these bacteria produce the same chemical language to synergistically enhance each other's virulence. QS in *Burkholderia cepacia* is density-dependent regulatory system. Protease production and repress synthesis of the ornibactin siderophore is regulated by the cep system. Mutation in the cepIR genes causes stopping production of AHL then biofilm formation of *Burkholderia cepacia* doesn't form (37). Many studies investigated inhibition of biofilm formation in *Burkholderia cepacia*. For example, Huber et al. have shown that, the AHL-antagonistic activities of EGCG (epigallocatechin gallate) had caused the

creation of thinner biofilms compared with thick biofilm in wild types (38). In another study, the amount killed by bacteria had been evaluated with combination antibiotics and inhibitors of biofilm formation, and antibiotics alone. Interestingly, combination of QS inhibitors such as baicalin hydrate or cinnamaldehyde with tobramycin for killing of Bcc were useful and these combination were more increase in killing bacteria than using antibiotic alone (39).

*QS and Listeria monocytogenes*

*Listeria monocytogenes* is an opportunistic gram-positive bacterium that can be cause severe infections. *Listeria monocytogenes* is zoonosis (illness or infection that is naturally contagious and can be transmitted from vertebrate animals to humans) and very important bacterium. Also, the fatality range of this bacterium is high ,especially in human with severe underlying disease such as, AIDS (40). QS in *Listeria monocytogenes* is composed of agrBDCA operon that agrD and agrA have key roles in it. Like other bacteria, in this bacterium QS causes coordinated control of gene expression (41). Also, the sensor kinase and response regulator are coded by agrC and agrA genes (42). Then, transcriptional regulation, metabolism, flagellum and peptidoglycan biosynthesis are engaged in biofilm formation of *Listeria monocytogenes*. In the first step of biofilm formation, this bacterium attaches to the surface of cells and produces extracellular polymeric substance (EPS). EPSs include proteins and nucleic acids (43). Complex Biofilm formation of *Listeria monocytogenes* and other bacteria, such as *Pseudomonas* spp is a big problem. *Listeria monocytogenes* in growth phase produces autoinducer 2 (AI-2) like molecule. AI-2 is important to cell attachment during biofilm formation of *Listeria monocytogenes*. AI-2 is encoded by LuxS that it is similar to LuxS in some bacteria such as, Vibrio harveyi, *Staphylococcus aureus*, *Helicobacter pylori*, *Clostridium perfringens*, and *Bacillus subtilis* (44, 45). Nguyen et al. in a study identified small molecules to inhibit biofilm formation and/or disperse established biofilms. These molecules include several classes of antibacterials (such as lactams, fluoroquinolones, quaternary ammonium compounds, biguanides, oligopeptides, and glycopeptides), antifungals, and vitamins. (43). Nguyen et al. in another study showed that, kinase inhibitors can inhibit the biofilm formation of *Listeria monocytogenes*. In that study they used the 80-compound (kinase inhibitors) and among those, 15-compound could inhibit the biofilm formation of *Listeria monocytogenes* (46). Wei et al. showed that the amount of attached cells and adherence of *Listeria monocytogenes* was reduced obviously by treated with phloretin (47). Furthermore, phloretin has antibacterial effect on Gram-positive bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium* (48). Also, phloretin leads to the death of inner cells and decreases the density of biofilm. The AI-2 concentration was reduced after phloretin regimen. These findings showed that phloretin has an impact on the generation of AI-2 and intervened with the function of LuxS system. consequently, phloretin prevented the biofilm formation of *Listeria monocytogenes* (47).

*QS and Staphylococcus aureus*

*Staphylococcus aureus* is one of the essential bacteria in the medical and generates considerable infections such as endocarditis, pneumonia, osteomyelitis, sepsis and pericarditis (49). Recently, resistant antibiotics *Staphylococcus aureus* converted to series problems in human health. Also, biofilm formation has an important role in *Staphylococcus aureus* infections (50). Then, QS of *Staphylococcus aureus* have two regulatory systems, the accessory gene regulator (Agr) system and the LuxS system. Agr is more important compared with LuxS system. There are

two transcriptional units in agr locus, RNAII and RNAIII that, RNAII locus has agrB, agrD, agrC and agrA genes. Agr targets are controlled by intracellular effector molecule that called RNAIII (4). P2 promoter and P3 promoter are in agr locus to encode RNAII and RNAIII, respectively (51). In the following, secreted autoinducing peptide AIP is encoded by agrBDCA. Then, AIP binds to AgrC and AgrA for activating the P2 and P3 promoters and finally causes expression and increased transcription of the RNAIII (Figure 1) (52, 53).

PIA/PNAG (PIA=encoded polysaccharide intercellular adhesion, PPNAG=poly-N-acetylglucosamine) production is important to biofilm formation in methicillin susceptible *Staphylococcus aureus* (MSSA). PIA has positive charge and causes attachment cells. Also, ica operon is in formation of *Staphylococcus aureus* biofilm and has icaA, icaD, icaB, icaC (biosynthesis genes) and icaR (transcribed repressor) genes (54, 55). Biofilm formation in PNAG-independent *Staphylococcus aureus* is mediated with biofilm associated protein (Bap) (56).

Although, biofilm formation in MSSA and methicillin resistant *Staphylococcus aureus* (MRSA) has similarity, there are differences between them. For example, NaCL induces biofilm formation of MSSA but doesn't have any effect on MRSA biofilm formation. Also, glucose only induces biofilm formation of MRSA (57). Opposite of MSSA, agr system in MRSA has a key role in biofilm formation (58). Importantly, more studies have been done about inhibition of biofilm formation in *Staphylococcus aureus* and agr quorum-sensing system identified as one of the targets of inhibition biofilm formations in *Staphylococcus aureus*. So, Baldry et al. have shown Solonamide B (a non-ribosomal depsipeptide of marine bacterial origin) inhibits biofilm formation of *Staphylococcus aureus* with interference in agr quorum-sensing system (59). Also, *Staphylococcus aureus* can be transferred to human and created infection by binding to metals surface and formes biofilm. Fortunately, Nan et al. have shown special stainless steel namely 304CuSS type, has strong restraint on the growth and cohesion of the biofilms (60). Furthermore, Zhao et al. in one study showed, Tet213 peptide could has an effect on biofilm formation of *Staphylococcus aureus* (61).

According to Ismaeil et al. study, the extraction of sumac both, prevents biofilm formation and decreases some virulence factors in *Staphylococcus aureus*. Additionally Ismaeil et al. noted that sumac extract has an essential role in modifying of *Staphylococcus aureus* infections (62).

The distinct composite of sumac is gallic acid. Borges et al. searched the mechanism action of gallic acid towards specific microorganisms and investigated this compound induced irreversible change in the cell membrane properties through hydrophobic modifications and pore-forming on the cell membranes causing leakage of essential components of the cell. The other active quinones compound of sumac is 1, 2-dioxo-6-hydroxycyclohexadiene4-carboxilic acid. The quinine compounds have some free radicals and can react with nucleophilic amino acids in the protein and generate constant complexes which cause loss function and rupture of the cell membrane (63).

*QS and Candida albicans*

*Candida albicans* is one of the important diploid pleomorphic fungi in medicine (64). This fungus is a member of normal microbiota in human body and also an opportunistic pathogen in medically immunocompromised patients (65, 66). Urinary tract infections (UTIs) are increasing in recent years and *Candida albicans* is one of the reasons of the creation of these infections (64). Luckily, *Candida albicans* biofilm formation study has been increased in the past years. These studies have shown biofilm formation of *Candida albicans* is important virulence factor of this fungus. Also,

attachment of yeast cells to a surface, growth of the yeast cells attached, maturation of the biofilm, and finally colonization in different places are steps of biofilm formation in Candida albicans (67, 68). Unfortunately, *Candida albicans* biofilm formation causes increasing minimum inhibitory concentrations (MICs) of antifungal compounds (69). Interestingly, *Candida albicans* could form biofilm on biotic or abiotic surfaces. The major heat shock protein Hsp90 plays a key role in the regulation of dispersion in *Candida albicans* biofilm formation (70). Also, Bcr1, Tec1 and Efg1 are transcription factors and control biofilm formation of *Candida albicans* (71, 72). Additional factor controls production of matrix. The major component of biofilm matrix is β-1, 3 glucan. Then, zinc-responsive transcription factor Zap1 controls β-1, 3 glucan, glucoamylases, glucan transferases and exo-glucanase (73).

It is gratifying, more studies have done about inhibition of biofilm formation of *Candida albicans*. First, Azevedo et al. have shown that, 7-hydroxycalamenene component has antifungal activity against several fungi such as *Candida albicans*. They showed the purified 7-hydroxycalamenene was able to inhibit *Candida albicans* activity with 58% ratio (74).

**Table 1.** QS bacteria and activity mentioned in this review.

| **Bacteria** | **QS molecules** | **Control biofilm formation** | **Formation of persister cells** | **Biofilm inhibitory activity** | **Anti- QS activity** |
|---|---|---|---|---|---|
| *E. coli* | | | Indole | | |
| *Pseudomonas aeruginosa* | N-(3-oxododecanoyl)-L-homoserine lactone synthesized by AHL synthase, LasI and RhlI AHL synthase synthesizes N-butyryl-L-homoserine lactone. 3-oxo-C12-HSL acts as virulence modulating the responses of the host's defense RsaL has acting as antagonist to the 3-oxo-C12-HSL-LasR complex and binds to lasI promoter, thus repressing the | Cyclic di-GMP | Phenazine pyocyanin and Acyl-homoserine lactone | -*Chamaemelum nobile*<br>-*Moringa oleifera* and *Hibiscus sabdariffa*<br>- *Cladodionen*<br>- *Natrinema versiforme* | -*Agaricus blazei*<br>-catechin, naringenin, and taxifolin of *Combretum albiflorum*<br>-*Natrinema versiforme*<br>- PUTY1 |

| | | | | | |
|---|---|---|---|---|---|
| | expression of LasI. | | | | |
| *Salmonella typhimurium* | | | Indole | | |
| *Streptococcus mutans* | | | CSP Pheromone | | |
| *Burkholderia cepacia* | QS is homologs of the LuxR and LuxI. LuxR/AHL complex is activated transcription of luxI. C8-HSL and C6-HSL are synthesized by CepI | | | EGCG (epigallocatechin gallate) | Baicalin hydrate or Cinnamaldehyde |
| *Listeria monocytogenes* | agrBDCA operon | AI-2 | | -Kinase inhibitors<br>-Phloretin(The AI-2 concentration was reduced after phloretin) | |
| *Staphylococcus aureus* | -Agr and LuxS. .autoinducing<br>-AIP | ica operon<br>-PIA/PNAG (biofilm formation in MSSA)<br>-Bap (Biofilm formation in PNAG-independent *S. aureus)* | | -Solonamide B<br>- Tet213 peptide<br>- Sumac | |
| *Candida albicans* | | Protein Hsp90 , Bcr1,Tec1 and Efg1 | | -7-Hydroxycalamenene<br>- Silver nanoparticles<br>- Shikonin | |

Second, Lara et al. have shown spherical silver nanoparticles (achieved by microwave-assisted techniques) have inhibitory effect (with an IC50 of 0.089 ppm) on biofilm formation of *Candida albicans*. Also, they showed silver nanoparticles have a good effect (with IC50 of 0.48 ppm) against pre-formed biofilm of *Candida albicans* (75). Third, based on Yan et al. study, cellular surface hydrophobicity (CSH) is an important factor for adherence to cell and biofilm formation. So, they investigated shikonin can be used to reduce the CSH of this yeast biofilms (76). Luckily, in the past years *Candida albicans* biofilm formation study has been increased. These studies have shown biofilm formation of *Candida albicans* is importantly virulence factor of this fungus. Also, attachment of yeast cells to a surface, growth of the yeast cells attached, maturation of the biofilm, and finely colonization in different places are steps of biofilm formation in *Candida albicans* (67, 68). Unfortunately, *Candida albicans* biofilm formation causes increasing minimum inhibitory concentrations (MICs) to antifungal in (69). Interestingly, *Candida albicans* could form biofilm on biotic or abiotic surfaces. The major heat shock protein Hsp90 play a key role in the regulation of dispersion in *Candida albicans* biofilm formation (70). Also, Bcr1, Tec1 and Efg1 are transcription

factors and control biofilm formation of *Candida albicans* (71, 72). Additional factor controls production of matrix. The major component of biofilm matrix is β-1, 3 glucan. Then, zinc-responsive transcription factor Zap1 controls β-1, 3 glucan, glucoamylases, glucan transferases and exo-glucanase (73).

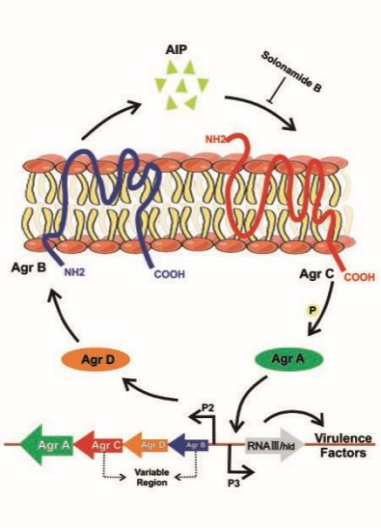


**Figure 1.** Quorum-sensing system in *Staphylococcus aureus*.

It is gratifying that, more studies have done about inhibition of biofilm formation by *Candida albicans*. First, Azevedo et al. shown that, 7-hydroxycalamenene component has antifungal activity against several fungi such as *Candida albicans*. They showed the purified 7-hydroxycalamenene was able to inhibit *Candida albicans* activity with 58% ratio (74). Second, Lara et al. were shown spherical silver nanoparticles (achieved by microwave-assisted techniques) have inhibitory effect (with an IC50 of 0.089 ppm) on biofilm formation of *Candida albicans*. Also, they shown silver nanoparticles have good efficancy (with IC50 of 0.48 ppm) against pre-formed biofilm of *Candida albicans* (75). Third, based on Yan et al. study, cellular surface hydrophobicity (CSH) is an important factor for adherence to cell and biofilm formation. So, their investigation can be used to inhibit the biofilm formation by yeasts (76).

## Conclusion

Inhibition of biofilm formation in microorganisms could be effective in treatment process of those infections. Happily, there are different ways to the inhibit the biofilm formation in different microorganisms.

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## Ethics approval and consent to participate

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## Conflict of interest

The authors declare that they have no conflict of interest.

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