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Biofilm versus Antibacterial Substances: An Evolving Battlefield in Bacterial Diversity

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A B S T R A C T

Biofilm is an aggregate of microorganisms forming multicellular communities where cells are stuck cooperatively by a produced by oneself Matrix Extracellularis. Biofilm formation by microorganisms is a ubiquitous, omnipresent & active mechanism. This vigorous ability of microbes to form biofilms proves to be a key technique for resisting and surviving in adverse climatic conditions as well as antibacterial compounds. Numerous features of biofilms contributed by component of extracellular matrix and microbial community allows in tolerating adverse conditions. Microbes in a biofilm have a remarkable property of resistance against antimicrobials, in contrast with free-swimming planktonic cells. Antimicrobial tolerance is the tendency of microbes like fungi, bacteria, or protozoans to evolve amid sensitivity to antimicrobial compounds engineered to prevent their proliferation. The development of resistance by microorganisms to antimicrobial drugs implies that these drugs are no longer effective in treating contagious illnesses which continues pioneering consequence of death rates broadly. It is believed that Coevolving bacteria with contagious microbes in biofilms have been establishing defense toward bioactive compounds from the atmosphere and to withstand their natural antibiotics and protection substances. This tolerance in infectious microorganisms Protects from chemotherapeutic interference and cause biofilm-based infections that are hard to heal. In this article, we briefly look into the characteristics of biofilms, the process of their development, and antibiotics tolerance of Biofilm-forming microbes. A vast variety of molecular pathways lead to biofilm-based antibiotic resistance have been proposed which include, effect of environmental stresses, slow growth response, restriction to antimicrobial penetration in biofilms, quorum sensing, etc. Any of these processes when acts alone only partially responsible for the increased antimicrobial resistance in biofilms. However, working together, such defenses surely enable biofilm cells longevity even against the most aggressive antimicrobial agents.

Introduction

Researches about biofilms was begun in the 1970s, however its very first existence was discovered by Antonie Philips van Leeuwenhoek through investigation of plague scratched by his tooth (1). A syntrophic consortium of microorganisms was defined in many ways by different scholars such as: (i) “Biofilms are composed of bacterial

communities attached to surfaces surrounded by a glycocalyx matrix."(2); (ii) "Biofilms are made up of single-cell and multiple cells forming microcolonies embedded in a hydrated anionic matrix of bacterial exopolymer." (2); (iii) "A community of microbial cells permeated by water channels allowing effective biomass exchange between the population and the environment" exclusively wet atmosphere. (1,3); (iv) "City of microbes" including 85percent of the maximum exopolysaccharides (EPS) as "house of the biofilm cells" (4); (vi) "Microbial communities consists of the large number of different bacterial cells living together enclosed in a self-secreted extracellular biopolymeric substances" (5).

Syntrophic consortiums of microorganisms possess several characteristics that are considered as their important tactic for sustainability. 3D structure, the existence of single or multiple microbial organisms, Coherence to each other, stick to substratum, and obedience to liquid/solid, air/ liquid & solid/air interfaces are some of the common features and decrease antibacterial resistance and hosts protection processes (1). The structural integrity of biofilm is critically maintained by the ECM secreted by the cells however the Depending on the residence microbiota including its atmosphere, the physiology, and chemistry of the biofilms can differ (1, 6). Biofilms may be comprised of multiple or Single or bacterial species. For instance, oral biofilms have been found to contain > 500 separate bacterial species. (7).

Microbes have been identified mainly as plankton based on their growth characteristics (8). It's now believed around 99percent of all microbes adhered to a surface and forms a biofilm is a ubiquitous microbial technique for their existence that in particular during unfavorable circumstances, (1). The number of cells present in biofilms is high ranges from 10^8 CFU/mg to 10^{11} CFU/mg. For example, dental plague on human teeth, chronic lung infections in cystic fibrosis (CF) patients by *Pseudomonas aeruginosa* biofilms are found in nature as well as in the industrial and clinical environment by connecting themselves to abiotic and abiotic surface. (9), in the intestine of a cow, on the submerged rock in a strong currents stream. Biofilms can colonize clinical equipment involve intrauterine contraception tools, appliances, prosthetic surgical tools, cannulas, dental implants, pulmonary devices, & contact lenses (10). For instance, *Staphylococcus aureus* biofilms can colonize pacemakers (11). It has just become evident that biofilms have an immense effect on medicines because the bacterial infections caused by them are difficult to successfully treat with antibiotics (12).

Biofilms have various features that distinguish them against planktonic cells, such as plankton cultures, whereby bacteria in a biofilm undergo a continuum of nutrition and surplus items (14). Another marked difference between planktonic and biofilms form is that their transcriptomes and proteomes are dissimilar thus providing different morphological features betwixt 2 lifestyles (15). Biofilms show separate developmental stages, unlike planktonic cells.16). In response to adverse environmental conditions, most of the microbes form biofilms, and an attribute to their elastic nature, they will contest and resist environmental forces like hunger & desiccation that proves to be advantageous characteristics for their existence (17). Biofilm formation occurs due to the regulated gene transcription of each cell through quorum sensing (18). Most of the bacterial biofilms are composed of multiple pathogenic and non-pathogenic microbial communities rather grow as a single microbial species (19). The most important morphologic distinction

betwixt planktonic cells and biofilms is that biofilm cells are much less susceptible than their genetically identical planktonic equivalents to antibacterial drugs (2). Cells can become 10-1000 fold highly robust to the influence of antibacterial drugs when embedded in biofilms. (20, 21, 22, 23). For instance, biofilm-forming microorganism *P.aeruginosa* grows on urinary catheters are about 1000 fold more tolerant to tobramycin than planktonic cells (24).

Resulting from increased biofilm tolerance to antibiotics, infections caused by biofilm-forming microorganisms continue chronically, despite antibiotic treatment (25). It is believed that mechanisms of antibiotic resistance vary depending upon the antimicrobial agent (drug) being applied, the strain as well as species of bacteria present in the biofilm, growth, and developmental conditions of biofilm (26).

The purpose of this review is to account for an insight into the mechanism used by biofilm-forming bacterial pathogens to develop resistance against antibiotics. We are focusing on bacterial species But it's supposed to be pointed out that biofilms of fungus also have resistance to antifungals than their equivalents in planktonic (27). A number of model systems have been used in previous studies to establish how and why biofilms are so tolerant to antibacterial drugs, including *P.aeruginosa* as a prototype model system for this research. Here, we also highlight the mechanisms that have been employed by other pathogenic bacterial species along with *P.aeruginosa*.

Biofilm formation

Biofilms might be either monolayered or multilayered relies on the association betwixt the substrate & the constituent cells (28). Instead of association betwixt the constituents' cells, the monolayer biofilm has the visible association between the cell & the surface. Formation of the monolayer biofilm increases or accelerates due to Various adhesive framework types, such as pilus and flagellum. Also, microorganisms usually form multilayer biofilms by adhering to a surface or each other (29). It has been noticed that repulsion takes place during biofilm formation due to the essence of bacteria's outermost layer. For example, attributed to the prevalence of O antigen, the cell wall of Gram-negative bacteria is charged negatively. This poses a problem in forming multilayer biofilms because of the repulsive force between the organisms. To form multilayer biofilms concealing and countering the repulsive force of negatively charged species arises by mutation mechanism, down regulation of very long O antigen synthesizing genes, incorporation of divalent cations, or development of extracellular polymeric compounds (28).

The development of biofilm is a cyclical and flexible procedure that encompasses transportation, diffusion, & chemical reactions occurring by several ecological and biological processes. Numerous mechanisms, like mass transport, adhesion, detachment, quorum sensing, cell death, and effective dispersal, can affect this. (1,30). However, the process of biofilm formation varies greatly among different bacterial species but it occurs in all bacterial species (31).

Biofilm formation begins with the Planktonic conformity of bacterial cells to the substratum with the help of flagella, pili, and fimbriae, the bacterial appendages (28) and also through the involvement of physical forces such as steric, Van der Waals forces, & electrostatic interactions (32). Following the preliminary stage of substratum binding, the process proceeds built and mature to the biofilm (33).

Biofilm development accomplishes through 3 common steps; in particular, Adherence, development &dispersal. In step one, planktonic bacteria transiently binds to a firm substrate through adhesion. In step two, aggregation initiates accompanied with secretion of biopolymeric substances leading to the formation of microcolonies encased by the same protective self-discharged substances called as extra polymeric substance (EPS) matrix. The final step is the dispersal stage that includes exuviates bacterial cells from biofilm. Such phase may lead to additional colonization of the biofilms with a host which eventually becomes beneficial to species because of restricted supply of resources and the deposition of waste (34). Bacteria are exposed to different stresses depending on their habitat like pH shifts, UV radiation, temperature, osmolarity, nutrient availability, and desiccation (35) that may hinder their growth and survival. The change from planktonic growth form to sessile life on a surface is a highly regulated process stimulated by these environmental signals and also genetic factors but these signals vary greatly among different organisms. For instance, *Escherichia coli* O157:H7 requires low-nutrient conditions to make biofilms (36). It is also revealed that organisms may use different genetic mechanisms to initiate biofilm formation (37). Like, *Vibrio cholera* uses different pathways for the initial stage of attachment according to the adherence surface of an organism. It has been shown from in vivo study that Tcppilus is needed for intestinal colonization of *vibrio cholera* (38) but not required for its attachment to abiotic surfaces. In *P.aeruginosa*, exposure to subinhibitory concentrations of aminoglycosides is one out of many signals that can stimulate biofilm formation (39, 40). Here, the development of biofilm occurs through five phases which are a sub-categorization of the above three basic stages (41, 42). These five phases begin with the formation of a film (layer) attached to a surface where the biofilm grows. Microorganisms then moves into the nearby surface and attached there via reversible and irreversible adhesion leading to the formation of microcolony after continuous division and growth. The change in the phenotype and genotype of the microbial cells in a biofilm observes. The process then completes with the dispersal of cells by swarming, clumping, and surface/clumping manner (43, 44).

*E. coli*is the predominant genetically diverse Gram-negative biofilm-forming bacterial strain (46,47) in which different structural phenotypes of biofilm can be observed such as simple flat, compact, and lose biofilm structures (48,49). Biofilm formation accomplishes through a series of developmental stages that are adhesion, proliferation, structural maturation, and final dispersal of cells. But, biofilm formation can be aided by certain factors such as appendages (flagella, pili, fimbriae), receptors proteins, autotransporter proteins, extracellular polysaccharides, and different genes depending on the environmental conditions and the particular strain of the bacterium (50, 51). These various contributing factors helps in different ways such as flagellar motility assists in surface contact and reversible attachment whereas irreversible attachment takes place through fimbriae and unbranched β-1,6-N-acetyl-D-glucosamine polysaccharide, microcolony formation and early development of biofilm structure is maintained by motility, curli, antigen 43 (autotransporter protein), colonic acid and extracellular polysaccharides, maturation process occurs with the help of colonic acid, curli conjugative pili, at last, final dispersion process takes place by flagella and motility (52). Biofilm formation in *S.aureus*, one of the most common nosocomial Gram-positive bacterium (53,54) provides several survival advantages such as

quorum sensing, increased protection against external stresses of the host immune system, and antimicrobials agents due to the defensive action of an extracellular polymeric matrix (55,56). In *S.aureus* biofilm formation occurs through five stages: attachment, multiplication, exodus, maturation, and dispersal. Bacterial surface molecules such as murein hydrolase AtlA (needed in cell division, cell wall turnover, and bacterial lysis) and fibronectin-binding proteins or teichoic acids involves in the initial surface attachment process (56). Hydrophobic interactions are required for attachment of cells to the abiotic surface whereas microbial surface adhesive matrix molecules help in attachment to biotic surfaces. This process leading to the development of a mat-like structure of cells made up of extracellular DNA and proteinaceous matrix. The cells shed from the biofilm by degradation of extracellular DNA with the help of nuclease allows the formation of three-dimensional microcolonies. Finally, the dispersal of cells is taking place via protease activation.

Biofilm matrix

Biofilm formation is regulated by the expression of polysaccharide molecule, which mediates cell to cell interaction. These molecules are encoded by icaADBC genes that are up-regulated in biofilms (57). Biofilm matrix is majorly composed of 97% water (58) and the rest is comprised of extracellular polymeric substances (EPS). The EPS varies in its composition, chemical, and physical properties (58). It has been observed that biofilms undergo phenotypic changes on altering environmental conditions in which it develops (58,59,60). EPS has several roles in biofilms as it protects against a variety of environmental stresses which was seen when conferring resistance against desiccation in mucoid strains of *E. coli* bacteria compared to non-mucoid strain (61). In adverse environmental conditions, bacterial species became adjusted due to the presence of EPS. To enhance the production of EPS in biofilms for adaptation bacteria attains slow growth. The mutants that are unable to synthesize the EPS are usually unable to form biofilms (39).

Antibiotic resistance

It has been observed that biofilm-forming cells exhibit a remarkable property distinct from planktonic cells that is an increased resistance to antimicrobial agents. Currently, several researches have started to emphasize on the mechanisms of how and why surface-attached microbial communities develop resistance to antimicrobial agents. Recent work suggested that multiple resistance mechanisms are there, which varies with the bacteria present in the biofilm and the drug or antimicrobials being applied. These mechanisms involve physical or chemical diffusion barriers to antimicrobial penetration into the biofilms, slow growth of the biofilm due to nutrient limitation, induction of general stress response and evolution of biofilm specific phenotypes could confer antimicrobial resistance. The physical and chemical nature of biofilm's extracellular polysaccharides or other architecture of biofilms could also contribute resistance by expulsion of antimicrobials from the bacterial community (62). The phenomenon of horizontal gene transfer in natural environments has been proved important due to the emergence of multidrug-resistance bacteria as this promotes the evolution and genetic diversity of natural microbial communities. (9, 63-65).

Failure of the antimicrobial to penetrate the biofilm

The important constituents of biofilm are glycocalyx capsule and exopolysaccharide matrix, both were found in both gram-positive

and gram-negative bacteria (66). It is believed that the matrix or capsule prevents the access of antibiotics to the bacterial cells forming biofilms in the community (67). Mathematical models suggest that many antibiotics face no barrier to their penetration into a biofilm, while some studies have shown a clear inability of certain antimicrobials agents to diffuse into the biofilm. For instance, Chlorine a commonly used disinfectant did not reach more than 20% of the bulk media's concentration of a mixed population of *Klebsiella pneumonia* and *P. aeruginosa* biofilm, this observation was measured by a chlorine-detecting microelectrode (68). Similarly, Suci *et al.,* used infrared spectroscopy to show that the rate of transport of the antibiotic ciprofloxacin to the colonized surface was reduced when compared to the transport to a sterile surface (69). This study suggested that the ciprofloxacin was binding to the biofilm components. Other researchers have taken different approaches to show whether the biofilm acts as a barrier to antimicrobial agents. On one hand where we have observed that P. aeruginosa biofilm formed on one side of a dialysis membrane prevented diffusion of antibiotic piperacillin through it (70). On the other hand, *Staphylococcus epidermidis* biofilms formed in the same way allowed rifampicin and vancomycin to diffuse across the membrane (71). These results showed that inhibition of diffusion cannot always prove to be the cause of resistance to antimicrobial compounds.

Several studies showed the difference between thick and thin biofilms and their resistance to antibiotics. Thin biofilm-covered beads allowed the penetration of hydrogen peroxide, although the cells comprising the biofilm were more resistant to the compound as compared to planktonic cells (72). On the other hand, thicker biofilms, grown on glass slides posed a barrier to the penetration of hydrogen peroxide but an interesting activity was observed that thick biofilm formed by a mutant strain of *P. aeruginosa* was penetrated by hydrogen peroxide. A mutant strain of bacteria lacked the enzyme catalase that neutralizes hydrogen peroxide, this result suggested that cells forming thick biofilms were protected from penetration of hydrogen peroxide by its catalase-mediated destruction. Another analysis for antibiotic diffusion from the agar plate through the colony by performing a standard zone of inhibition assay with the filter. This study revealed that ampicillin was unable to penetrate the biofilm due to the production of ampicillin-degrading enzyme β-lactamase, as the ampicillin was able to penetrate a biofilm formed by a β-lactamase mutant (73). Interestingly, it was found that β-lactamase mutants grown in a biofilm were still resistant to ampicillin suggesting the presence of other mechanisms contribute to the resistance of these cells.

From these studies, it is clear that the exopolysaccharide matrix (or other components of biofilms) does not form an impenetrable barrier to the diffusion of antimicrobial agents, and other multiple mechanisms are required for overall antimicrobial resistance.

Low growth as well as the stress response

Cell culture of bacteria undergoes a physiological change in growth rate from logarithmic to less or sometimes no growth due to complete starvation, which can account for an increase in resistance to antibiotics (74,75). Bacteria are observed to show slow growth (76,77). Planktonic forms and biofilms of *E. coli*, *P. aeruginosa*, &*S. epidermidis* have some effect due to the changes in growth rate under controlled growth conditions as was reported by several researchers (78,79,80). They observed that both free-swimming cells and biofilm cells show increased sensitivity toward

ciprofloxacin or tobramycin, with an enhancing rate of growth which indicates that the lower biofilm cell rate of growth protects cells against antibacterial activity. However, some experimental studies based on relativity of growth rate with resistance to antimicrobials indicate that certain important properties of the biofilm, and not slowly increasing was attributable to reported tolerance of biofilms to antimicrobial treatment (78). Also, Certain experiments have shown that the processes for various antibiotics vary. For instance, the slow growth of *P. aeruginosa* biofilm accounted for the sensitivity of biofilm to tetracycline and, it did not show up to impact tolerance in the tobramycin case. (81).

Non-uniformity in growth rate and metabolism

There is a strong assumption that individual cells comprising the biofilm, experiencing different environmental conditions with others in the same biofilm which results in their differential growth rate and metabolism. The presence of variations in signaling factors, nutrients, and oxygen availability is responsible for the phenomenon of heterogeneity among the biofilm-forming microorganisms (77). Clostridium bacterium have been reported to present a remarkable view regarding activity and growth under diverse culture conditions (82). To envisage the nonuniformity in a biofilm, a simple stain technique was employed that utilizes acridine orange dye to demarcate the region where the growth of microbes are high or where it is low based on the comparative content of their nucleic acid. The area of colonies of bacteria that carry out orange have huge content of RNA, thus having a faster growth rate, in contrast with the region turned yellow/green shows slow growth rate. An observation reported by seven-day-old biofilms shows that periphery of biofilm stained orange marked high metabolic activity and fast proliferation of bacteria due to the presence of the enormous amount of essential nutrient & O_2, whereas microbes at the biofilm center undergoes slow growth and metabolism as a result of less diffusion of nutrients, thus the region marked yellow/green in color as shown in the figure below (77).

The diversity in growth and metabolism is due to the cellular enzyme synthesis in a biofilm (83). The level of enzyme synthesis which depends upon the biomass, changes by the stages of the bacterial growth cycle (84), and synthesis gets stopped on entering the stationary phase or for slow-growing bacteria (85). Metabolically active bacteria are more susceptible and killed by antibiotics whereas at their inactive phase they are less susceptible to antimicrobial agents and have high resistance against them (86). Moreover, it is very well known that oxygen availability within a biofilm is responsible for metabolic activities. For instance, *Pseudomonas aeruginosa* forming biofilms exposing to ciprofloxacin and tobramycin antibiotics gets killed under high oxygen conditions, and its antibiotic resistance property increases on reduction of oxygen level (87,88). It is seen that under anaerobic conditions resistance of bacterial biofilm against antibiotics enhances through specific gene expression.

Quorum sensing

The process of regulation of bacterial behavior by cell to cell interaction is termed quorum sensing. It is accomplished by the production of extracellular signaling molecules, autoinducers, and their detection by another cell. Both gram-positive and gram-negative bacteria possess this mechanism of regulation (89). Quorum sensing involves the secretion of acyl-homoserine lactone (AHL), which diffuses outside the cell through the cell wall in the surrounding medium (90) and

enables the bacteria to sense and regulate the increased cell density. It also occurs through the secretion of peptides as signal compounds and two regulatory systems in gram-positive bacteria to sense the changes in gene expression pattern (91). It has been demonstrated in many bacterial species that quorum sensing plays a role in biofilm formation. The process regulates the cellular enzyme synthesis by controlling the diversification of the biofilm community. Also, quorum sensing mediates phenotypic expression under suitable nutrient and environmental conditions which in turn important for the migration of cells within a biofilm and protects the effect of new modes of growth pattern (92). It was observed that deficient quorum sensing mechanism among the bacterial species is responsible for information of thinner biofilm and lesser EPS production, and this phenotype of biofilm is susceptible to kanamycin (93). But, the direct role of quorum sensing in antibiotic resistance is not yet clear. Scientist Davies and colleagues from their previous work suggested that a mutant quorum sensing system last-last in *P.aeruginosa* was deficient information of normal structure of biofilm (94). Their presented data showed that the last mutant biofilms were sensitive to SDS treatment, but whether this mutant had any alteration regarding antibiotic resistance or not was not answered (94). Further research is required to address the (direct or indirect) effect of quorum sensing on antibiotic resistance.

Fig.1 Electron micrograph scanning of the staphylococcal biofilm of inhabited clinical tools

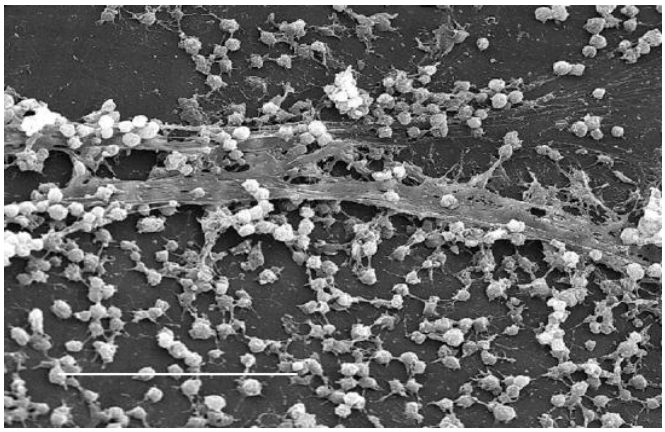


Fig.2 Schematic diagram of the main stages of biofilm formation (45)

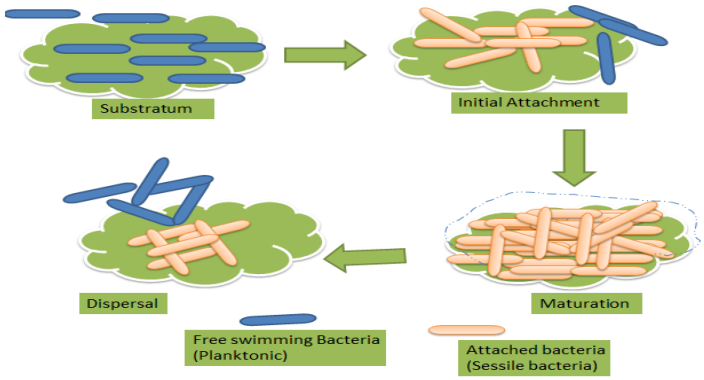


Fig.3 Heterogeneity in Biofilms (77)

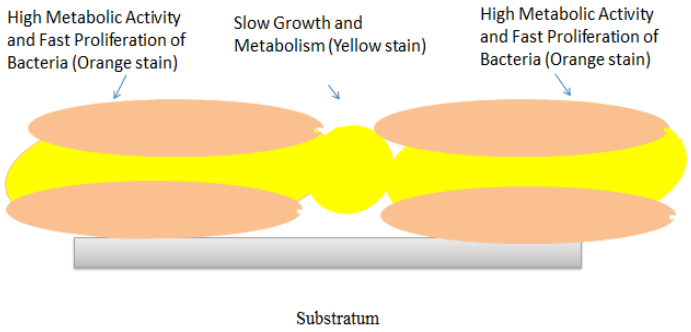
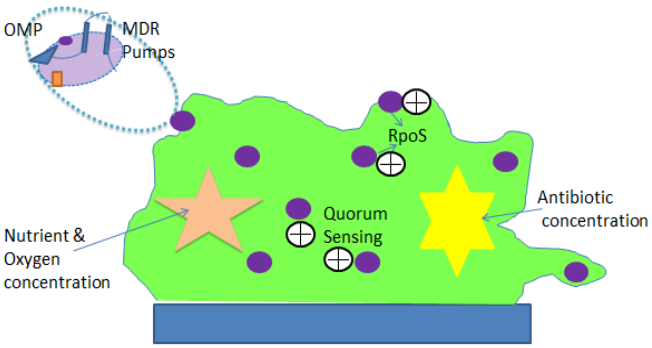


Fig.4 Schematic representation of mechanisms of antimicrobial resistance in biofilms (109)



General stress response

As the cells grow to high density within biofilms they experience various environmental stresses, towards which cells seem to have undergone stress response. The stress response is pivotal to combat the cells from the effect of heat shock, cold shock, alteration in pH, and other chemical agents (95). The cell in a biofilm shows stress response by expressing σRpoSfactor which is an essential factor in RNA polymerase. The RpoS factor normally expresses during the stationary phase of the microbial growth cycle (95). A link between RpoS factor and biofilm was identified by observing the mutant of *E. coli* that lacks rpoS results in the inability to form normal biofilms whereas planktonic cells remain unaffected by the deficiency of rpoS factor (96). In *P. aeruginosa*, another factor AlgT, plays a role in stress response (97,98). It was found that when the mutants lacking these factors formed thick biofilms on glass slides, they showed resistance to oxidative biocides as the wild forms do. Though evidence suggests that these factors have a role in biofilm resistance to oxidative biocides, other factors are also required to contribute to this resistance (99).

Resistance conferred by Enzymes

Enzyme causes detoxification of bactericide by converting it into nontoxic form resulting in resistance to biofilm. Degradation of toxic substances includes aromatic, phenolic, and heavy metals (Cu, Ag, Zn, Pb, etc) was reported in several bacteria (100). Heavy metal found in biofilms enhanced the wider range of resistant phenotypes (101).

Biofilm specific phenotype mediated resistance

Microbial cells forming biofilm are believed to express biofilm specific phenotype that could increase resistance to antimicrobial agents (72). The factors responsible for the induction of these resistant phenotypes might be nutrient limitation, high cell density, and various stresses. Currently, several works have started to find out genes that are activated or repressed in biofilms as compared to planktonic forms. (102). Multidrug resistance phenotype is mediated by multidrug efflux pumps which can involve in expulsion of antimicrobial agents from the cell. The efflux pump AcrAB is believed to be responsible for contributing multidrug resistance in *E. coli*, which occurs by activation of mar operon. Several studies were performed to find out the role of multi-drug resistance system as involved in resistance of biofilm to antimicrobial agents (103). Researchers made mutant strains of *E. coli* lacking mar- and acrAB genes to know the effect of this deletion upon antimicrobial resistance by biofilms (104). But the results do not support the assumption of attaining antimicrobial resistance by the activation of mar operon. Some other results suggested by using *Pseudomonas* strain is that they show resistance against antibiotic ofloxacin on inducing one of the efflux pumps (105). However, like *E. coli* there is no confirmation of these efflux pumps to play a role as the key factors of contributing resistance or not, therefore more research is needed in this area. The change in the composition of membrane protein induced in response to antimicrobial agents also contributes as a defense system due to the impermeability of the cell to these agents. It was observed in *E. coli* that mutation in the ompB gene encodes for outer membrane protein results in resistance against β-lactam antibiotic (106). *E. coli* mutant strain lacking ompF gene has been found to exhibit more resistance against chloramphenicol and tetracycline (107). It was also suggested that due to changing environmental conditions within the biofilm microbes altered the composition of their cell membrane which protects them from harmful effects of antimicrobial agents (108).

In conclusion biofilm forming microorganisms possess remarkable characteristics that contributed them the surviving ability. Different microorganisms form biofilms in various organs of the human body where they cause severe infections. Illness induced by Biofilm constituting microorganisms is tough to eradicate because of antibiotic resistance property. They can survive in those quantities of antibiotics that would destroy free-swimming cells. As we have discussed several general mechanisms of antibiotic resistance contributed by most common biofilm microorganisms *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus*. Researches are ongoing to elucidate more mechanisms of antibiotic resistance contributed by different entities of microorganisms. This biofilm mediated antibiotic resistance mechanisms belonging to unique pathogen allows for the development of therapeutics to impair specific tolerance mechanisms.

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