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Chapter

Development of Biofilms for Antimicrobial Resistance

Asma Bashir, Neha Farid, Kashif Ali and Kiran Fatima

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Abstract

Biofilms are a unit referred to as assemblage of microbial cells growing as surface-attached microbial communities within the natural surroundings. Their genetic and physiological aspects are widely studied. Biofilm development involves the assembly of extracellular compound substances that forms the most bailiwick network. Quorum sensing is one more crucial development specifically connected with biofilm formation in several microorganism species. In ecological purpose, the biofilm offers protection against unfavorable conditions and provides a platform for the genetic transfer. A biofilm-forming bacterium area unit is medically necessary, as they are resistant to several antibiotics and might spread resistant genes. This chapter provides the summary of microorganism biofilm formation and its significance in ecology.

Keywords: biofilms, resistance, microbes, disease, antimicrobial agents

1. Introduction

In the years which pursued the historical backdrop of microbiology, microscopic organisms have been for the most part contemplated as planktonic (free-floating) forms, the investigation of which contributed particularly to the comprehension of fundamental physiological procedures. It was just late 1960s and mid-1970s when the broad physical and chemical examinations of surface-attached microbes began coming up and the prevalence of surface-related microorganisms (biofilms) was perceived. A significant part of the prior work on biofilm characterization depended on the instruments, for example, scanning electron microscopy and standard microbiological culture procedures. The utilization of scanning electron microscopy by scientists uncovered that the biofilms are made out of a blend of various microorganisms; and the matrix material was predominantly made out of polysaccharide. The first genuine examination of biofilm was made by Costerton JW and KJ in 1978 when their examinations demonstrated that numerous microorganisms spend their most part of life inside surface-attached, sessile networks encased in a polymer network [1].

Initially the studies on biofilm were mostly focused on the structure of the polymer network or "glycocalyx" which was later portrayed by Costerton as an ion exchange network, thought to trap supplements from the surroundings [1]. Costerton found that the glycocalyx was a hydrated polyanionic polysaccharide network created by the polymerases inserted in the lipopolysaccharide part of bacterial cell wall [2]. In a watery situation (at the strong/fluid interface), biofilm generation assumes a noteworthy role in the assimilation and convergence of natural and

inorganic supplements. In addition, the biofilm provides a physical barrier that ensures incomplete protection against antibacterial substances.

During the 1990s, researchers started to comprehend the complex association of bacterial biofilm network. With the quick advances in the molecular technologies and microscopic techniques and systems, empowering extensive investigations of the biofilm method of life, there has been a striking advancement of biofilm understanding in late years. The biofilm can be framed by a solitary bacterial species; be that as it may, in many biological systems, biofilm comprises of heterogeneous networks of microorganism including bacteria, fungi, algae, and protozoa. Biofilm arrangement usually happens when microorganisms attach to surfaces in fluid conditions and begin discharging extracellular fluid like slimy material that can anchor them to a variety of materials including metals, plastics, soil particles, medical implant materials, and tissue. Microbial biofilm arrangement is known to be a successive bacterial development process and is managed by a progression of hereditary and phenotypic determinants. Accurate screening strategies, for example, isolation of biofilm defective mutants, have contributed incredibly to understanding the hereditary qualities of biofilm formative procedure; furthermore, noteworthy data is included in the hereditary premise of biofilm development.

A biofilm is known to have the involvement of many associations of microorganisms which leads to the adherence of the cells to one another and also to the surface where they are growing [3]. These adherent cells become installed inside a slimy extracellular network that is made out of extracellular polymeric substances (EPS). The cells inside the biofilm produce the EPS components, which are ordinarily a polymeric aggregation of extracellular polysaccharides, proteins, lipids, and DNA [3].

Biofilms may form on living or nonliving surfaces and are common in natural, industrial, and hospital settings [4]. The microbial cells developing in a biofilm are physiologically distinct from planktonic cells of a similar life form, which, on the other hand, are unicellular which have the ability to buoy or swim in a liquid medium. Biofilms can also grow on the teeth structure of many creatures in the form of dental plaque. This dental plaque then leads to the oral diseases of tooth decay and gum illness.

Microbes form a biofilm by the contribution of many different factors which somehow help in the recognition of sites of attachments on a surface, help them to find the nutritional sources, or, in some cases, help to develop resistance to

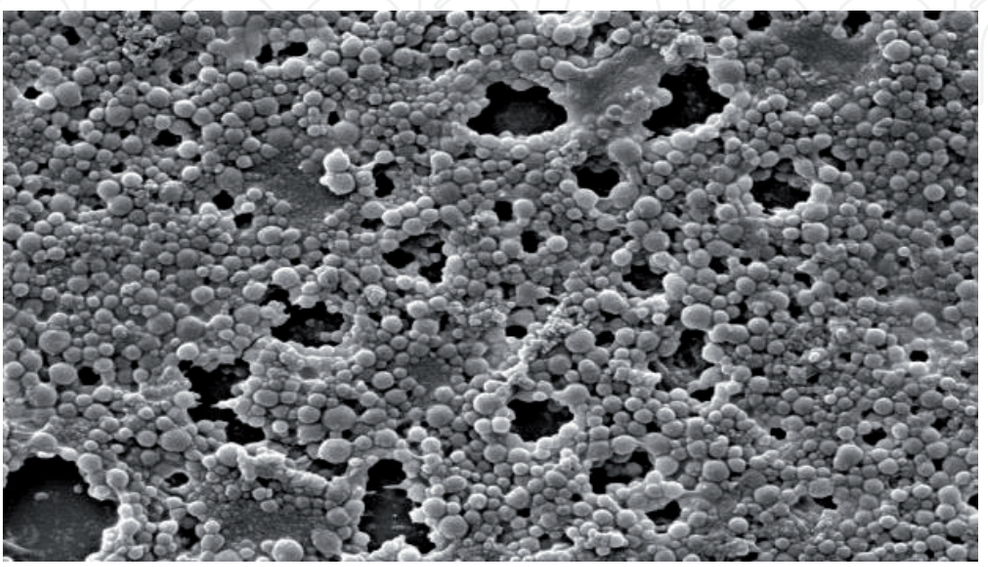


Figure 1.
Biofilm on the septum.

antibiotics. When a bacterial cell develops the property to form biofilm, it then undergoes phenotypic changes. These phenotypic changes also bring a change in the functioning of the genes.

A biofilm structure can be elucidated as hydrogel, made up of polymer which contains the dry mass enclosed in the water. Biofilms are layers formed of bacterial sludge along with the naturally occurring frameworks. This whole organization of network gives a look of well-structured meshwork of cells. Biofilms can connect to a surface, for example, a tooth, rock, or surface, and may incorporate a single microorganism category or various gatherings of microorganisms. The biofilm microscopic organisms can share nutrients and are shielded from harmful factors in the environment, for example, antitoxins, and a host body's insusceptible framework. A biofilm for the most part starts to frame whenever a free-swimming bacterium appends to a surface (**Figure 1**).

2. Origin and formation

2.1 Origin

Biofilms are known to have emerged on the primitive Earth for the purpose of defense for the prokaryotes at that time because the condition of the Earth in the early ages was very harsh and difficult for the survival of prokaryotic organism. Biofilms provide the prokaryotic cells with homeostatic conditions which empowers them with the advancement of complex interactions between the cells having biofilm.

2.2 Formation

The arrangement of a biofilm starts with the connection of free-skimming microorganisms to a surface [5]. Initially, the microbes of a biofilm may adhere tightly to the surface with the help of hydrophobic interactions and van der Waals forces. If the other colony-forming microbes are not isolated from the surface instantly, then they quickly attach themselves to the surface permanently by utilizing their cell griping structures such as pili.

Hydrophobicity has been observed to have effect on the ability of the microbes in the formation of biofilms. Microorganisms which have high amount of hydrophobicity are seen to have low amount of repulsive forces between the adherent surface and the attaching bacterium. In some cases, the microbes face difficulty in binding to the surface properly. This is because of their restricted motility, but however they can still adhere themselves to the matrix surface and to the other microbes which were initially present. The microbes having nil motility can neither attach to the surfaces nor have the ability to aggregate with each other effectively as that seen in the case of bacteria having motility.

In the process of surface colonization, the microbes have the ability to communicate by using the products of quorum sensing (QS). One of these products is N-acyl homoserine lactone (AHL). Once the cellular colonization starts, the development of biofilm also initiates by the combined effect of cell division and cell recruitment. The bacterial biofilms are mostly enclosed in the matrices made up of polysaccharides. Apart from the polysaccharides, these adherent matrices may also contain some other components such as different substances from the surrounding environment such as blood segments including fibrin and erythrocytes, minerals, particles of soil, and many other small substances. After all this comes the last phase of the arrangement of biofilm. This last stage is known as dispersion. Dispersion has been recognized as the stage in which the biofilm completely forms and may undergo some variations in shape and size.

3. Stages in the formation of biofilm

There are three stages in biofilm formation: initial attachment events, the development of complex biofilms, and separation events by clumps of microorganisms or by a "swarming" phenomenon within the interior of bacterial clusters, bringing about the so-called "seeding dispersal." Once a biofilm has fully formed, it frequently contains diverts in which supplements can flow. Cells in various locales of a biofilm additionally display diverse examples of gene expression. Since biofilms regularly build up their very own metabolism, they are in some cases contrasted with the tissues of higher creatures, in which firmly packed cells cooperate and make a system in which minerals can stream.

The biofilm life cycle is observed in three different stages: attachment, growth of colonies (advancement, and occasional detachment of planktonic cells: Free-drifting, or planktonic microorganisms experience an immersed surface and then within few minutes, they can become attached. They start producing slimy EPS and eventually begin to colonize the surface [1–4]. The formation of EPS allows the biofilm network to develop a three-dimensional and complex structure which is affected by various environmental factors. These complex networks of biofilm structures can be formed within few hours [5]. Biofilms have the sections of cluster of small or large portions of cells. It can also be observed by the process of "seeding dispersal" which helps to discharge the cells which are in singular property. Both the types of cellular separation allow the microbes to get connected either to a surface or to a unique network of biofilm [6, 7] (**Figure 2**).

3.1 Properties

Biofilms are mostly found on the solid substrates which are either submerged in or exposed in an aqueous environment. They are present in these environments apart from the fact that they can function as floating mats on liquid surfaces and also on the external surface of the leaves, which are present especially in the

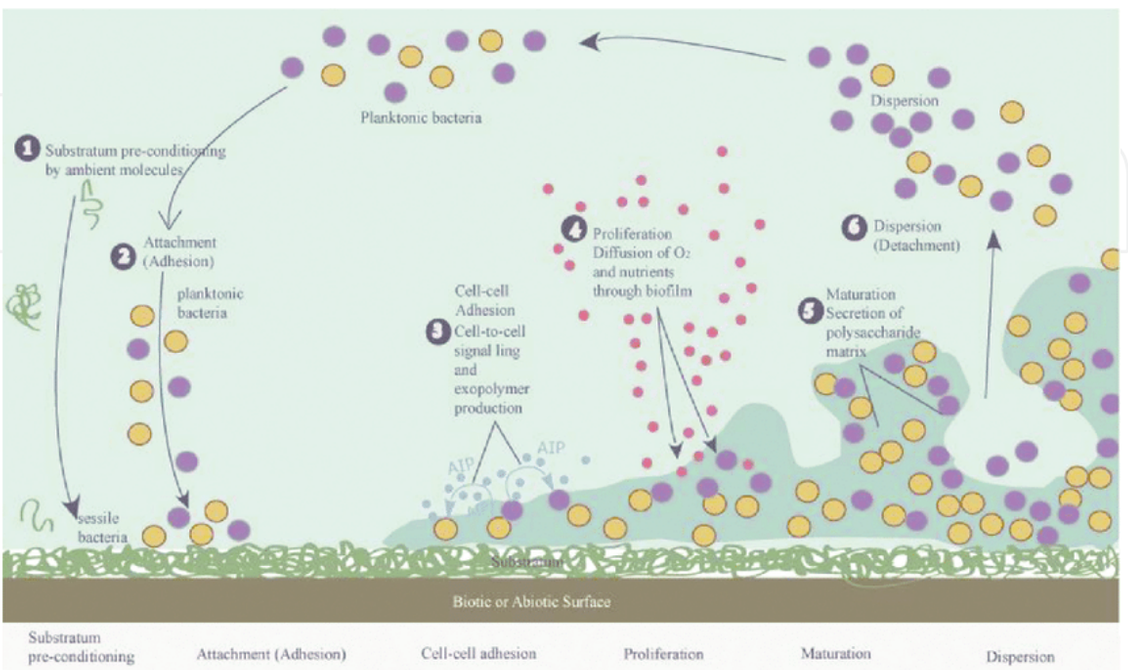


Figure 2.
Stages of biofilm development [8].

environment of high moisture. When the adequate resources for development are provided, there will be rapid development of a biofilm naturally in such a way that it will be visible clearly. Biofilms have the property to provide surface for the growth of a wide range of microorganisms which includes archaea, protozoa, bacteria, algae, and fungi with each organism having its own specific metabolic properties [9, 10].

3.2 Extracellular matrix

The EPS matrix is made up of exopolysaccharides, nucleic acids, and proteins. A major proportion of the EPS is somewhat hydrophilic along the hydrophobic portion. The example of such combination is cellulose which is made by many microbes. This matrix encloses the bacterial cells at intervals and also provides them the ability to communicate with each other through the biochemical signals and more importantly through gene exchange. The EPS matrix facilitates to trap the extracellular enzymes and then encloses them near the cells. This process shows that the EPS matrix has the ability of external digestion and it leads to the process of stable synergistic between various microbial species. There are some biofilms which have water channels. These water channels help them in the distribution of food and nutrients along with the signaling molecules [11].

Bacteria having the property of biofilm production are different from those which are free-floating bacterium of the same species. This is because of the dense and guarded setting of the biofilm which permits them to stick together [12]. The biofilm gives the microbe the advantage of resistance to different chemicals such as detergents and antibiotics. Thus, the dense matrix along with the external layer of cells provides a shield to the internal environment of the cells. In some instances, the biofilms increase the resistance several folds in the microbes [13]. It also helps in the lateral gene transfer in the normal microorganisms and the archaeal biofilms. This eventually makes a more stable biofilm structure [14]. But in some cases the biofilms have no contribution in the antimicrobial resistance. This can be seen in *Pseudomonas aeruginosa* which has no increased resistance to any antimicrobials as compared to the stationary-phase microbial cells which do not produce the biofilms. The biofilm production is seen in high rate in microbial cells present in the logarithmic phase of life cycle. This antimicrobial resistance seen in both the cells of the stationary phase and those of the biofilms may be contributed by the presence of persisted cells [15].

3.3 Quorum sensing

The role of quorum sensing in the regulation of biofilm has been first reported by Davies which initiated the dynamic research in the cell-to-cell signaling in biofilms [16, 17]. He demonstrated that lasI-mutant cells of *P. aeruginosa* that were unfit to blend the QS signaling molecule [3OC12-HSL (3-oxododecanoylhomoserine lactone)] created undifferentiated biofilm architecture and are additionally delicate to biocide SDS. Supplementation of lasI- mutant cells with 3OC12-HSL brought about a design similar to the wild sort biofilms. The procedure of cell-to-cell correspondence in bacterial populace is known to happen through small diffusible signaling molecules perceived as autoinducer. These signal molecules are created by the bacterial cells, and their concentration in the environment relies upon the density of the population. At the point when a limit focus is achieved, the signal can initiate other microbes leading to the induction or restraint of certain target genes [18].

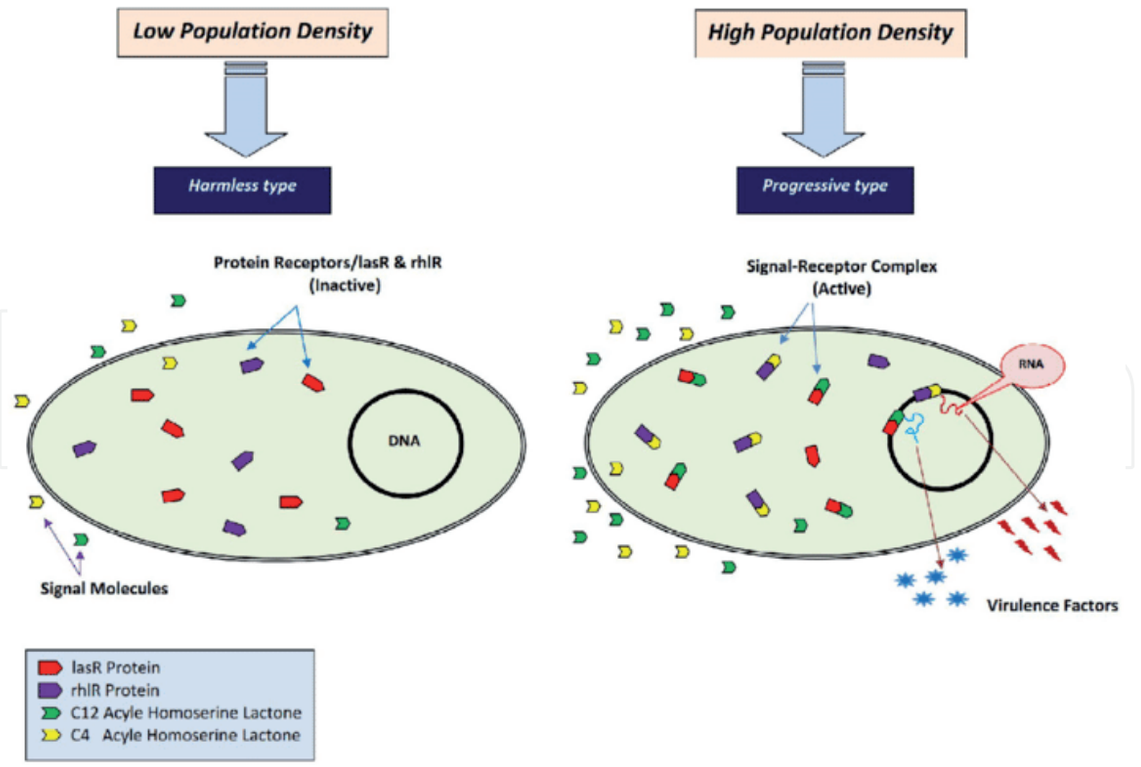


Figure 3.
Quorum sensing.

Cell density-dependent gene regulation phenomenon is otherwise called quorum sensing (QS). The chemical properties of signaling molecules associated with QS are differing; however gram-negative microbes most regularly utilize N-acylhomoserine lactones (AHLs). For instance, types of *Acidithiobacillus*, *Acinetobacter*, *Aeromonas*, *Agrobacterium*, *Brucella*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Chromobacterium*, *Hafnia*, *Mesorhizobium*, *Methylobacter*, *Paracoccus*, *Pseudomonas*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Rhanella*, *Serratia*, *Sinorhizobium*, *Vibrio*, and *Yersinia Williams* are referred to utilize AHLs as their major signaling molecules. In the biofilm arrangement as well as in the dispersal, QS assumes a noteworthy job. In *Rhodobactersphaeroides* (mutant cells), the addition of 7,8-cis-tetradecenoyl-HSL to the cell total brought about cell scattering prompting the development of free individual cells in suspension (**Figure 3**).

4. Taxonomic diversity

There are many different types of microorganisms which are known for their property to form biofilm. These include both the gram-positive and gram-negative species. The gram-positive bacteria include *Listeria monocytogenes*, *Bacillus species*, *Staphylococcus species*, and *lactic acid bacteria*, which includes *Lactobacillus plantarum* and *Lactococcus lactis*. And the gram-negative species include *Escherichia coli* and *Pseudomonas aeruginosa*. It is also been observed that other bacteria such as *Cyanobacteria* have the ability to form the biofilms in the aqueous environments. The production of biofilms is also the property of microbes which are known to colonize the plants. These microbes include *Pseudomonas putida*, *Pseudomonas fluorescens*, and connected *pseudomonads*. They are mostly the plant-associated microorganisms and are known to be present on roots, leaves, and within the soil. This is the reason which gives them the property of producing

biofilms in botanical areas. Other than these microbes, there are many other nitrogen-fixing symbionts found in legumes such as the genus *Rhizobium leguminosarum,* and *Sinorhizobium meliloti* form biofilms on legume roots and different inert surfaces. Along with microorganisms, biofilms also are generated by archaea by a variety of eukaryotic organisms including fungi, e.g., *Cryptococcus laurentii* and *microalgae* [19].

5. Biological importance

5.1 Safety from the environment

The biofilm gives a safe house and homeostasis to the living beings living inside it, and the imperative segment of this safe house is the extracellular polymeric substance network. This network can possibly forestall the flood of certain antimicrobial operators in this way confining the dissemination of these mixes from the environment into the biofilm.

EPS has appeared to have metal binding property and consequently can sequester lethal metal particles and give defensive functions. In addition to metal binding capacity, the EPS can likewise sequester nutrients and minerals from the environment. This coupling property of EPS is basically because of the nearness of ionizable functional groups, for example, carboxyl, phosphoric, amine, and hydroxyl groups. Researchers found that the sanitized EPS from the container of a freshwater sediment bacterium is fit for restricting copper. Farag detailed the concentration of metals (Ar, Cd, Pb, Hg, and Zn) in various nourishment web segments [20]. Likewise, different authors have announced the stimulatory impact of metal particles on the biofilm development. Researchers in 1997 observed an enlistment of biofilm in the developing colony of *Archaeoglobus fulgidus* when exposed to high grouping of copper and nickel. Bereswill explained the creation of amylovoran: the fundamental polysaccharide of EPS in *Erwinia amylovora*, in the presence of copper [21]. Ordax demonstrated that the EPS removed from *E. amylovora* can bind copper cations and in this manner inferred that the EPS favors the survival of *E. amylovora* under copper pressure [22]. Comparable perceptions of increment in EPS generation within the sight of metal pressure have been accounted for other bacterial species. EPS is additionally known to give a certain level of assurance to the biofilm cells from different natural stresses, for example, UV radiation, pH shifts, osmotic shock, and desiccation.

5.2 Nutrient absorption

The developed biofilm regularly contains voids and water channels that give an expanded surface zone to nutrient trade. As the water channels are interconnected and dive deep into the biofilm, it guarantees supplement accessibility to microbial networks dwelling somewhere inside the biofilm. The biofilm traps the follow component and supplement from outside condition through physical trapping or electrostatic interaction. The complex biofilm design additionally gives the chance to metabolic cooperation, and specialties are framed inside these spatially composed structures. The microcolonies created in these specialties vary in their structure removal and redistribution of metabolic end product. As these microcolonies are orchestrated one next to the other, it gives a great chance to the trading of substrate, evacuation, and redistribution of metabolic finished result [23].

5.3 Gene transfer

Biofilm offer an appropriate niche during which bacterium of various microbial community will grow in shut proximity to every possible vicinity. This provides associate in nursing area for the exchange of extrachromosomal genetic parts like plasmid inclusion body. Indeed, the transfer of inclusion body deoxyribonucleic acid via conjugation occurs at higher frequency within the biofilm cells as compared to their planktonic counterparts. The horizontal transfer of conjugative plasmid adds to the event and stabilization of biofilm. Since inclusion body could have genes that provide resistance to several antimicrobials agents, biofilm formation also offers a mechanism for the unfolding of microorganism resistance to antimicrobial agents [24]. Conjugal transfer of deoxyribonucleic acid (plasmid) is not the sole mechanism of factor transfer in a very microbial biofilm; another mechanism like transformation also can be expected, as an amount of deoxyribonucleic acid is additionally found in the biofilm structure. This deoxyribonucleic acid is assumed to be discharged within the biofilm matrix by the lysis of microorganism cells as found within the case of *Streptococcus pneumonia* and *Acinetobacter calcoaceticus*. The dense population within the microcolonies of biofilm conjointly provides a wonderful chance for the uptake of this extracellular matrix deoxyribonucleic acid [25]. Researchers observed a high frequency transformation within the young and actively growing biofilms of *Acinetobacter sp.* BD413 and correlative enlarged transformation frequency with the deoxyribonucleic acid concentration and located no saturation [26].

5.4 Disease

The role of biofilm forming microorganism in mediating numerous infectious diseases is changing into rather more necessary with an increasing numbers of infections in humans. Biofilm infection in human includes microorganism endocarditis (infection of heart valves), otitis (infection of the middle ear), chronic microorganism inflammation (infection of the prostate gland), cystic fibrosis (infection of lower metabolic process system), dentistry diseases, and most medical device-connected infections [27]. These diseases are well reviewed by researchers. *Vibrion* infectious disease which is the causative agent of infectious disease has been famous to endure transition to conditionally viable environmental cells, once discharged into the environment. Recently, researchers showed that this process involves assemblage sensing dependent biofilm formation, the factors that enhances the waterborne unfold of infectious disease epidemic [28]. In *Acinetobacter baumannii*, a medical building pathogen, biofilm formation on abiotic and biological surfaces is understood to influence its virulence. Biofilm microorganisms are consistently resistant to the antimicrobial stress, and so their demolition with antibiotic treatment could be a prime concern of medical analysis [29, 30].

6. Conclusion

The nature of biofilm structure and therefore the physiological attributes of biofilm organisms have inherent resistance to antimicrobial agents, no matter these antimicrobial agents are antibiotics or disinfectants. From the results obtained from the study, it can be concluded that the microbial strains that have the ability to produce biofilms become methicillin resistant. This supports the argument that biofilms play major role in providing the antibiotic resistance to bacteria.

Conflict of interest

There is no conflict of interest.

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Author details

Asma Bashir, Neha Farid*, Kashif Ali and Kiran Fatima
Department of Biosciences, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST), Karachi, Pakistan

*Address all correspondence to: neha_farid@hotmail.com

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