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RESEARCH ARTICLE

THE ROLES OF BIOFILM IN ANTIBIOTICS RESISTANCE

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Abstract

Biofilms are composed provinces of microscopic organisms, growths, or yeasts that structure heterogeneous substances on a natural or non-organic surface. Biofilm arrangement is a noteworthy issue in the clinical, food, and marine industries and can prompt significant economic and medical issues. The complex microbial community of a biofilm is exceptionally impervious to antibiotics and created persistent survival that is a challenge to survive. There are various approaches to control biofilms, physical and additionally mechanical expulsion, synthetic evacuation, and the utilization of antimicrobials and nanoparticles to kill biofilm organisms.

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Introduction:-

Biofilms are organized colonies of bacteria, fungi, or yeasts that form heterogeneous entities on biotic or abiotic surfaces by secreting extracellular polymeric substances (EPS). These substances protect individual cells from hostile factors, such as immunologic defense systems, nutrient limitations, and antibacterial agents (Priya et al., 2015). The genotypic and phenotypic characteristics of cells in biofilms differ from those of their free-floating counterparts, and these differences make them strongly resistant to antibiotics. This resistance has been attributed to the failure of antibiotics to penetrate biofilms, the induction of multidrug efflux pumps of biofilm-specific phenotypes, and the presence of persister's cell (Verderosa et al., 2019).

Essentially, microorganisms can adhere to surfaces, including those of dormant materials, engineered polymers, and inhabiting clinical gadgets, and this prompts colonization and mature biofilm development. Besides, cell separation from mature biofilms prompts infection spread and transmission (Fernando et al., 2018; Verderosa et al., 2019). Because of the vicinity of cells, they exchange their quorum-sensing molecules, extra chromosomal plasmids and indicated an alternate character in each biofilm community (Fernando et al., 2018).

The biofilm resistance of the microorganisms has several economic and environmental implications, including medical implants, oil recovery, drinking-water distribution, papermaking, metalworking and food-processing (Shritiet al., 2017). The bacterial attachments in the food and dairy industries are also well known related problems caused by the biofilm mechanism (Windler et al., 2015). Antimicrobial agents target a range of functional hereditary material, enzymes, respiratory system, and other cellular loci. However, due to genetic exchanges and inherent discrepancies such as exclusive cell envelope composition and non-susceptible protein different bacteria react

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differently to bactericides. Bacterial biofilm has increased antibiotic resistance and involved in many persistent diseases. Inside biofilm, several mechanisms confer the multi-factorial resistance to antibiotics.

Biofilm formation has been correlated with increased resistance to treatment with antibiotics. *K. pneumoniae* has been found to form biofilms, especially in hospital settings, and in particular on catheters. The biofilm protects *K. pneumoniae* from antibiotic treatments. This protection, thought initially to be a result of limited penetration of antibiotic molecules, is instead a result of slow growth of cells at the center of the biofilm (Vuotto et al., 2014).

Quorum sensing (cell-to-cell communication):

This is a complex regulatory process of cell to cell communication which prevents biofilm cell density from reaching an unsustainable level (Dickschat, 2010). Biofilms population density are control by cell-to-cell signaling mechanism known as quorum sensing (Hannan et al., 2010). Quorum sensing is depend on signaling molecules known as auto inducers. These auto inducers are constantly being produced by the bacterial cells, and thus, as cell density increases, so does the level of auto inducers (Figure 1). At a specific cell density, a critical threshold concentration of auto inducers is reached, which is known as the quorum level (Annouset al., 2009). During this time, auto inducer receptor binding leads to the repression or activation of several target genes. This modulation of the quorum sensing process allows bacteria to display a unified response that benefits the entire bacterial community by maintaining the optimal biofilm size and co-ordinating virulence phenotypes (Nadellet al., 2008; Annouset al., 2009; Dickschat, 2010). This unified response allows the biofilm to behave more like a multicellular organism, which enables the bacterial community to adapt to changing environmental conditions. The benefit of quorum sensing is not limited to controlling population density. In fact, quorum sensing has also been shown to aid the spread of beneficial mutations throughout the biofilm colony, enhance access to nutrients, and contribute to antibiotic tolerance (Hannan et al., 2010).

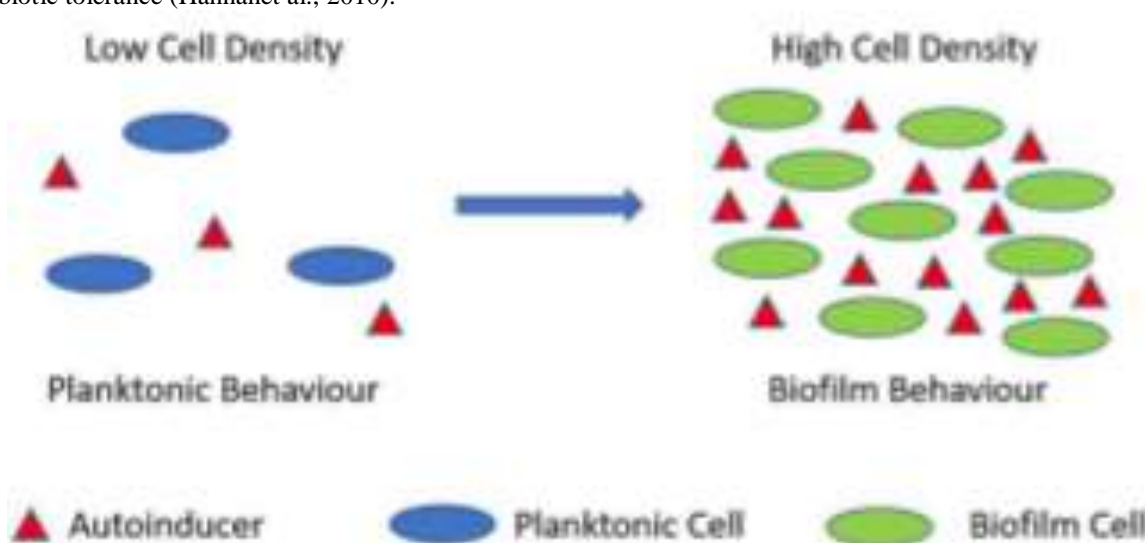


Figure 1:- Quorum sensing illustration. During planktonic cell growth (blue ovals), the relative amount of auto inducers (red triangles) is proportionally low. As cells enter a densely populated mode of growth (green ovals) the relative proportion of auto inducers increases.

Biofilm antibiotic tolerance (bat):

Biofilms are inherently more tolerant to antimicrobial treatment when compared directly to planktonic cells of the same strain. Some studies have shown that bacteria growing in biofilms are often thousands of times more tolerant to antimicrobial treatment than their planktonic counterparts (Stewart and William Costerton, 2001; Luppenset al., 2002; Davies, 2003). While, the mechanisms of antibiotic resistance in planktonic bacteria are generally well-understood (Munita and Arias, 2016), those same mechanisms (mutations, efflux Pumps, and antibiotic modifying enzymes) do not appear to be the main cause of biofilm-mediated antibiotic tolerance. For example, inherently drug-susceptible bacterial strains often exhibit significant antibiotic tolerance when in the biofilm mode of life, however, when biofilm-residing cells are dispersed (released) from the main community, antimicrobial susceptibility is quickly restored for these cells (Anderlet et al., 2000). Thus, biofilm antibiotic tolerance (BAT) is thought to involve alternative mechanisms to bacterial antimicrobial resistance.

Mechanisms of antibiotics resistance to biofilms:

Different mechanisms have been explored that are considered to be critical factors in the high resistance nature of biofilms as shown in figure 2. These mechanisms include Limited diffusion, enzyme causing neutralizations, heterogeneous functions, slow growth rate, presence of persistent (non-dividing) cells and Biofilm phenotype such as adaptive mechanisms, e.g., efflux pump and membrane alteration (Hogan and Kolter, 2002; Poole, 2002).

Limited Penetration of Antibiotics:

Diffusion of antibiotics can take place through the matrix of the biofilm. Distribution or penetration of antibiotics to deeper layers of biofilm is affected by exopolysaccharide acting as a physical barrier. When molecules directly interact with this matrix, their movement to the interior of the biofilm is slow down, resulting in antibiotic resistance. This may also act as a hindrance for high molecular weight molecules such as complement system proteins and lysozyme, and in liquid culture, bacterial cells are readily exposed to antibiotics as compared to compact structure biofilm. Bacteria escape from biofilm that does not produce polysaccharide and are easily attacked by immune system cells. Inactivation of antibiotic takes place when binding to the biofilm matrix. *P. aeruginosa* has alginate exopolysaccharide, which is anionic. Presence of this matrix explains the slow penetration of fluoroquinolones and aminoglycosides (Fernando et al., 2018).

Neutralization by Enzymes:

Antibiotics resistance in the biofilm may be due to the presence of neutralizing enzymes which degrade or inactivate antibiotics. These enzymes are proteins which confer resistance by mechanisms such as hydrolysis, modification of antimicrobials by different biochemical reactions. Accumulations of these enzymes occur in the glycocalyx from the biofilm surface by the action of antibiotics. Neutralization by enzymes is enhanced by slow penetration of antibiotics and also antibiotics degradation in the biofilm. In cystic fibrosis which is caused by *P. aeruginosa*, overproduction of cephalosporinase AmpC enzymes is responsible for resistance to different antibiotics. This enzyme confers resistance to β -lactam in the presence of even much more concentration of carbapenems (Rojas and Del Valle, 2011).

Heterogeneous Nature:

The biofilms are mixed in nature both metabolically and structurally, and both processes such as aerobic and anaerobic occur at the same time. So, response against antibiotics may be different in different areas of the biofilms. On the surface of the biofilm there is a high level of activity of antibiotics while inside the biofilms, slow or absent growth reduces the sensitivity of the cells to antimicrobials (Stewart and Franklin, 2008). In the various sub layers of biofilm, aerobic or facultative anaerobic microbial populations help us to know the differential susceptibility to various antibiotic therapies. Antibiotics response to the planktonic forms is different from the adhered cells. The action of aminoglycosides is affected by the limitation of oxygen and anaerobic growth of microorganisms, which is affected by the presence of oxygen and pH gradients.

Cells Slow Growth Rate:

Slow growth of microorganisms occurs due to limited availability of nutrients which confer resistance to antibiotics. Bacterial cells are attacked by both penicillin and ampicillin only when they are growing. Some other antibiotics that attack cells in the stationary phase are β -lactams, aminoglycosides, cephalosporin and fluoroquinolones (Muhsinet al., 2015).

The existence of Persistent Cells:

After a purling antibiotics treatment of biofilm, a minimal number of bacterial cells remain viable, called persistent cells. These cells may or may not give this resistance to their progeny and return to their normal state after the release of the applied stress or pressure. The persistent cells stop their replication for a small duration for the survival of the community. There is specific evidence for the presence of persistent cells in a biofilm:

1. There is an existence of a biphasic dimension in biofilms which means that a large number of cells population is attacked while the rest of the population is not attacked (resistant) even with an extensive antibiotics treatment
2. Persistence gene description function as a circuit of regulation
3. Bacteriostatic antibiotics contribute to the growth of persistent cell and biofilm preservation by inhibiting the growth of sensitive cells and
4. Reshaping of biofilm into original form when the antibiotics therapy is withdrawn (Gefenet al., 2017).

Biofilm Phenotype:

During biofilm formation, bacteria produce some products called secondary metabolites. These products are not required by the cell for their growth. These metabolites function as signaling molecules thus enhancing the formation process of biofilms (Estela and Alejandro, 2011). Biofilm phenotype is regarded as community cells that confer no response to antibiotics treatment. These characteristics have proposed the presence of specific genes.

Efflux Pumps:

Efflux pumps are protein structures, either expressed constitutively or intermittently. These pumps may have substrate specificity. Similar compounds can be transported by these pumps that may be involved in multidrug resistance (Estela and Alejandro, 2011).

Efflux pumps, inside the bacteria in the periplasmic area, are involved in the antagonized accumulation of antibiotics. The show is resistant to multiple antibiotics such as tetracycline, macrolides, fluoroquinolones, β -lactam and thus reducing these antibiotics concentration at a low toxic level. In several pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Enterobacter aerogenes* and *Klebsiella pneumoniae*, the efflux pump slows down the penetration of hydrophilic solutes that decrease the transmembrane diffusion of lipophilic solutes by down-regulating the 'porin' production (Li and Nikaido, 2004), (Pagès et al., 2008). Five families of efflux transporters have been identified in prokaryotes such as the major facilitator superfamily (MF), the resistance-nodulation-division family (RND), the small multidrug resistance family (SMR), the ATP-binding cassette family (ABC) and the multidrug and toxic compound extrusion family (MATE) (Kumar et al., 2005). The ABC family system hydrolyses ATP to drive antimicrobial agent efflux, whereas the MF family, MATE family, and the RND family functions as secondary transporters, catalyzing drug ion anti-proton (H^+ or Na^+) (Poole, 2005). RND family transporters are the first line of defense in bacteria by serving the target mutation or drug modification. Exposure of the bacterial biofilm to lower concentrations of antibiotics, such as chloramphenicol and tetracycline and to xenobiotics such as salicylate and chlorinated phenols, induces the expression of multi-drug resistance operons and efflux pumps (Poole, 2005).

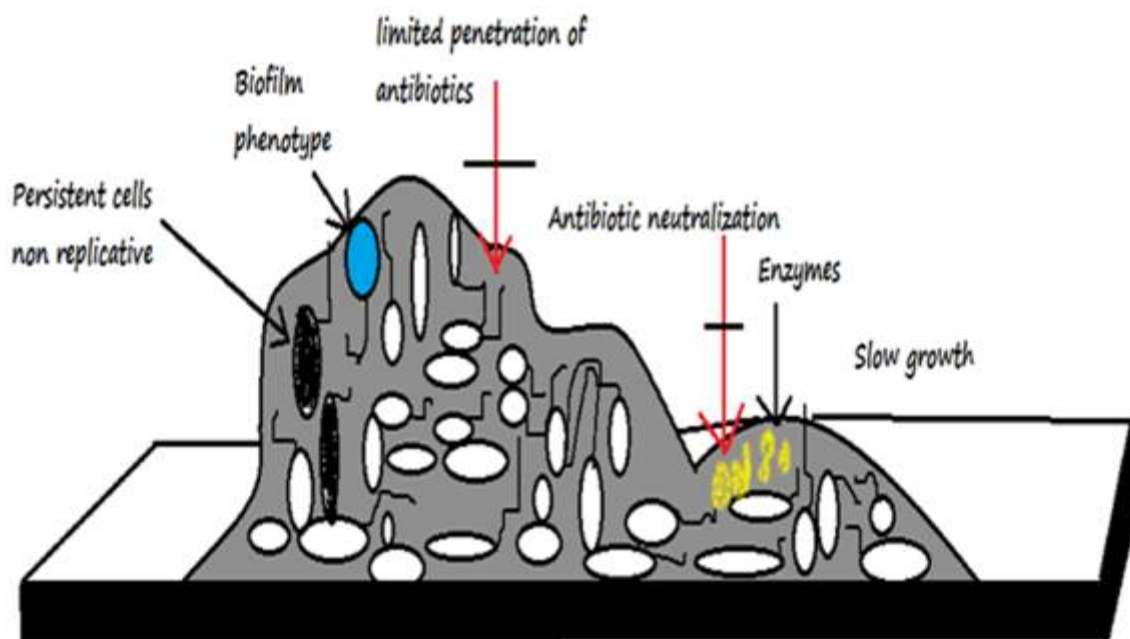
ANTIBIOTIC RESISTANCE ASSOCIATED TO BIOFILMS

Figure 2:- Antibiotic resistance associate to biofilm. Description of the critical mechanisms involved in antibiotic resistance such as enzyme causing neutralization, the presence of persistent (non- dividing) cells and biofilm phenotype.

Biofilm inhibition strategies:

The material matrix of implanted medical devices and biomaterials provide an ideal site for bacterial adhesion promoting mature biofilm formation (Arciola et al., 2012). Thus, methods which prevent bacterial attachment to these materials represent an obvious preventative strategy. The most common method for preventing bacterial adhesion is surface modification. Here, the exterior surface of the implanted medical device or biomaterial is altered, either directly or with the aid of a coating, to produce a barrier which is inhospitable to bacteria (Bazaka et al., 2012). This strategy has shown significant promise for preventing biofilm-related infections resulting from Orthopedic implants (Arciola et al., 2012).

The use of small molecule biofilm inhibitors is another approach used to prevent biofilm formation. In fact, the antibiofilm properties of a biofilm inhibitor are often employed to passivate the surface of an implanted medical device or biomaterial (Nablot et al., 2005; Boase et al., 2018). The use of biofilm inhibitors is one of the largest areas in biofilm remediation research with a plethora of unique biofilm inhibitors currently described (e.g., phenols, imidazoles, furanone, indole, bromopyrrole, etc.) (Simões et al., 2010; Worthington et al., 2012; Rabin et al., 2015; Rabin et al., 2015).

Strategies for prevention of biofilm formation on implant surfaces by use of three different approaches:

1. Use of non-adhesive coatings over surfaces to inhibit the microbial attachment to the surface.
2. Use of nanoparticles and antibiotics to disrupt the survival of attached bacteria.
3. Use of compounds like dispersion and DNase to disrupt preformed biofilm

Conclusion:-

Biofilm formation by bacteria and their subsequent resistance to antibiotic is a slow but progressive process that constitute a serious threat to public as well as domestic health. Biofilm formation has become a ubiquitous phenomenon not only for human infections, but also on non-biological aspects. Biofilms are formed on food items and water are considered as the basic necessities of daily life. Current therapeutic approaches for prevention of biofilms is limited to use of antimicrobial agents and post infection remedy lies in surgical removal of the biofilm followed by continued antibiotic administration.

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Conflict of interest:

The authors declared no conflict of interest.

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