

Multidrug Resistance Bacterial and Fungal Biofilms Become A Life Threat

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I. Introduction

For quite long time we have known that bacteria can adhere to solid surfaces and form a slimy, slippery coat. These bacterial and fungal biofilm are prevalent on most wet surfaces in nature and can cause environmental problems. Perhaps because of many biofilms are sufficiently thick to be visible to the naked eye, these microbial communities were among the first to be studied by the late-developing science of microbiology. Anton van Leeuwenhoek scraped the plaque biofilm from his teeth and observed the "animalculi" that produced this microbial community with his primitive micro-scope. However, it was not until the 1970s that we began to appreciate that bacteria in the biofilm mode of existence, sessile bacteria, constitute a major component of the bacterial biomass in many environments¹ and it was not until the 1980s and 1990s that we began to appreciate that attached bacteria were organized in elaborate ways.² For example, different bacterial species specifically attach to different surfaces or co-aggregate with specific partners in the mouth.³ Often one species can co-aggregate with multiple partners, which themselves can aggregate with other partners to form a dense bacterial plaque. Advances in light microscopy coupled with developments in microelectrode technology have led to an appreciation that bacterial biofilms consist of micro colonies on a surface, and that within these microcolonies bacteria have developed into organized communities with functional heterogeneity.

Bacterial and fungal biofilms can cause environmental problems and studies of biofilms have required the development of new analytical tools, many recent advances have resulted from collaborations between microbial ecologists, environmental engineers, and mathematicians. These efforts have led to our current definition of a bacterial biofilm as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface whereas Fungal biofilm-associated infections are frequently refractory to conventional therapy because of resistance to antimicrobial agents. This resistance could be in part due to the surface-induced upregulation of drug efflux pumps⁴.

II. What Is A Biofilm?

Biofilm is a community of microorganisms surrounded by the extracellular polysaccharides (EPS) secreted by them, attached to either an abiotic or a biotic surface. More properly known as biofilm, everyday examples are slimes which flourish in places wherever there is water such as wash hand basins in the kitchen, on teeth, contact lenses and in the gut epithelium.⁵

Understanding biofilm is a means by which complex issues associated with microbiota can be unveiled and those involving pathogenic organisms can be resolved particularly the health related problems. Over the years, studies regarding bacterial structure and behaviour have used planktonic cells that are cultivated in liquid or solid media. However, recent studies have shown that, naturally most bacteria are attached to surfaces as sessile form especially in biofilm.⁶ Now, while it is true that many bacteria can exist in diverse forms (planktonic or sessile) state biofilm formation is associated with large aggregates of these sessile bacteria^{1,7} where the bacteria adhere to surfaces by means of adhesins and EPS produced by the organisms themselves to form micro-colonies.

III. Formation And Mechanism Of Biofilm

A biofilm forming organisms, transforms from a planktonic to a sessile form and a community of organisms living in a polymeric matrix is derived. Biofilm formation comprises of three main phases ;

1. Attachment Phase (Reversible and Irreversible).
2. Micro colony formation.
3. Detachment of biofilm.

1. Attachment phase

Surfaces and interfaces are important in biofilm formation because they facilitate the acquisition of nutrients. There is always an initial attachment of a pioneer bacterium onto the surface. Initial attachment is mainly dependent on electrostatic attraction and physical forces, rather than the chemical attraction.⁸ There are two types of attachment during biofilm formation, the reversible and irreversible attachments. Some of the bacteria that finally form biofilm become irreversibly attached and produce a matrix material composed of polymeric sugars, protein or DNA.⁹

2. Microcolony formation

This matrix helps the bacterium to secure itself to the surface and helps to protect the colonising microbiota from environmental and host stress. As the bacteria begin to grow and multiply they form an aggregate of cells called a microcolony. Bacteria within the microcolony continue to divide until a critical density of bacteria or quorum forms that allows the microcolony to develop further. Biofilm bacteria continue to produce extracellular polymeric substances (EPS), a sticky substance that hold the bacteria within the biofilm together and attach the biofilm to a surface. In addition, the EPS trap scarce nutrients as well as protect the bacteria from chemical substances such as antibodies and antimicrobials. The bacteria within biofilm multiply and the daughter cells extend outwards giving the biofilm a mushroom-like which Mittelman^{10,11} termed spider - web structure. In a mature biofilm, the most of the content is water which is usually (75-95%), and the organisms (5-25%) of the biofilm.¹² This composition gives the slipperiness that is often observed in biofilm.

3. Detachment phase

At maturity, a biofilm microcolony attains a critical density of microbial population when the signalling molecules released by the colonisers portray the overpopulation of the matrix and the need for some members to disperse.^{1,13} Although quorum-sensing molecules control biofilm formation, it is also important to biofilm detachment as a means of regulating biofilm population.^{14,15} In response to the signalling molecules some biofilm colonisers transform to planktonic form and disperse from the biofilm thereby reducing the population within the matrix. An enzyme often produced by biofilm organism which aid dispersion is alginate lyase which breaks the matrix and the organisms disperse to other locations to initiate biofilm.¹⁶

Mature biofilm

The mature biofilm is a fully functional system made up of different species or genera, almost like a human community with a complex, metabolically cooperative interaction with each organism living in an adapted niche. Biofilm can form within hours of colonization by an organism or may take several weeks depending on the particular organism and the environmental factors.^{10,11} According to studies by researcher's biofilm organisms can adhere to stainless steel within of exposure¹⁷ and was found to form biofilm within 8 hour of infection in thermally injured mice.¹⁸

Quorum-sensing

Microbiologists have proved that bacteria do communicate through a process known as quorum sensing.¹⁹ Quorum sensing is a phenomenon whereby microorganisms communicate amongst themselves through chemical substances called cell signalling molecules or auto-inducers. It has been demonstrated that production of signalling molecules accumulate in the medium as the bacteria multiply and at a certain density (critical density) the molecules triggers the expression of various genes²⁰ such as production of toxins and biofilm formation^{15,21} thereby coordinating their activities. There are various types of signalling molecules which are often group related. Gram positive bacteria utilize short chain amino acids called peptides²² whereas Gram negative bacteria make use of acyl-homoserine lactones (AHL).²¹ It is now believed that these signalling molecules play an important role in diseases caused by pathogenic bacteria in animals, plants and human.²³

Mechanism of antibacterial drugs resistance of biofilm.

Microbial biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy. This also helps in the spread of antibiotic resistant traits in nosocomial pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance. Antibiotic therapy against device associated biofilm organisms often fails without the removal of the infected implant. An elevated expression of the efflux pump is another mechanism for the development of antibiotic resistance in biofilm bacteria. The specific up regulation of genes which encode antibiotic transporters, has been seen in biofilms which are formed by *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. Physiological heterogeneity is another important characteristic which is observed in biofilm bacteria. This phenomenon affects the rate of growth and metabolism of the bacteria and is reflected by

interbacterial quorum signals, the accumulation of toxic products and the change in the local micro environment. These so called persister cells are not resistant to antibiotics per se, but become resistant when associated with the biofilm.²⁴ The overall healthcare mechanisms of the underlying antimicrobial resistance of biofilms are:

1. Trapping of antibiotics in the exopolysaccharide matrix – The exo polysaccharide slime causes a diffusion barrier by restricting the rate of molecule transport to the interior of the biofilm, or by chemically reacting with the molecules themselves. The exopolysaccharide which is negatively charged, restricts the penetration of the positively charged molecules of antibiotics by chemical interactions or by molecular binding. This also dilutes the concentration of the antibiotics before they reach to the individual bacterial cells in the biofilm, thus making the antibiotics less effective against microorganisms.^{25,26}
2. Bacteria which are coated with biofilm escape the host immune system – Biofilm bacteria escape the damaging effect of the antibodies which are produced by the host immune system in response to infections.²⁷
3. Quorum sensing and genotyping adaptations alter the metabolism and decrease the growth rate of bacteria- A cell to cell communication in bacterial biofilms is established through chemical signaling. Small, diffusible molecules of class of N-acylated homoserine lactones (AHLs) are liberated by biofilm bacteria into their surrounding environment. These AHLs are associated with DNA binding proteins. As the amount of AHLs reaches a threshold level, it induces the transcription of specific genes throughout the population. The regulation of this type is known as quorum sensing (Requirement of a specific population of bacteria that is necessary for the activation of the AHL – responsive genes). The cells lying deep within the biofilm have less metabolic activity and growth rates. This makes the biofilm organisms inherently less susceptible to antibiotics. Due to the consumption of oxygen and glucose, relative anaerobiosis is created at the deeper layers of the biofilm, where in order to survive, the microorganisms transform into slow growers and non growers. Older biofilms are relatively more resistant than newer biofilms.²⁸ After the attachment to a biotic or an abiotic surface, the bacteria undergo further adaptation, i.e, increased synthesis of exopolysaccharide and increased antibiotic resistance. They also develop an increased resistance to UV light, increased genetic exchange, altered metabolism and increased secondary metabolism production.

Mechanism of antifungal drugs resistance of biofilm

Antifungal drug resistance is quickly becoming a major problem in the expanding population of immunocompromised persons. It has resulted in a drastic increase in the incidence of opportunistic and systemic fungal infections. Clinical resistance is defined as persistence or progression of an infection despite appropriate antimicrobial therapy. Resistance is considered primary when an organism is resistant to the drug before exposure, whereas secondary resistance is that which develops in response to exposure to the drug.²⁹

1. This latter mechanism of resistance accounts for the emergence of resistance to azoles seen over the last few years. Azole antifungal agents have become important in the treatment of mucosal candidiasis in HIV patients. Specifically, fluconazole is considered the drug of choice for the most common HIV-associated opportunistic infections in the oral cavity.³⁰
2. Increased use of the azoles, coupled with the fact that they are fungistatic drugs, has likely resulted in the emergence of resistance to azoles. Major genes that contribute to drug resistance are those coding for multidrug efflux pumps, the upregulation of which can result in a multidrug-resistant phenotype.^{31,32,33,30,34}
3. *C. albicans* and *C. dubliniensis* possess two different types of efflux pumps: adenosine triphosphate-binding cassette (ABC) transporters encoded by the *CDR* genes (*CDR1* and *CDR2*) and major facilitators encoded by the *MDR* genes.^{31,35,30,36}
4. Genes for both types of efflux pumps have been recently demonstrated to be upregulated during biofilm formation and development.^{31,32,33}
5. The ABC transporters *CDR1* and *CDR2* in *C. albicans* and *C. dubliniensis* constitute a multigene family with a demonstrated role in resistance.
6. The *MDR1* gene encodes a major facilitator, the over expression of which leads exclusively to fluconazole resistance.^{32,33,35}

IV. Biofilms In Human Diseases

In human host, there are various surfaces available for attachment particularly the surfaces that are exposed to the external environment such as the skin, mouth, respiratory and gastrointestinal tract (GIT). These sites and other parts of the body support the planktonic as well as the sessile populations, some of which are commensal bacteria. The exception of the situation is found with teeth, a situation that encourages formation of biofilm which results in dental plaque.^{37,38,39} Advancement in medical treatment has resulted in the use of implants and other devices such as catheters and prostheses that often encourage the formation of biofilm.^{40,41}

The association of these devices with biofilm formation has posed a lot of problems to the medical practitioners and the patients.

Biofilms have been found to cause serious health related infections because of their resistance to antibiotics and antifungal drugs in host defence mechanism and such diseases are often persistent and difficult to effectively eradicate.⁴² However the majority of human diseases associated with biofilm are usually linked with the presence of some implantable devices (e.g. catheters, heart valves, prostheses) or impairment of the host defence systems such as cystic fibrosis patients.⁴³ It has been shown that 65% of human infections involve biofilms.⁴⁴ *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Candida albicans* and *C. dubliniensis* are the commonly isolated etiological agents producing biofilm.

V. Conclusion

We concluded that in next coming years biofilms will become a threat which causes chronic health problems as mentioned above. These chronic health issues difficult to treat which leads to multiple drug resistance. By using various models of both bacterial and fungal biofilm several studies have shown uniform resistance to the organisms. In the biofilm use of wide spectrum conventional antibacterial resistance to cephalosporins and fluoroquinolones and antifungal agents resistance to the new triazoles, which have been shown to be fungicidal with extended activity against many resistant organisms. Therefore, biofilm-associated infections are difficult to treat, which emphasizes the need to develop antimicrobial drugs that show activity against biofilm-associated organisms and specifically target biofilm-associated infections. The overall healthcare costs which are attributed to the treatment of biofilm associated infections are much higher due to their persistence. Besides, a longer hospital stay is another factor for higher costs. Early detection of biofilm associated infections and newer treatment options for the management of the same are needed.

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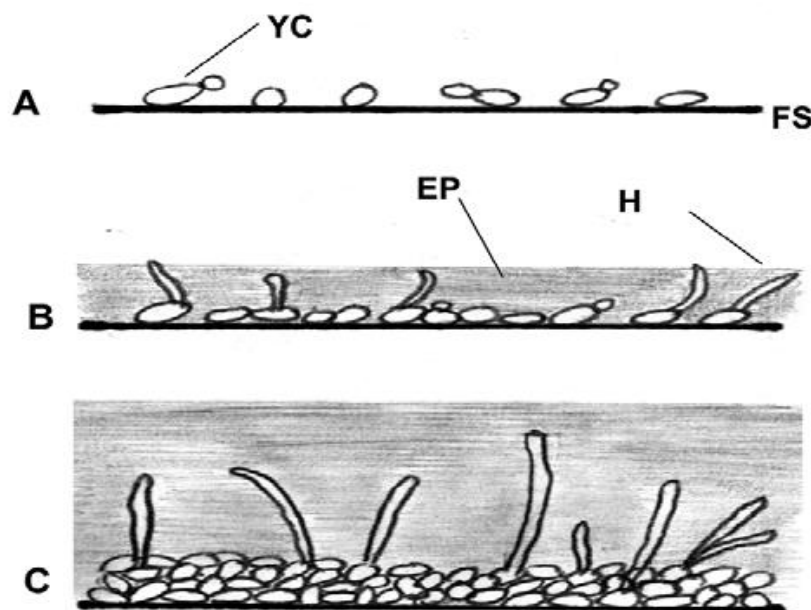


Figure 1. Illustration of biofilm development in *Candida albicans* and *C. dubliniensis*; A, early 0–11 h; B, intermediate 12–30 h; C, mature 38–72 h; FS, flat surface; YC, yeast cell; H, hyphae; EP, exopolymeric matrix.