MINI REVIEW

Bacterial Biofilms - A Therapeutic Challenge

Dixit K. Parasana[1*], Bhavesh B. Javia[2], Dhaval T. Fefar[2], Dilipsinh B. Barad[2], Sanjay N. Ghodasara[2], Irsadullakhan H. Kalyani[1]

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Abstract

A bacterial biofilm is a community of bacteria or colony, adhered to a stationary living or non-living surface within a matrix of self-produced extracellular polymeric material and microbial cells. Bacterial biofilms can result in nosocomial infections and are typically harmful in nature. According to the National Institutes of Health (NIH), biofilm formation is the cause of 80% of chronic illnesses and 65% of all microbial infections. Bacterial biofilms exhibit resistance to both the host immune system and antibiotics. Infection linked to biofilms can result in significant productivity losses for the livestock industry. Treating chronic mastitis with commonly available antibiotics is exceedingly challenging when it is caused by biofilm-producing *Streptococcus* spp. and *Staphylococcus aureus*. Staphylococci that produce biofilm are important contributors to wound infection because they hinder wound healing, which increases the risk of chronic infection and subsequent bacterial infection. Additionally, biofilm bacteria may act as zoonotic agents. Therefore, we should try with alternate management techniques to combat biofilm microorganisms. In this overview, emphasis has given on status of biofilm associated diseases in animal, zoonotic importance, probable reason for antibiotic resistance and therapeutic approaches against biofilm infections

Keywords: Antibiotic resistance, Bacterial biofilm, Chronic infection, Therapeutic approach.

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Introduction

A biofilm is a microbial community made up of microbial cells that attach to one another on living or non-living surfaces and are encased in an extracellular polymeric substances (EPS) matrix (Jamal *et al*., 2017). The sluggish growth rate, up and down regulation of genes, and production of extracellular polymeric matrix distinguish biofilm-associated cells from other types of cells (EPM). In veterinary field biofilm producing bacteria are also involved in many conditions including pneumonia, liver abscess, enteritis, wound infections and mastitis(Kokare *et al.,* 2009). Proteins, DNA, polysaccharides, and RNA are among the extracellular polymeric molecules that make up biofilm. The water makes up the majority of biofilm (up to 97%) and is responsible for the movement of nutrients throughout the biofilm matrix, and it is also a significant component (Vinodkumar *et al*., 2008).

Biofilm and Antibiotic Resistance

Antibiotic resistance in biofilms is 1000-1500 times higher than in planktonic stages (Shakibaie, 2018). Low antibiotic penetration, enzyme neutralisation, heterogeneous nature, the presence of persistent cells, and the slow pace of cell development are a few mechanisms that have been studied and are thought to be important contributors to the biofilm's high resistance (Jamal *et al*., 2017).

Low penetration of antibiotics

Antibiotics may be able to penetrate the biofilm's matrix. Exopolysaccharide acts as a physical barrier, which affects the diffusion or penetration of antibiotics into deeper levels of biofilm. Antibiotic resistance arises when molecules directly contact with this matrix, which slows down their migration to the biofilm's centre. The alginate exopolysaccharide, which is anionic in nature, is present in *Pseudomonas aeruginosa*. The presence of this matrix explains why fluroquinolones and aminoglycosides only are partially penetrate the skin (Lewis, 2001).

[1]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Navsari, Gujrat, India.

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Junagadh, Gujrat, India.

***Corresponding author:** Dixit K Parasana, Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Navsari, Gujrat, India, Email: dkpatel784@gmail.com.

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Neutralization by enzymes

The presence of neutralising enzymes, which break down or render inactive antibiotics through hydrolysis and other biochemical processes, which may be the cause of antibiotic resistance in biofilm. Cephalosporinase Ampc enzyme overproduction in cystic fibrosis induced by *Pseudomonas aeruginosa* results in antibiotic resistance even in the presence of significantly higher antibiotic concentrations (Jamal *et al*., 2017).

Heterogeneous nature

The biofilms are diverse both in terms of metabolism and structure. Since aerobic and anaerobic metabolism coexist inside the biofilm, various regions of the biofilms may have varied responses to antibiotics. Antibiotics are highly active on the surface of biofilms, but inside of them, where growth is slow or nonexistent, the sensitivity of the cells to antimicrobials is reduced (Stewart *et al*., 2008).

Existence of persistent cells

A relatively small fraction of the bacteria, known as persistent cells, are still alive after the biofilm has been treated with conventional antibiotics. For a shorter period of time, persistent

cells stop replicating in order to ensure the community's survival. When bacterial cell density in stationary phase reaches its maximum, persistent cells become more numerous, indicating their primary function in survival (Lewis, 2008). As soon as the antibiotic therapy is stopped, persistent cells reconstruct the biofilm into its original structure.

Slow growth rate of cells

Microorganisms develop slowly because there aren't enough resources available to them, which makes them resistant to antibiotics. A gradient of nutrients creates metabolically active cells and dormant cells in biofilms (Brown *et al*., 1988). Penicillin and ampicillin both target developing bacterial cells for destruction whereas beta lactams, aminoglycosides, and fluroquinolones are antibiotics that target stationary phase cells (Costeron *et al*., 1999).

Biofilm and Zoonosis

Microorganisms that create biofilms are frequently responsible for human and animal infections, and they can spread from one another. The dog bites can spread bacteria that forms biofilms to humans (Zambori *et al*., 2013). Biofilm forming *Campylobacter* and *Salmonella* in poultry can transmit infection to human through consumption of poultry meat (Tram *et al*., 2020; Lapierre*et al*., 2020).

Infections Related to Biofilms

Bacterial biofilms accompany bacterial illnesses in about 65% of cases (Lewis, 2001). Both device and non-device associated infections are included in this.

Device related biofilm infections

The majority of the time, biofilms develop on or inside of numerous devices, including peritoneal dialysis catheters, central venous catheters, mechanical heart valves, milking machines, and urine catheters (Donlan, 2002). The formation of biofilm on central venous catheters is typical, but the location and intensity of the biofilm depend on how long the catheterization was in place. The long-term catheterization (>30 days) have stronger biofilm growth in the catheter lumen, short-term catheterization (10 days) have a higher affinity for biofilm formation on the exterior surface. The bacteria like *E. coli, Enterococcus faecalis, S. epidermidis, P. aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and other gram-negative bacteria frequently contaminate and create biofilms on these devices (Stickler, 1996).

Non-device related biofilm infections

The bacteria like *Staphylococcus, Streptococcus, Pseudomonas, E. coli, Pasteurella* and *Corynebacterium* are major infectious agents responsible for various biofilm associated conditions in veterinary field (Abdullahi *et al*., 2016). The staphylococci produces biofilm play a significant role in hindering wound healing and increases the risk of secondary bacterial infection. In addition to antibiotic resistance, *Pseudomonas aeruginosa* has ability to build biofilms which makes it a chronic source of infection for the host and inhibits host defence by destroying polynuclear immune cells through the creation of rhamnolipid. (Jamal *et al*., 2017). *E. coli* are the predominant commensal bacteria in human, animal and poultry, and some strain of Enterohaemorrhagic *E.coli* are capable to form biofilm (Puttamreddy *et al*., 2010). *Listeria monocytogenes* biofilms are a real problem since they have been found to be more resistant to disinfectants and sanitizers than planktonic cells (Amalaradjou *et al*., 2009). The ability to build biofilm and the presence of several virulence genes in *Streptococcus* spp. isolated from mastitis affect the course of the illness and its management(Kaczorek *et al*., 2017). Some important biofilm related conditions in veterinary field are listed in Table 1.

Therapeutic approaches of biofilm

antibiofilm and antimicrobial agents must be used for effective treatment of biofilm infection (Romling *et al*., 2012). For control of biofilmseveral approaches like phage therapy, electric current, enzymes, ultrasound therapy and anti adhesion agents are commonly used (Kostakioti*et al.,* 2013).

Phage Therapy

Although not frequently used in veterinary biofilm therapy, this is a strong treatment strategy. However, it requires the usage of a protein that binds to the DNA or RNA genome in order to strongly activate bacteriocidal activities at the location of a biofilm infection. By producing enzymes, phage produces its antibiofilm activity. This enzyme breaks down and hydrolyzes the biofilm's extracellular matrix. Perhaps the combining ~~an~~ of antibiotic with a bacteriophage will be successful (Verma *et al*., 2010).

Electric current

The antibacterial action of antibiotics, which are often resistant to biofilm organisms, is increased when low level electric current is used in conjunction with them. By using electric current, cationic antibiotitics can boost their antibacterial efficacy against bacterial biofilm.Against *S. aureus* biofilmadministration of gentamicin with simultaneous release of electromagnetic impulse can increases drugs effect (Kasimanickam *et al*., 2013).

Enzymes

The enzymes DNase I and Dispersin B function as excellent antibiofilm agents for Gram-positive infections. DNase I's actions are based on its capacity to break down the eDNA

Table 1: Biofilm associated conditions In veterinary field

Type of infection	*Organism*	*Reference*
Mastitis	*Staphylococcus* spp.	Fox *et al.* (2005); Szweda *et al.* (2012)
	Streptococcus spp.	Kaczorek *et al.* (2017); Parasana *et al*. (2023)
Otitis externa	*Staphylococcus* spp.	Moreira *et al.* (2012)
	Pseudomonas aeruginosa	Chan *et al*. (2019)
Wound infection	*Pseudomonas aeruginosa*	Seth *et al.* (2012); (Pastar *et al.* (2013)
Urinary tract infections	*E. coli*	Oliveira *et al.* (2014); Kern *et al.* (2018)
Pyometra	*E. coli*	Fiamengo *et al.* (2020)

contained within the biofilm structure (Qin *et al*., 2007). *Staphylococcus* and *Enterococcus* biofilm development was prevented by DNase treatment (Guiton *et al*., 2009). *Actinobacillusactinomycetemcomitans* produces Dispersin B, a glycoside hydrolase that breaks down beta 1-6 N-acetylglucosamine polymers (PNAG) in the bacterial peptidoglycan layer (Fey, 2010). Treatment with Dispersin-B against *S. aureus* and *S. epidermidis* biofilms has demonstrated to be effective (Izano *et al*., 2008).

Anti Adhesion Agents

The anti-adhesion surface of implanted devices helps to lessen the pathogenic bacterial attachment, which significantly reduces biofilm formation. *Staphylococcus aureus* adherence on the titanium surface is significantly decreased by applying a polyethylene glycol coating (Harris *et al*., 2004). Simentenously monomeric biphenyl mannosides have been demonstrated to destroy already-formed biofilms and to stop the growth of uropathogenic *E. coli* biofilms in vitro (Cusumano *et al*., 2011).

Ultrasound Therapy

This gadget boosts the antimicrobial agent's ability to kill bacteria by sending non-invasive acoustic energy waves through the skin to the location of the biofilm. Ultrasonic energy is also utilised to speed up the release of medication from delivery systems and to promote the degradation of biofilm cell membranes, which promotes antibacterial action and facilitates the active or passive uptake of antibiotics (Kasimanickam *et al*., 2013).

Conclusions

A biofilm is a microbial community that forms when microbial cells cling to one another on living or inert surfaces within a self-made EPS matrix. It is extremely resistant to antibiotic and host immune system. It is responsible for chronic infections like mastitis, pneumonia, wound infection, urinary tract infection etc. Biofilm can be occuron various devices like venous catheter, urinary catheter etc.Because of the high level of antibiotic resistance, biofilm infections can be treated using phage therapy, ultrasound therapy, electric current, enzymes, and anti-adhesion medicines. The current situation of biofilm-associated infection in veterinary medicine is becoming a serious problem, posing a substantial risk to human health and causing significant financial loss. It is possible to prevent the biofilm infection by managing the indiscriminate use of antibiotics and upholding appropriate biosecurity and biosafety procedures.

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