Review Article

Impact of Bacterial Biofilm in Veterinary Medicine: An Overview

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A B S T R A C T

Biofilms are communities of microorganisms that are attached to a surface and play a significant role in the persistence of bacterial infections. Biofilm bacteria are highly resistant to antimicrobial agents and host immune responses resulting in chronic infection. There is paucity of information regarding biofilm bacteria of veterinary importance and their role in disease pathogenesis. Biofilm associated infection can cause huge economic loss in livestock industries in terms of production. For example, chronic mastitis due to biofilm producing Staphylococcus aureus and Streptococcus spp. is almost incurable with common antibiotics. Moreover, biofilm bacteria are potential zoonotic agents. So we should ventured alternative managerial practices to fight against biofilm bacteria.

Introduction

Since their birth, bacteria are constantly modified and adopted themselves to combat all the hostile environment of planet. As a result, for self-defense, multidrug-resistant (MDR) and extremely resistant (XDR) bacteria emerged throughout the world pose a threat to global health. The situation of antibiotic resistance is increased up to 1000 times if the infections are associated with biofilm producing bacteria compared to their planktonic counterparts (Rasmussen and Givskov, 2006). Biofilm is a well-organized, cooperating community of microorganisms embedded in self producing extracellular polymeric substance (EPS). Biofilm associated cell is differentiated from
suspended counterparts by reduced growth rate, up and down regulation of gene and generation of extracellular polymeric matrix (Kokare et al., 2009). The high resistance to harsh conditions, including pollutants (Joubert et al., 2006), desiccation (Queck et al., 2006), protozoan grazing, antimicrobial agents in nature and host defences (Taylor and Webster 2009) in chronic infections, is some/one of the most important features of biofilms. It is estimated that 99% of bacteria in nature exist in biofilms, while biofilms account for more than 65% of nosocomial infections (Böhme et al., 2009) and approximately 61% zoonotic human infection (Garcia and Percival, 2011). Biofilm bacteria are also involved for many diseases in veterinary medicine including pneumonia, liver abscesses, enteritis, wound infections and mastitis infections (Olson et al., 2002; Clutterbuck et al., 2007). In this overview, emphasis has given on biofilm architecture, mechanism of biofilm formation, status of biofilm associated diseases in animal, zoonotic importance, probable reason for antibiotic resistance, possible diagnostic approaches and control strategies.

Structure of biofilm

The structure of the extracellular polymeric substance (EPS) matrix of bio-films is composed of one or more of extracellular polysaccharides, DNA and proteins (Flemming et al., 2007). Channels in the biofilm allow for water, air and nutrients to get to all parts of the structure (Zhang et al., 1998). Exopolysaccharides are high molecular weight polymers of sugar synthesized extra cellulary or intra cellulary and secreted into the outside environment (Nwodo et al., 2012).

Extracellular proteins are another major EPS matrix component (FrØlund et al., 1996). Some proteins are attached to cell surfaces and polysaccharides to help with biofilm formation and stabilization. For example, glucan binding proteins (Gbps) of Streptococcus mutans play an important role in biofilm architecture maintenance by linking bacteria and exopolysaccharides. Biofilms produced by Gbps mutants have significantly reduced height (Lynch et al., 2007). Amyloids are also insoluble fibrous proteins that play a supportive role in biofilm architecture. Overexpression of Fap amyloids in Pseudomonas spp. leads to increased biofilm formation (Dueholm et al., 2013).

Extracellular DNAs (eDNAs) play an important role in biofilms formation. eDNA also helps to get attached bacterial cells to substratum surface by receptor mediated interaction (Das et al., 2010). eDNA also coordinates the twitching motility of Pseudomonas aeruginosa leads to biofilm formation (Gloag et al., 2013). Due to its negative charge, eDNA is able to chelate metal cations and some positively charged antibiotics leading to antimicrobial resistance in biofilm bacteria (Lewenza, 2013).

Process of biofilm formation

Formation of a biofilm begins with the attachment of free floating microorganisms to a stable surface. The microorganisms adhere to surface through weak van der Waals forces and hydrophobic effects (Briandet et al., 2001; Takahashi et al., 2010). Biofilm formation can be described in three stages: attachment, maturation and dispersion. The attachment step could be further categorized as a two-stage process: initial reversible attachment and irreversible attachment. The irreversibly attached biofilm can tolerate stronger physical or chemical shear forces (Renner and Weibel, 2011). In the initial
attachment, flagella and type IV pili-mediated motilities play important roles. Flagella help to attach bacterial cells with surface, whereas Type IV pili-mediated twitching motilities enable attached cells to aggregate (Rabin et al., 2015). Once the attachment becomes irreversible, the biofilm begins to grow and mature. Maturation involves significant genetic upregulation results in marked difference between planktonic and sessile bacteria (Sauer et al., 2002). Biofilm grows from a thin layer to a ‘mushroom’ or ‘tower’ shape structure. In a thick biofilm (>100 layers), bacteria are arranged according to their metabolism and aerotolerance, in which anaerobic bacteria prefer to live in deeper part to avoid exposure to oxygen (Rabin et al., 2015).

Once maximum thickness attained, the final stage of biofilm development occurs. This is called the dispersion stage and it involves the release of planktonic cells from the biofilm. Biofilms disperse because of several factors, such as lack of nutrients, intense competition, outgrown population, etc. Dispersal could occur in the whole biofilm or just a part of it. Several enzymes help in the dispersal process, viz., dispersin B, deoxyribonuclease etc. (Kaplan 2010; Izano et al., 2008).

Biofilm producing bacteria of veterinary importance

**Staphylococcus spp.**

*Staphylococcus* spp. cause wide array of diseases in animals including mastitis, wound infection, septic arthritis, otitis, urinary tract infection etc. Mastitis is an economically important and frequently occurring disease of dairy cows. *S. aureus* is the primary pathogen isolated from mastitis cases (Oliveira et al., 2007). During infection, pathogens associated with mastitis form biofilms, which facilitate their persistence in the udder leads to chronic infection (Melchior et al., 2006). Approximately one-third (37.5%) of *S. aureus* and *S. epidermidis* isolates from subclinical mastitis recorded as biofilm producer (Oliveira et al., 2006). It is also recorded that *S. aureus* associated with milk are more likely to produce biofilms, when compared to *S. aureus* extra-mammary sources (Haveri et al., 2008) Several genes have been associated with biofilm formation by staphylococci. These genes encode the accessory gene regulator agr, icaADBC which encodes the PIA/PNAG producing enzyme and transporter, and the biofilm-associated protein Bap (Melchior et al., 2009).

Biofilm producing staphylococci plays a significant role in wound infection by impairing the healing of wounds leading to chronic infection and increase the chance of secondary bacterial infection. Moreover it also minimizes the treatment options showing high resistance to commonly used systemic and topical antibiotics. The first evidence of wound infection by biofilm bacteria comes in 1996, where *S. aureus* was inoculated onto a wound in a mouse skin and only within 6 hours biofilm was developed (Akiyama et al., 1996). Methicillin-resistant *S. aureus* (MRSA) could also form dense biofilm within the inoculation of 24 hours tested in murine wounds model (Roche et al., 2012). Moreira et al., (2012) demonstrated *S. intermedius* and *S. simulans* biofilm infection from canine otitis. Association of staphylococcal biofilm and septic arthritis is well established in experimental animal model (Colavite and Sartori, 2014).

**Pseudomonas aeruginosa**

*P. aeruginosa* is considered as the most potent member of multidrug-resistant (MDR) and extremely resistant (XDR) gram-negative pathogens (‘ESCAPE’ group) emerged throughout the world with the property to
‘escape’ the treatment with antibiotics. Biofilm formation is a significant virulence property of *P. aeruginosa* generating not only antibiotic resistance, but also it acts as a constant source of infection in the host and it can prevent host defence such as chemotaxis of polynuclear immune cells. In animals, *Pseudomonas aeruginosa* is associated with wound infection in majority of species; respiratory infection, mastitis, enteritis in cattle; pneumonia, mastitis, fleece rot in sheep; embryonic death in poultry; and otitis, urinary tract infection in companion animals (Samanta 2013). In India, Rashid et al., (2000) could detect *P. aeruginosa* biofilms on a murine burn. Schaber et al., (2007) also demonstrated *P. aeruginosa* biofilm in a thermally injured mouse model. Seth et al., (2012) could demonstrate *P. aeruginosa* biofilm in dermal punch wounds of white rabbit ears where, they found delayed healing compared to uninfected wounds. Synergistic effect of MRSA and *P. aeruginosa* biofilm in delaying reepithelialisation of experimental porcine wound is well documented (Pastar et al., 2013).

**Escherichia coli**

*E. coli* are frequently used as indicator bacteria to monitor the trends in antimicrobial resistance because they are the most prevalent commensal enteric bacteria in humans and animals, can be cultured easily and inexpensively (Van Den Bogaard et al., 2000) and they can acquire and preserve antimicrobial resistance genes from other organisms in the environment and in animal populations (Murray 1997). *E. coli* are also considered as a good indicator of the selective pressure imposed by antimicrobial use in food animals (Talukdar et al., 2013) and the situation is worsened if they are capable to produce biofilm. It has been observed that established *E. coli* biofilms were difficult to treat with some antibiotics, which was supported by observations made in clinical cases involving pig, cattle and poultry (Olson et al., 2002). Some strains of enterohaemorrhagic *E. coli* O157: H7, a worldwide food borne pathogen, are able to form biofilms. A genome-wide transposon mutagenesis of *E. coli* O157: H7 strain EDL933 revealed that virulence plasmid pO157 plays an essential role during biofilm formation (Puttamreddy et al., 2010). Other studies indicated that the biofilm negative strains of *E. coli* O157: H7 can also be associated with pre-established biofilms generated by commensal *E. coli* strains (Uhlich et al., 2010). Nandanwar et al., (2014) isolated extraintestinal pathogenic *E. coli* (ExPEC) isolated from human and birds and were characterized in vitro using adhesin, invasin, biofilm formation and serum bactericidal assays. All the isolates were found to be equally capable of adhering to and invading the mammalian kidney cell lines. Similarly, the isolates were also able to form strong biofilms in M63 medium. Furthermore, they were recorded as resistant to the bactericidal activity of human and avian serum. Oliveira et al., (2014) demonstrated biofilm producing uropathogenic *E. coli* isolated from urinary tract infection of dog which were resistant against fluoroquinolone group of antibiotics.

**Other animal pathogens**

Biofilms of *Listeria monocytogenes* are of particular concern as they are recorded more resistance to disinfectants and sanitizing agents than planktonic cells. Many disinfectants, including quaternary ammonium compounds and hypochloride do not effectively kill the biofilms of *L. monocytogenes* (Amalaradjou et al., 2009). In New York State, biofilms of milking equipments of a dairy farm have been implicated as a potential source of bulk tank milk contamination with *L. monocytogenes*.
Biofilm formation usually occurs in both natural and man-made environments. Biofilm forming microorganisms are frequently cause infection in human and animal and can be transmitted from each other. Biofilm forming bacteria in oral cavity of dogs can transmit infection to human through bites (Zambori et al., 2013). Similarly, biofilms formed in water tank, drinker and farm equipments can be transmit easily to the farm animal as well as animal handlers and workers. P. aeruginosa, Acinetobacter baumannii, Staphylococcus aureus are the notable examples, which can cause wound infection and implanted device related infection in man and animal (Percival 2011; Abrahamian and Goldstein, 2011). Mycobacterium avium and Mycobacterium intracellulare are also potentially zoonotic and can form biofilm in drinking water system (Percival, 2011).

Biofilm and antibiotic resistance

Bacteria in a biofilm are more resistant to antibiotics than planktonic bacteria. It has been estimated that biofilm cells can be up to 10,000 times more resistant to antibiotics than planktonic cells (Nickel et al., 1985; Costerton et al., 1995). It is highly probable that multiple factors work together to protect biofilm cells from antibiotic treatment. The exopolysaccharide matrix (EPS) prevents the penetration of antibiotic inside the biofilm. Charged polysaccharides and eDNA of the matrix can trap several kinds of antibiotics (Rabin et al., 2015). Due to slow growth rate there is limited availability of oxygen and nutrients inside biofilms, so biofilm cells, especially those in the deep layers. These features make biofilm bacteria insensitive to antibiotic that target dividing cells. For example, the targets of β-lactams are dividing cells, so when they are used on E. coli biofilms, their bacteriolytic activity is.
diminished (Ashby et al., 1994). In biofilms, there is a small subpopulation of cells called persister cells (Keren et al., 2004; Lewis 2007). Their growth rate is zero or extremely slow. Most of the antibiotics acts at this stage of microbial cell growth or division are not effective against persister cells. Efflux pumps are another important factor, which allow bacterial cells to pump intracellular toxins out, including antibiotic drugs. Efflux pumps are also expressed in planktonic cells, but some efflux pump genes are upregulated in biofilm, indicating that they contribute to antibiotic resistance (Zhang and Mah, 2008).

Plasmid borne antibiotic resistance could also be possible in single-species or multi-species biofilm by horizontal gene transfer. In biofilms, the frequencies of horizontal plasmid transfer are much higher than between planktonic cells. Studies on S. aureus biofilms showed that biofilms promote the spread of plasmid-borne antibiotic resistance genes by conjugation/mobilization (Savage et al., 2013).

**Diagnostic approaches of biofilm infection**

For achieving accurate diagnosis of biofilm associated infection bacterial culture remained as ‘gold standard’ of clinical microbiology for identification of pathogen is not sufficient (Hall-Stoodley et al., 2012). Aggregation of bacteria (biofilm) to specific tissue of host often give negative culture result and false positive culture result is obtained due to free floating planktonic microbes (Abdullahi et al., 2015).

The new generation molecular approaches have good reproducibility including 16S rRNA polymerase chain reaction, loop-mediated isothermal amplification (LAMP), fluorescence in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM) (Wu et al., 2014; Abdullahi et al., 2015).

**Therapeutic approaches of biofilm infection**

As the biofilm producing bacteria are badly resistant to antimicrobial agent, incorporation of antibiotic agents with conventional antibiotics are the need of the hour for effective control of biofilm associated infection. Till date, most of the published literatures were reported the following approaches in therapy against biofilm producing bacteria.

Altering the surface properties of implanted devices can minimize biofilm associated infections (Pavithra and Doble, 2008). Generally, antibiotics, heavy metal silver or silicon or their combination are used as bactericidal or bacteriostatic agents for surface coating of indwelling devices (Chen et al., 2013). Anti-adhesion surface of implanted devices also helps to reduce the attachment of pathogenic bacteria leading to significantly decreased in biofilm formation. For example, coating with poly ethylene glycol to the titanium surface markedly reduces S. aureus adhesion (Harris et al., 2004).

Bacteriophage therapy is a robust technique to control bacterial infections especially the multi-drug resistant bacteria. The mechanism through which phage achieves its antibiofilm action is by enzyme production, which hydrolyses and degrades the extracellular matrix of biofilm (Fu et al., 2010; Verma et al., 2010). Bacteriophages alone or combined with antibiotics might give fruitful result to check the biofilm infection.

Combined use of electric current and antibiotics greatly enhances the antimicrobial activity against biofilm (Abdullahi et al., 2015). Low frequency electric current can enhances the efficacy of polyionic antibiotics, viz., gentamicin has improved activity against
**S. epidermidis** (Kasimanickam et al., 2013). Synergistic effect of low frequency ultrasound and antibiotics can effectively increase the antimicrobial action by releasing the antibiotic in a triggered manner, enhances the cell membrane permeability leading to disruption of biofilms (Kasimanickam et al., 2013). Dual action of LASER and antibacterial agents can enhances the killing properties by better penetration inside the biofilm. For example, helium/neon laser light in presence of toluidine blue killed 95% bacteria in oral polymicrobial biofilm (Soukos and Goodson, 2011). Combination of antimicrobial drugs with nano carrier can kill the biofilm bacteria by prolonging the action of active molecules of drugs and increasing solubility and bioavailability (Kasimanickam et al., 2013).

Emergence of multidrug resistance bacteria is global public health concern. Situations may be further worsened when MDR bacteria also produce biofilms. Present scenario of biofilm associated infection in Veterinary medicine becoming a serious challenge leading to serious health hazard and economic loss. Control of indiscriminate use of antibiotics and maintaining proper biosecurity and biosafety measures might reduce the biofilm infection. In the ‘post antibiotic era’ the alternative therapeutic and managemental strategies should be ventured to combat the biofilm producing MDR bacterial pathogens.

**References**


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