PNEUMOCOCCAL BIOFILMS AND THEIR INTERVENTION STRATEGIES

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ABSTRACT

Pneumonia is a fatal infection with hard time breathing, cough, and fever. The children are at high risk worldwide due to pneumonia. This is responsible for childhood mortality and morbidity worldwide. It is mainly caused by bacteria. Pneumonia-causing bacteria are resistant to most of the antibiotics and therapeutic agents due to the formation of biofilms. Laboratories around the world are trying to develop strategies to combat pneumococcal biofilms. This review deals with the formation of pneumococcal biofilms and their different intervention strategies.

Keywords: Pneumonia, Biofilms, Streptococcus pneumoniae, Intervention Strategies

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INTRODUCTION

Pneumonia is a lung infection with a cough, fever and hard time breathing. Traditionally pneumonia is cured at home and often clears up in 2 to 3 w. But old age people, babies, and medically challenged people need special care and medication. Pneumonia is mainly caused by bacteria like Streptococcus pneumoniae, Klebsiella pneumoniae, Hemophilus influenzae, Staphylococcus aureus, Mycoplasma pneumoniae, Legionella pneumoniae and Chlamydia pneumoniae.

According to WHO pneumonia is the single largest cause of death in children worldwide. It kills an estimated 1.1 million children under the age of five years, accounting for 18% of all deaths of children every year worldwide. Pneumonia is most prevalent in South Asia and Sub-Saharan Africa. In 2013, WHO and UNICEF launched an integrated Global Action Plan for Pneumonia and Diarrhea (GAPPD). The aim was to accelerate pneumonia control with a combination of interventions to protect, prevent, and treat pneumonia in children with actions to:

- Protect children from pneumonia include promoting exclusive breastfeeding and adequate complementary feeding.
- Prevent pneumonia with vaccinations, soap hand washes, reducing household air pollution, HIV prevention and cotrimoxazole prophylaxis for HIV-infected and exposed children.
- Treat pneumonia which is focused on making sure that every sick child has access to the right kind of care (either from a community-based health worker or in a health facility if the disease is severe) and can get the antibiotics and artificial oxygen they need to get well [1]

Due to biofilm formation and associated horizontal gene transfer, the microbes (pathogenic species) are becoming resistant to the commercially available antibiotics. Biofilms are the accumulation of microbial cells which grow on surfaces with a matrix of extracellular polymeric substances (EPS) on them. Naturally, biofilms consist of mixed microbial species. Bacterial cells secrete EPS using quorum sensing mechanism and lead to the formation of biofilm [2]. Biofilms help bacteria to protect them from antibiotics, host immune response and predation [3]. Naturally, biofilms can be formed by most of the microorganisms. Biofilm protects microorganisms from various environmental challenges such as metal toxicity, salinity, and pH [4]. It has been estimated that the frequency of infections caused by biofilms, especially in the developed world, lies between 65% and 80% as per reports from Centres for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) [5]. The pathogenic potential of carcinogenic bacteria in plaque biofilms is found to be modulated [6]. Most pathogenic organisms like Streptococcus, E. coli, Klebsiella, Pseudomonas, S. aureus, Enterococcus faecalis which grow on catheters, artificial joints, mechanical heart valves, etc lead to persistent infections [7]. EPS is composed of surfactants-lipids, extracellular DNA, extracellular proteins and exopolysaccharides. The composition of EPS determines the blooming and distribution of biofilms and antibiotic response.

![Fig. 1: Showing different stages of biofilm formation (self-drawn in MS Powerpoint)](image-url)
Virulence and pneumococcal biofilms

Naturally, biofilms have a complex, dynamic and heterogeneous structure. Biofilm formation in *S. pneumoniae* is influenced by the presence of both extracellular DNA and certain proteins. The encapsulated *S. pneumoniae* is found to be virulent because of the presence of capsular polysaccharide. Some researchers found out that under continuous culture conditions, biofilm formation is accompanied by an increase in the concentration of various kinds of proteins involved in virulence, adhesion, and resistance [9]. Furthermore, it has been shown that there is an overexpression of pneumolysin-coding gene under continuous culture conditions and repression under polystyrene grown biofilms. [10, 11]. The role in biofilm formation of choline binding proteins, which anchor to the choline residues of the cell wall teichoic acids was studied in various mutants. It was found that Lyt lysoyzyme, Lyt Amidase, Lyt B Glucosaminidase, Cbpa adhesin, PcpA putative adhesin and PspA (pneumococcal surface protein A) mutants were poor biofilm formers compared to Pce phosphocholinesterase or CbpD putative amidasate mutants [12]. Recently, about 69 mutants with insertions in 42 different genes and 8 promoters have been identified with altered biofilm formation [13]. More recently using genetic dissection of developmental stages of biofilm revealed that biofilm formation involves multiple, convergent signaling pathways and a genetic program for the transition from planktonic growth state to the biofilm mode of growth. In *Streptococcus pneumoniae*, the induction of genetic competence favors the growth of biofilm. The induction and maintenance of genetic competence are regulated by a CSP-mediated quorum sensing system in transformable streptococci [14].

Pneumococcal biofilms and antimicrobial chemotherapy

A current estimation reveals that 60 % of all bacterial infections are the result of biofilms of microorganisms and of the resistance of these communities to antibiotic agents and host immune defense mechanisms. However, the most significant evidence of the pathogenic relationship between humans and biofilms is based on the microscopic observations that have revealed the presence of these communities at the site of infection (otitis caused by pneumococci, endocarditis by *Staphylococcus*, *Pseudomonas* in the lungs of patients with cystic fibrosis, etc) or in implants recovered from patients [15]. The most effective procedure for control of such infections is to prevent or stop the colonization of the organisms and its biofilm formation. Many antibiotics have been used for the purpose but most of these strategies proved ineffective because the use of antibiotics further provoke secondary nosocomial infections leading to morbidity and mortality worldwide and is a matter of further concern with increasing economic and human impact because of population density. Antimicrobial drugs are very frequently administered against nosocomial infections and through selection and change of genetic resistance elements; antibiotics promote the appearance of multidrug resistant, as well as extremely drug-resistant strains of bacteria. Microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital environment. The widespread use of antimicrobials for therapy or prophylaxis (including topical) is the major determinant of resistance. Many strains of *Pneumococci*, *Staphylococci*, *Enterococci*, and *Mycobacterium tuberculosis* are currently resistant to most or all antimicrobials which were once effective. Multi resistant *Klebsiella* and *Pseudomonas aeruginosa* are prevalent in many hospitals [16]. Some researchers have found out that the phage lytic enzyme (Cpl-1) prevent the formation of the biofilm of *Streptococcus* in the middle ear during otitis media [17]. A genetically engineered phage lysis had been remarkably used in the treatment of biofilm of *E. coli* which has been found that 99% of the biofilm has been eliminated [18]. It has been reported that an organic compound cis-2-decanoic acid induces the dispersion of biofilm of *P. aeruginosa* cells and some gram-positive species [19]. A study of the concomitant DNA and quorum sensing system via genetic transformation has been done in planktonic cultures [20]. It would enable the study of issues such as the passive resistance to antibiotics of the communities as well as the antibiotic tolerance. Alternatively, it is thought that the honeycomb structure of the EPS is a distinct feature of each organism indicating the role of genetic control in their formation [21]. The emergences of infectious diseases are still the causes of many deaths and tragedies [22]. Along with the emergence of new causal agents of infectious diseases (AIDS, Lyme, Ebola), old acquaintances, such as *P. aeruginosa*, *Staphylococcus*, *Pneumococcus* and many more, as well as apparently innocuous bacteria, such as *Legionella* started to reveal their extraordinary versatility and giving

**Fig. 2:** Showing host-pneumococci interaction (self-drawn in MS powerpoint)
rise to complex structures of biofilms. It is now known that today the most common mode of infection is the biofilm [9].

**Intervention strategies**

One of the prime thirsts of modern-day medical microbiology is to look for agents that can destroy biofilms. Laboratories around the world are trying to develop anti-biofilm agents against biofilms of different organisms. Categorically these are as follows:

1. **Antimicrobial agents:** Studies have previously reported that high resistance to penicillin, tetracycline, rifampicin, amoxicillin, erythromycin, clindamycin, and levofloxacin is manifested by pneumococcal biofilms [23, 24]. Pneumococcal biofilms formed on the nasopharyngeal tissue of mouse were more resistant to gentamicin and penicillin G than planktonic cell [25].

2. **Quorum sensing inhibitors:** In *S. pneumoniae*, quorum sensing (QS) signaling regulates biofilm communities and plays a key role in coordinating the spatial disposition, aggregation of cells, and exopolysaccharide formation. Sinefungin, a nucleoside analogue of 5-adenosylmethionine, has shown a significant effect on pneumococcal biofilm formation in vitro and inhibit colonisation of pneumococcal biofilm in vivo by decreasing the AI-2 production and down-regulating gene expression [26]. In bacteria, the alteration of pathogenic gene expression and the methylation of adenine in the DNA duplex and of macromolecules are executed by DNA adenine methyltransferase (Dam) during the activated methyl cycle (AMC). AMC is involved in the biosynthesis of quorum sensing molecules that regulate competence and biofilm formation in pneumococci. The effect of a small molecule Dam inhibitor, pyrimidinedione, on *Streptococcus pneumoniae* biofilm formation and evaluated the changes in global gene expression within biofilms [27]. Their study reported that pyrimidinedione inhibits pneumococcal biofilm growth in vitro at concentrations that do not inhibit planktonic cell growth and downregulates important metabolic-, virulence-, competence-, and biofilm-related genes. Macrolides or quinolones alone or in combinations may be used to target not only intracellular pathogens but also their quorum sensing mechanisms and reduce the host inflammatory response [28].

3. **Novel organic and inorganic chemicals:** *Streptococcus pneumoniae*, a Gram-positive bacterium is a human respiratory tract pathogen which depends on a conserved β-carbonic anhydrase (CA, EC 4.2.1.1) for in vitro growth intracellularly and extracellularly. So, it is to be expected that the transmission and pathogenesis of the bacterium pneumococcal carbonic anhydrase (PCA) which is a potential therapeutic target. Organic anions such as cyanate, bromide, selenium, selenocyanate, chloride, trithiocarbonate, iodide and cyanide were effective inhibitors of PCA. Sulfamate, sulfamic acid, phenylboronic, phenylarsonic acid, diethyldithiocarbamate and sulfonamide acetazolamide showed a significant effect on PCA [29]. A compound named cis-2-decanoic acid released from Dornase alpha is a highly purified form of recombinant human DNase I (rhDNase I) that has been shown to be effective against the established biofilms of *Streptococcus pneumoniae*. It was reported that DNase treatment resulted in significant degradation of a biofilm (by 66.7% to 95%), even though the biofilm were grown for 6 d [40, 4].

4. **Biocides:** Biocides are amphiphilic compounds of biological origin containing a hydrophilic region (polar or non-polar) and a hydrophobic region (lipid or fatty acid). Biocides have been identified in many biological processes as the components of cellular metabolism, motion, and defense. They are found abundantly in bacteria, in biofilms as quorum-sensing molecules, lubricants, promoting the uptake of poorly soluble substrates, as virulence factors, antimicrobial compounds, immune modulators and secondary metabolites [30]. Lipopeptide, a class of biocides which is released from *Bacillus tequilensis* was found to inhibit biofilm formation of *E. coli* and *S. mutans* [31]. Mixed biocides like lunasan extracted from *Lactococcus lactis* and *Streptococcus thermophilus* was reported to be effective against *Streptococcus pneumoniae*. Rubus ulmifolius, *Rubus idaeus*, and *Cimicifuga Schott.* rich in ellagic acid, and ellagic acid derivatives, inhibited the formation of pneumococcal biofilms in a dose-dependent manner. As measured by viability assay, 100 and 200 mg/ml of 220D-F2 had significant bactericidal activity against pneumococcal planktonic cultures as early as 3 h post-inoculation having MIC’s 80 mg/ml of 220D-F2 which completely eradicated overnight cultures of planktonic *Pneumococcus* [39].

5. **Nanoparticles:** Silver coated polyvinyl pyrrolidone nanoparticles was reported to be effective against capsular polysaccharide influenced bacteriocidal effect against *Streptococcus pneumoniae* [34]. The *Aspergillus flavus* mediated silver nanoparticles is found to be effective against many human pathogens like *E. coli*, *B. subtilis*, *L. reuteri*, *E. faecalis*, *K. pneumoniae*, *S. epidermidis* and *P. mirabilis* [35].

6. **Natural products including phytochemicals:** Two prenylated flavonoid derivatives, sanganogen G and sanganogen A was reported to inhibit pneumococcal NAs and, in contrast to the approved NA inhibitor oseltamivir, as well as also the planktonic growth and biofilm formation of *Pneumococcus* [36]. Bioactive compounds of aqueous fraction of the dried fruit of *Magnolia officinalis* and *S. pneumoniae* ATCC 49619 were also as significantly suppressed the biofilm formation by different *S. pneumoniae* clinical isolates also [38]. The ethyl acetate and methanol extract of *Gymnema sylvestre* have an antibiofilm effect on *Streptococcus pyogenes* from upper respiratory tract patients [41]. The methanol extract of *Plectonema ambionicus* is reported to have an antibiofilm effect on *Streptococcus pyogenes* isolated from pharyngitis patients [42].

In another study of the plant extract of *Rubus ulmifolius* Schott., rich in ellagic acid, and ellagic acid derivatives, inhibited the formation of pneumococcal biofilms in a dose-dependent manner. As measured by viability assay, 100 and 200 mg/ml of 220D-F2 had significant bactericidal activity against pneumococcal planktonic cultures as early as 3 h post-inoculation having MIC’s 80 mg/ml of 220D-F2 which completely eradicated overnight cultures of planktonic *Pneumococcus* [39].

**EXTRACELLULAR POLYMERASE SUBSTANCE DEGRADING ENZYMES:**

Dornase alpha is a highly purified form of recombinant human DNase I (rhDNase I) that has been shown to be effective against the established biofilms of *Streptococcus pneumoniae*. It was reported that DNase treatment resulted in significant degradation of a biofilm (by 66.7% to 95%), even though the biofilm were grown for 6 d [40, 4].

**CONCLUSION**

Pneumonia-causing Bacterial biofilms are responsible for childhood mortality worldwide. The recent trend on biofilm research not only aims at the intervention strategies and combating pneumococcal biofilm formation by antimicrobial chemotherapy which indirectly promotes the growth of bacteria but also many strategies are used to stop colonization of the bacteria and its biofilm formation. Such strategies are quorum sensing inhibitors like sinefungin and pyrimidinedione, inorganic and organic chemicals like cyanate, bromide, selenocyanate, chloride, trithiocarbonate, iodide and cyanide are effective inhibitors of PCA. Sulfamate, sulfamic acid, phenylboronic, phenylarsonic acid, diethyldithiocarbamate and sulfonamide acetazolamide showed a significant effect on PCA [29]. A compound named cis-2-decanoic acid released from Dornase alpha is a highly purified form of recombinant human DNase I (rhDNase I) that has been shown to be effective against the established biofilms of *Streptococcus pneumoniae*. It was reported that DNase treatment resulted in significant degradation of a biofilm (by 66.7% to 95%), even though the biofilm were grown for 6 d [40, 4].

**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally

**CONFLICT OF INTERESTS**

Declared none

**REFERENCES**

1. [www.who.int/mediacentre/factsheets/fs331/en/](http://www.who.int/mediacentre/factsheets/fs331/en/) [WHO Fact sheets Nov 2013]; 2013.
2. Nadell CD, Xavier JB, Levin SA, Foster KR. The evolution of quorum sensing in bacterial biofilms. PLoS Biol 2008;6:14.
3. Johnson LR. Microcolony and biofilm formation as a survival strategy for bacteria. J Theor Biol 2008;251:24-34.

4. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95-108.
5. Kerkisek K. A life in slime biofilms rules the world. Infection Microbiol 2004;2:95-108.

6. Costerton W, Veeh R, Shirillo M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 2003;112:1466-77.
7. Granich RM, Mount H, Holmes RM, Poiesz B, Roos D, Walker J, Wilson S, Ackland S, Zaki S, Chaisson R, et al. Farnesol on planktonic and biofilm cells of Staphylococcus aureus. Antimicrob Agents Chemother 2006;50:1463-9.

8. Lawrence JR, Swerhone GD, Kuhlicke U, Neu TR. In situ evidence for microdomains in the polymer matrix of bacterial microcolonies. Can J Microbiol 2007;53:450-8.

9. Moscoso M, Garcia E, Lopez R. Pneumococcal biofilms-review article. Int Microbiol 2009;12:77-85.
10. Allegrucci M, Hu FZ, Shun K, Hayes J, Ehrlich GD, Post JC, Sauer K. Phenotypic characterization of Streptococcus pneumoniae biofilm development. J Bacteriol 2006;188:2325.

11. Oggoni MR, Trappetti C, Kadioglu A, Cassone M, Iannelli F, Ricci S, et al. Switch from planktonic to sessile life: a major event in pneumococcal pathogenesis. Mol Microbiol 2006;61:196-10.

12. Moscoso M, Garcia E, Lopez R. Biofilm formation by Streptococcus pneumoniae: role of choline, extracellular DNA, and capsular polysaccharide in microbial adhesion. J Bacteriol 2006;188:7785.

13. Munoz-Elías EJ, Marcano, Camilli A. Isolation of Streptococcus pneumoniae biofilm mutants and their characterization during nasopharyngeal colonization. Infecc Immun 2008;76:5049-61.

14. Civitkovitch DG, Li YH, Ellen RP. Quorum sensing and biofilm formation in streptococcal infections. J Clin Invest 2003;112:1626-32.

15. Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med 2008;59:415-28.

16. WHO/CD/CSR/DFH/2002.12; 2002.

17. McCullers JA, Karlström A, Iverson AR, Leffler JM, Fischetti VA. Novel strategy to prevent otitis media caused by colonizing Streptococcus pneumoniae. PLoS Pathog 2007;3:e28.

18. Lu TK, Collins J. Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci USA 2007;104:11197-202.

19. Davies DG, Marques NH. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 2009;191:1393-403.

20. Moscoso M, Claveres JP. Release of DNA into the medium by competent Streptococcus pneumoniae: kinetics, mechanism and stability of the liberated DNA. Mol Microbiol 2010;45:187-97.

21. Schaudinn C, Stoodley P, Kainovic A. Bacterial biofilms, other microcolonies. Can J Microbiol 2007;53:450-8.

22. Lawrence JR, Swerhone GD, Kuhlicke U, Neu TR. In situ evidence for microdomains in the polymer matrix of bacterial microcolonies. Can J Microbiol 2007;53:450-8.

23. Carillo G, Morosini MI, Valverde A. Differences in biofilm development and antibiotic susceptibility among Streptococcus pneumoniae isolates from cystic fibrosis samples and blood cultures. J Antimicrob Chemother 2007;59:301-4.

24. Marks LR, Parameswaran GJ, Hakansson AP. Pneumococcal interactions with epithelial cells are crucial for optimal biofilm formation and colonization in vitro and in vivo. Infecc Immun 2012;80:2744-60.

25. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature 2004;430:422-9.

26. Susana P, Jesus RL, Juan BL. Review of probiotics for use in bivalve hatcheries. Vet Microbiol 2010;145:231-7.

27. Fahimi S, Balasubramanian V, Narasimhan S, Sivarakumar S. Aspergillus flavus mediated silver nanoparticles synthesis and evaluation of ITS antimicrobial activity against different human pathogens. Int J Appl Pharm 2016;8:43-6.

28. Griemeck U, Richter M, Waltner E, Hoffmann A, Kirchmair J, Makarow V, et al. Discovery of prenylated flavonoids with dual activity against Influenza virus and Streptococcus pneumoniae. BioMed Res Int 2016. 9:27156.

29. Murlidrty LY, Naradddy SM, Aka ST. Phytochemical screening, antibacterial and antivirus activity of Lagenaria siceraria fruit growing in Kurdistan Region, Iraq. J Pharmacogn Phytochem 2015;4:45-9.

30. Minami M, Konishi T, Takase H, Makino T, Shiniseihaito (Xinyiqingfeiting) Suppresses the biofilm formation of Streptococcus pneumoniae in vitro. BioMed Res Int 2017. https://doi.org/10.1155/2017/4575709.

31. Takekar S, Chocua S, Nelsen K, Kugman KP, Quave CL, Vidal JE. 2008-F2 from Rubus ulmifolius kills Streptococcus pneumoniae planktonic cells and pneumococcal biofilms. PLoS One 2014;9:e93734.

32. Kaplan JB, Volvetti K, Cardona ST, Madhyastha S, Sadowski K, Jabbouri S, et al. Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in Staphylococci. J Antimicrob Chemother 2012;65:73-7.

33. Manimekalai K, Srinivasan P, Dineshbabu J, Gun A, Teepica Priya Darsini D. Antibiofilm efficacy of Plectranthus amboinicus against Streptococcus pyogenes isolated from pharyngitis patients. Asian J Pharm Clin Res 2016;9:348-54.

34. Dineshbabu J, Srinivasan P, Manimekalai K, Teepica Priya Darsini D. In vitro antibiofilm activity of Gymnema sylvestre extract against biofilm forming Streptococcus pyogenes from upper respiratory tract. Asian J Pharm Clin Res 2016;9:83-6.