

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.

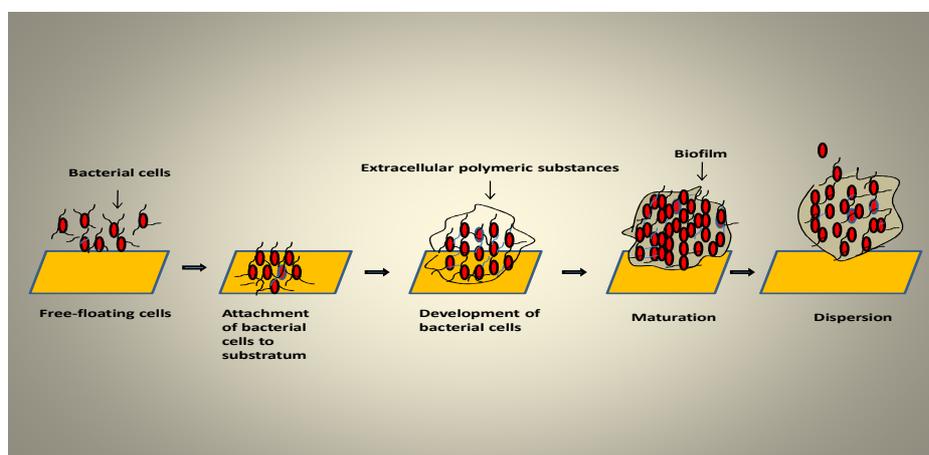


Fig. 1: Showing different stages of biofilm formation (self-drawn in MS Powerpoint)

Virulence and pneumococcal biofilms

Naturally, biofilms have a complex, dynamic and heterogeneous structure. Biofilm formation (fig. 1) imparts cells an adaptive strategy for survival in adverse conditions. The biofilm formation and maintenance depends on the production and amount of Extracellular

Polymeric Substances (EPS). The environment surrounding the cells in a biofilm is known as the microenvironment. This microenvironment is determined by the physicochemical properties like concentration, adhesion, charge, sorption capacity, specificity and nature of the individual components of EPS as well as the three-dimensional distribution of the matrix [8].

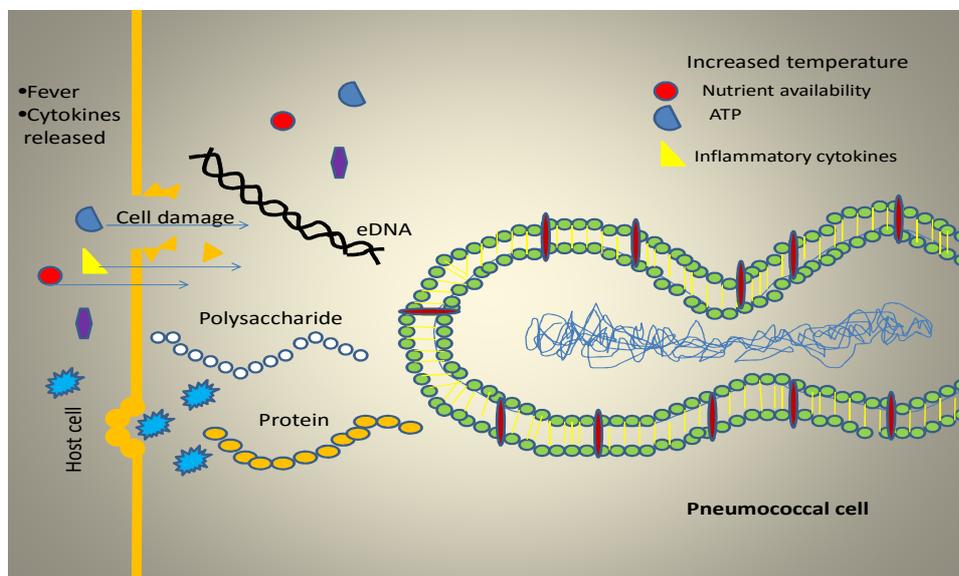


Fig. 2: Showing host-pneumococci interaction (self-drawn in MS powerpoint)

Fig. 2 shows the factors that are responsible for host-pneumococci interaction. 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 lysozyme, 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 amidase 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 *Staphylococci*, *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 exchange 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 lysin 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

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 higher 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 did 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 S-adenosylmethionine, have 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 it is a potential therapeutic target. Inorganic anions such as cyanate, bromide, selenocyanate, chloride, trithiocarbonate, iodide and cyanide were effective inhibitors of PCA. Sulfamide, 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 *P. aeruginosa* can disperse biofilms of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. pyogenes*, *B. subtilis*, *S. aureus* and *C. albicans* [19].

4. **Biosurfactants:** Biosurfactants are amphiphilic compounds of biological origin containing a hydrophilic region (polar or non-polar) and a hydrophobic region (lipid or fatty acid). Biosurfactants 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 biosurfactants which is released from *Bacillus tequilensis* was found to inhibit biofilm formation of *E. coli* and *S. mutans* [31]. Mixed biosurfactants like lunasan extracted from *Lactococcus lactis* and *Streptococcus thermophilus* was reported to be effective against *Staphylococcus*, *Streptococcus*, *Rothia* and *Candida* sp. [32]. A nonspecific class of biosurfactants like rufisan extracted from *Candida lipolytica* was effective against *Streptococcal* biofilms [33].

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*, *S. flexneri*, *E. faecalis*, *K. pneumoniae*, *S. epidermis* and *P. mirabilis* [35].

6. **Natural products including phytochemicals:** Two prenylated flavonoid derivatives, sanggenon G and sanggenol 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 *Pneumococci* [36]. Bioactive compounds of aqueous fraction of the dried fruit of *Lagenaria siceraria* was evaluated *in vitro* and found to be highly active against *Streptococcus pneumoniae* biofilm with (MIC 2.5 mg/ml and MBC 5 mg/ml). The phytochemical investigation shows the presence of flavonoids, hydrolysable tannin, sterol, quinine, and phenols [37]. A study showed that Shin'iseihaito a Japanese herbal remade consists of rhizome of *Anemarrhena asphodeloides*, rhizome of *Cimicifuga heracleifolia*, leaf of *Eriobotrya japonica*, *Gypsum fibrosum*, fruit of *Gardenia jasminoides*, bulb of *Lilium lancifolium*, flower of *Magnolia salicifolia*, tuber of *Ophiopogon japonicus*, and the root of *Scutellaria baicalensis* significantly inhibited the formation of biofilm from *S. pneumoniae* ATCC 49619 as well 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 *Plectonthus amboinicus* 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 *Pneumococci* [39].

7. 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 biofilms 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, sulfamide, sulfamic acid, phenylboronic, phenylarsonic acid, diethyldithiocarbamate and sulfonamide acetazolamide and cis-2-decanoic acid, biosurfactants like lipopeptide, lunasan and rufisan, many natural products like phytochemicals and extracellular polymerase degrading enzymes like Dornase alpha and nanoparticles are reported to have activity against pneumococcal biofilms. Advance research for establishing some of the assured strategies for the effective intervention of pathogenic biofilm requires further in-depth research in the subject.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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