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(RESEARCH ARTICLE)

Biofilm-Associated Immune Vulnerability in *Streptococcus pneumoniae*: Virulence-Factor Remodeling and Therapeutic Implications for Invasive Disease

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Abstract

Streptococcus pneumoniae biofilms contribute to persistent carriage, immune evasion, and progression to invasive disease. Recent studies indicate that the biofilm state alters the display and function of key surface-associated virulence factors, including PspA, PspC, and LytA, and may reduce susceptibility to complement-mediated clearance and phagocytosis. At the same time, biofilm-dispersed pneumococci appear to elicit stronger innate immune activation than planktonic cells, suggesting that transition out of biofilm may expose a biologically vulnerable state. This review synthesizes recent peer-reviewed evidence on pneumococcal biofilm-associated immune interactions, focusing on virulence-factor remodeling, host recognition, and implications for therapy. Literature supports a state-dependent model of pathogenesis in which biofilm growth promotes persistence, whereas dispersion may increase immune visibility. However, current studies are heterogeneous in model system, assay design, and outcome measures, limiting direct quantitative synthesis. Standardized biofilm phenotyping and clinically linked validation studies are needed to determine whether biofilm-targeted or state-aware interventions can improve outcomes in invasive pneumococcal disease.

Keywords: *Streptococcus pneumoniae*; Biofilm; Immune Evasion; Complement; Phagocytosis; PspA; PspC

1. Introduction

Streptococcus pneumoniae remains a leading cause of pneumonia, bacteremia, meningitis, and other forms of invasive pneumococcal disease despite vaccination and antimicrobial therapy. The success of this bacterium is intimately linked to its ability to establish a biofilm-associated community within the nasopharynx, from which it can later disseminate to sterile tissues. The formation of biofilms by *Streptococcus pneumoniae* is a dynamic process rather than a static aggregation of bacteria that changes its expression profile and interactions with the host immune system (Vilhena et al., 2023).

In recent years, numerous studies have established that biofilm-forming *S. pneumoniae* cells resist innate immune recognition more effectively than their planktonic counterparts (reference). It was originally found that biofilm-grown *S. pneumoniae* cells showed decreased deposition of C3b (Gil et al., 2022), as well as poor engulfment due to the activity of PspC proteins that recruited factor H and led to the inhibition of the classical pathway (Domenech et al., 2012). Subsequent studies showed that biofilm-forming pneumococci delayed complement activation and restricted C1q and C3b deposition, preventing opsonophagocytic destruction (Domenech et al., 2013). At the same time, biofilm-associated cells were found to trigger a weak inflammatory reaction while maintaining the ability to damage epithelial structures, further reinforcing the idea that biofilms are immunomodulatory as well as protective (Mathew et al., 2023). Collectively, these observations suggest a "state-aware" model in which biofilm growth dampens certain immune

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responses, whereas dispersal may expose a more immunogenic phenotype. If validated, this model could inform state-specific or biofilm-targeted strategies that complement conventional antibiotics and vaccines.

Complementary studies have centered around the impact of biofilm-related virulence factors on host interactions. It has been demonstrated that choline-binding protein such as PspA, PspC, and LytA impact biofilm structure and matrix formation, hemolysis, and endothelial damage, pointing toward the notion that the virulence factors required for the persistence of the biofilm architecture also act as mediators of pathogenesis (Vilhena et al., 2023). More recently, biofilm-dispersed *S. pneumoniae* has been shown to induce greater activation of neutrophils, monocytes, and platelets than conventional planktonic bacteria. Biofilm-derived populations, particularly dispersed bacteria, promoted enhanced expression of leukocyte activation markers, platelet activation, and platelet–neutrophil complex formation, suggesting that dispersal from biofilms is associated with a heightened innate inflammatory response. (Chao et al., 2024). These findings point towards the concept of "state-awareness", whereby biofilms dampen certain immune responses, while biofilm dispersion enhances these responses. Such a concept, if proven true, might provide us with novel strategies that target specific states and complement the use of traditional vaccines and antibiotics. However, the current evidence base remains narrative, heterogeneous, and only partially clinically linked.

Therefore, this review synthesizes evidence from peer-reviewed studies published from 2016 onwards, with particular emphasis on research conducted between 2020 and 2024, to examine biofilm-specific immune vulnerabilities in *S. pneumoniae*. The emphasis is on how variations in virulence-factor expression and biofilm physiology interact with complement, phagocytosis, and innate immune activation, as well as on the translational implications for invasive pneumococcal disease.

2. Methodology

This review followed PRISMA guidelines. The databases searched include PubMed, Scopus, and Web of Science, using the following keywords: *Streptococcus pneumoniae*, *pneumococcus*, biofilm, dispersion, carriage, complement, phagocytosis, immune evasion, PspA, PspC, LytA, pneumolysin, antibiotic tolerance, and invasive disease. Keywords were connected via Boolean logic for inclusion of literature focusing on both biofilm formation characteristics and host interaction

Eligible articles consisted of peer-reviewed original articles and reviews, published in English from 2016 onwards with emphasis on studies published after 2020. Studies directly pertaining to biofilm development, immunity, virulence factor regulation, antimicrobial resistance, and invasive infection were included. Excluded studies included those published as preprints or presented only in conference abstracts; editorials, and articles without any focus on the interaction between the pneumococcal biofilms and their hosts. After screening titles and abstracts for potential eligibility, full texts were subsequently reviewed. Data included study design and bacterial strain or isolate details, biofilm model used, immune assays employed, virulence factors assayed, and the key results found in each study. Due to differences in model systems and endpoints between the various studies analyzed, a qualitative narrative synthesis was preferred over meta-analysis. No quantitative measure of effect is included as comparable quantifiable results could not be obtained across all studies.

3. Results and Discussion

Table 1 Core mechanistic themes from verified recent studies

Theme	Key finding	Implication	Source
Complement evasion	Biofilm formation diverts complement activity and impairs phagocytosis	Biofilm state reduces innate clearance	(Domenech et al., 2013)
Capsule modulation	Pneumococci biofilms downregulate cps locus, reducing capsule expression and production	Capsule remodeling contributes to persistent colonization and immune adaptation.	Marks et al. (2012); Domenech et al. (2013)
Surface protein multifunctionality	PspA, PspC, and LytA affect biofilm matrix and host-cell injury	Surface proteins are potential anti-virulence targets	(Vilhena et al., 2023)
Dispersion and immunity	Biofilm-derived and dispersed bacteria induce greater activation of	Dispersion may create a therapeutic window	(Chao et al., 2024 ;

	neutrophils, monocytes, and platelets than planktonic cells		Mathew et al., 2023)
Host antimicrobial peptide escape	PspA confers resistance to indolicidin-mediated killing	Surface remodeling contributes to innate resistance	(Waz et al., 2024)
Ecological competition	Commensal streptococci can inhibit growth and disrupt pneumococcal biofilms	Live biotherapeutics may reduce carriage	(Borralho et al., 2025)

3.1. Biofilm-associated immune evasion

Accumulating evidence indicates that *S. pneumoniae* biofilms exhibit reduced complement recognition and are less susceptible to phagocytic clearance than their planktonic counterparts. Domenech et al. (2013) demonstrated that biofilm-grown pneumococci display impaired deposition of the central complement opsonin C3b, reduced binding of C1q and C-reactive protein, and diminished opsonophagocytic killing by human neutrophils. Fluorescence microscopy further revealed that C3b was deposited in a sparse and patchy pattern across the biofilm surface rather than uniformly coating the bacteria, suggesting that biofilm formation limits efficient complement opsonization, thereby impairing complement-mediated recognition and subsequent phagocytic clearance by innate immune cells (Hyams et al., 2010).

The complement-related phenomena are directly associated with PspC and factor H. PspC-producing biofilms exhibit increased factor H binding, leading to redirection of the alternative pathway and reduced deposition of C3b fragments, thus inhibiting opsonization and phagocytosis (Domenech et al., 2012). Meanwhile, bacteria in carriage-associated biofilms trigger a decreased inflammatory reaction when compared with planktonic cells, despite being able to induce injury and epithelial sloughing, meaning that these two processes can occur independently of each other. Nevertheless, immune evasion is not always complete. Biofilm formation can lead to the development of an imbalance in immune recognition in terms of both time and space. For example, biofilm-associated bacteria can release autoinducer-like substances, including autolysins, which contribute to the modulation of the immune reaction, showing that biofilm formation can have both immune-evasive and immune-modulating effects (Minhas et al., 2023)

3.2. Virulence-factor remodeling in biofilm states

Surface-associated virulence factors, especially PspA, PspC, and LytA, appear central to bridging biofilm physiology and host interaction. Recent work on the choline-binding proteins demonstrates that LytA limits robust biofilm formation, whereas PspA and PspC strongly enhance hemolytic effects on human red blood cells and endothelial injury. Specifically, lytA-deficient mutants are more likely to form biofilms, suggesting that LytA constrains biofilm mass, while *pspA* and *pspC* mutants show reduced hemolysis and endothelial damage, highlighting the importance of these proteins in overall pathogenicity(Vilhena et al., 2023).

These findings imply that virulence factors are not functionally restricted to either biofilm architecture or host-cell interaction; instead, they participate in both. PspA and PspC, for example, have been shown to mediate attachment, aggregation, and resistance to host antimicrobial peptides, further broadening their role in persistence and immune evasion. Similarly, PspA has been implicated in binding lactoferrin and protecting pneumococci from lactoferricin and indolicidin, providing a mechanistic link between surface protein function and innate peptide-mediated killing (Waz et al., 2024).

From a therapeutic perspective, this multifunctionality creates a dual-edged target. Disrupting PspA, PspC, or LytA could weaken biofilm stability, reduce hemolytic capacity, and impair endothelial injury, but may also alter inflammatory signaling (e.g., autolysin-driven NF-κB and cytokine modulation). The optimal intervention will therefore need to balance reduction of immune evasion with control of immunopathology(Vilhena et al., 2023).

3.3. Biofilm-derived and dispersed pneumococci are immunologically distinct

One of the most conceptually important recent observations is that biofilm-derived and dispersed pneumococci can induce stronger innate immune activation than conventional planktonic cultures. Indeed, in a series of studies using an ex vivo whole blood and platelet-rich plasma assay, bacteria derived from the biofilm elicited strong immune response of the neutrophils, monocytes, and platelets, while planktonic bacteria caused minimal or no activation at all. Platelet activation was inversely proportional to the degree of initial activation, with the bacteria being associated with immune cells depending on the state of bacteria themselves (Chao et al., 2024; Mathew et al., 2023).

This particular finding supports the state-dependent theory, whereby the formation of the biofilm may increase chances of persistence or evasion of the immune system, but switching to the dispersed or planktonic-like form will expose the bacterial surfaces, which would lead to greater immune recognition. This hypothesis corresponds to the old finding of the increased biofilm-associated adherence and decreased invasiveness during the initial stages of colonization and the ability of the dispersed descendants to disseminate and elicit an immune reaction (Blanchette-Cain et al., 2013). However, this particular mechanism can potentially be applied for therapeutic purposes only, as long as increased inflammatory reaction does not cause additional harm or complications. (Blanchette-Cain et al., 2013).

3.4. Metal homeostasis as an emerging regulator of biofilm and capsule remodeling

Emerging evidence suggests that transition metals, particularly manganese (Mn^{2+}), play an important role in regulating *S. pneumoniae* biofilm formation and may indirectly influence immune vulnerability through virulence-factor remodeling. Beyond their established roles in bacterial metabolism and oxidative stress defense, metal ions are increasingly recognized as regulators of biofilm development by affecting bacterial adherence, extracellular matrix production, and overall biofilm architecture. Because biofilm formation is accompanied by extensive phenotypic remodeling, including altered expression of surface virulence determinants, disturbances in metal homeostasis(McFarland et al., 2021) have the potential to reshape the pneumococcal lifestyle and its interactions with the host immune system(Martin & Waters, 2022) (McFarland et al., 2021; Martin & Waters, 2022).

Recent studies have further linked metal homeostasis to capsule regulation, a major determinant of pneumococcal virulence. It has been demonstrated that intracellular manganese and zinc balance influences phosphoglucomutase activity and capsule biosynthesis, while our previously published paper showed that manganese intoxication resulting from deletion of the manganese efflux transporter *mntE* impaired biofilm formation and simultaneously increased capsule production (Opoku et al., 2024) (Opoku et al.,2024). Since reduced capsule expression favours biofilm-mediated colonization whereas increased capsule synthesis promotes immune evasion during invasive disease, these findings suggest that metal availability may function as an upstream regulator coordinating the transition between colonization and invasion. Consequently, targeting bacterial metal homeostasis represents a promising strategy to disrupt biofilm persistence while modulating capsule-dependent virulence.

3.5. Antibiotic tolerance, persistence, and ecological interactions

Beyond immune evasion, biofilm formation in *S. pneumoniae* plays a role in the development of phenotypic tolerance, which refers to the enhanced survival of bacteria in communities compared to free-floating bacterial cells when exposed to antibiotics, without genetic-based resistance. This mechanism is especially significant in invasive infections caused by biofilm-associated or biofilm-derived pneumococci at sites like the nasopharynx, middle ear, and other implantable medical devices (Lane et al., 2023; Mayanskiy et al., 2015; Yu et al., 2026).

Interestingly, ecological interactions between pneumococci and other bacteria have recently emerged as a possible strategy for reducing biofilms. Certain commensal streptococcal species, which do not cause infection in hosts, were shown to inhibit both the growth and biofilm formation of *S. pneumoniae*. This result suggests that it is possible to exploit the microbial community in the nasopharynx, thereby destabilising biofilms without eliminating the bacterial microbiome (Borralho et al., 2025). Thus, ecological competition between microorganisms may provide a promising tool to prevent biofilm formation in the nasopharynx without disturbing the natural microbiota. At the same time, the two concepts of resistance and persistence cannot be treated as mutually exclusive, even though biofilm-specific immune vulnerability could be claimed as an advantage overcoming antibiotic resistance (Li et al., 2024).

3.6. Mechanistic and translational controversies

Despite considerable advances in understanding *S. pneumoniae* biofilm biology, several mechanistic and translational controversies remain. One of the principal challenges is the lack of a universal molecular signature for pneumococcal biofilms. Differences among in vitro, ex vivo, and in vivo biofilm models produce distinct transcriptional and phenotypic profiles, making direct comparisons difficult. Furthermore, substantial strain-to-strain variability exists, with serotype and clonal background influencing the expression of surface proteins, extracellular matrix components, and other virulence determinants. Consequently, no single gene expression profile or virulence factor can currently be considered representative of all pneumococcal biofilms, limiting the translation of experimental findings into broadly applicable therapeutic strategies.

Another unresolved issue concerns the therapeutic exploitation of biofilm-associated immune vulnerabilities. Although biofilm-dispersed pneumococci induce greater activation of neutrophils, monocytes, and platelets than planktonic bacteria, excessive inflammatory responses may contribute to host tissue injury, particularly during invasive disease.

Similarly, LytA exemplifies the complexity of targeting biofilm-associated virulence factors. While LytA promotes extracellular DNA release, biofilm maturation, and pathogenicity, it is also the major pneumococcal autolysin responsible for cell-wall turnover and the release of pro-inflammatory components, giving it both protective and pathogenic functions. Therefore, therapeutic inhibition of LytA may reduce biofilm persistence but could also alter immune activation in unpredictable ways. More broadly, the optimal strategy for targeting pneumococcal biofilms remains uncertain. Preventing biofilm formation, disrupting established biofilms, or inhibiting biofilm dispersal may each confer therapeutic benefit, yet the most appropriate approach is likely to depend on the clinical setting. Strategies aimed at reducing asymptomatic carriage may differ substantially from those required for treating pneumonia, bacteremia, or meningitis, highlighting the need for disease-specific anti-biofilm interventions that exploit immune vulnerabilities while minimizing collateral tissue damage.

Future Directions and Research Gaps

Growing evidence has expanded our understanding of pneumococcal biofilm biology; however, several important research gaps remain. A major priority is the standardization of biofilm phenotyping through harmonized experimental models, clearly defined biofilm developmental stages, and integrated transcriptomic, proteomic, metabolomic, and immunological analyses of the same bacterial isolates. Such approaches would improve reproducibility and enable more meaningful comparisons across studies.

Future research should also clarify the temporal and molecular mechanisms governing host immune responses to biofilm-dispersed pneumococci. Longitudinal infection models, in vivo imaging, and multi-omics analyses are needed to determine when dispersed bacteria become most susceptible to immune clearance. From a translational perspective, biofilm-associated surface proteins and emerging regulators such as metal homeostasis pathways represent promising anti-virulence and vaccine targets, although their effects on biofilm architecture and bacterial adaptation require further investigation. Combination therapies integrating biofilm-disrupting agents, antibiotics, immune modulation, and ecological approaches using commensal bacteria may offer greater therapeutic benefit than single-target interventions. Finally, integrating patient-derived isolates with biofilm phenotypes, virulence-factor expression, immune responses, and clinical outcomes will be essential for identifying biomarkers and developing precision therapies for invasive pneumococcal disease.

4. Conclusion

The available evidence supports a state-dependent model of pneumococcal pathogenesis in which biofilm growth promotes persistence and immune evasion, while dispersion may expose a more immunologically visible bacterial phenotype. Biofilm-associated pneumococci show reduced complement deposition and phagocytic clearance, whereas biofilm-derived and dispersed cells provoke stronger activation of neutrophils, monocytes, and platelets than planktonic bacteria. Surface-associated virulence factors, especially PspA, PspC, and LytA, participate in both biofilm architecture and host-cell interaction, making them attractive but context-dependent nodes for intervention

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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