



International Journal of Innovative Technologies in Social Science

e-ISSN: 2544-9435

Operating Publisher
SciFormat Publishing Inc.
ISNI: 0000 0005 1449 8214

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ARTICLE TITLE	THE ROLE OF BACTERIAL BIOFILMS IN RECURRENT TONSILLITIS IN THE PEDIATRIC POPULATION: PATHOGENESIS, ANTIBIOTIC RESISTANCE, AND FUTURE THERAPEUTIC PERSPECTIVES
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DOI	https://doi.org/10.31435/ijitss.2(50).2026.5568
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RECEIVED	23 March 2026
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ACCEPTED	27 June 2026
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PUBLISHED	30 June 2026
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THE ROLE OF BACTERIAL BIOFILMS IN RECURRENT TONSILLITIS IN THE PEDIATRIC POPULATION: PATHOGENESIS, ANTIBIOTIC RESISTANCE, AND FUTURE THERAPEUTIC PERSPECTIVES

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ABSTRACT

Recurrent tonsillitis (RT) in children is a major therapeutic problem. It has historically been managed with systemic antibiotics or surgery. For decades, medical understanding relied entirely on the planktonic model of bacterial infection. Modern microbiology and advanced imaging techniques changed this view. Recurrent exacerbations are actually rooted in structured bacterial communities known as biofilms. This review analyzes the pathogenesis of biofilm formation within the palatine tonsils. We unravel the exact mechanisms of biofilm-mediated antimicrobial resistance and evaluate new therapies. The data draws upon an extensive body of recent literature, including scanning electron and confocal laser microscopy findings. These studies map the anatomical predilection of tonsillar crypts for biofilm sequestration. The extracellular polymeric substance (EPS) and metabolically dormant persister cells explain the frequent failure of standard beta-lactam therapies like Penicillin V. This happens even when bacteria appear susceptible in vitro. We explore non-surgical antibiofilm strategies. These include physical disruption, bacteriophage therapy, endolysins, and nanotechnology. We view these against evolving clinical indications for adenotonsillectomy. A transition toward biofilm-targeted therapeutics is an absolute clinical necessity to reduce the socio-economic and physiological burden of recurrent pediatric upper respiratory tract infections.

KEYWORDS

Recurrent Tonsillitis, Bacterial Biofilms, Pediatric Otorhinolaryngology, Antimicrobial Resistance, Palatine Tonsils, Waldeyer's Ring

CITATION

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Introduction

Recurrent tonsillitis (RT) in children is a lasting clinical dilemma. It bridges general pediatrics, otorhinolaryngology, and clinical microbiology. Despite progress in diagnostic tools and pharmacotherapy, upper respiratory tract infections remain one of the most frequent reasons for pediatric ambulatory visits globally. The epidemiological scale is massive. Up to 11.7% of the pediatric population will experience recurrent episodes of tonsillitis during their development. This translates into a heavy burden on healthcare systems. Primary care consultations multiply. Prescriptions for broad-spectrum antibiotics increase exponentially, and surgical interventions often follow. The socio-economic impact is severe. Affected children miss many school days. Caregivers concurrently lose working days.

To understand this pathology, we must look at the local anatomy and immunology. The palatine tonsils sit directly at the gateway of the respiratory and alimentary tracts. Along with the pharyngeal, lingual, and tubal tonsils, they form Waldeyer's lymphatic ring. This ring is the primary immunological defense against inhaled or ingested pathogens. The architecture of the palatine tonsils maximizes antigen capture. They feature deep, branching epithelial invaginations called crypts, which extend deep into the tonsillar parenchyma. An average human palatine tonsil contains between 10 and 30 crypts.

The crypt epithelium is highly specialized. It is a reticular epithelium rich in M cells (microfold cells). These cells handle the transcytosis of luminal antigens to the underlying lymphoid follicles. This process initiates the adaptive immune response. However, this evolutionary adaptation has a structural flaw regarding chronic bacterial colonization. The deep crypts create a highly protected microenvironment. It is warm and moist. Nutrients are abundant. It is largely shielded from the mechanical clearance of swallowing or salivary flow. The base of the crypts is relatively hypoxic. This anatomy is perfect for surveying passing antigens, but it inadvertently gives opportunistic bacterial pathogens a secure place to settle and alter their phenotype.

Historically, the medical approach to tonsillitis leaned heavily on classical microbiology based on Koch's postulates. Bacteria were viewed strictly as planktonic—free-floating, rapidly dividing cells. When a

child presents with a fever, a sore throat, or tonsillar exudates, the standard procedure involves a physical exam. Doctors often use clinical scoring systems like the Centor or McIsaac criteria. They take a superficial swab of the tonsillar surface. If *Streptococcus pyogenes* (Group A Streptococcus, GAS) grows on the culture plate, a beta-lactam like Penicillin V or Amoxicillin is prescribed. The acute symptoms usually resolve in a few days. The clinician assumes the infection is eradicated.

In cases of recurrent tonsillitis, the clinical reality contradicts this assumption. Children return weeks later with identical symptoms. Culturing the collected material frequently isolates identical bacterial strains. For a long time, doctors interpreted these episodes as new, independent infections acquired from asymptomatic carriers. This classical explanation fails to account for the chronicity of the disease. It also fails to explain a highly frustrating clinical phenomenon. Bacteria isolated from these recurrent episodes often demonstrate complete susceptibility to penicillin in standard laboratory minimum inhibitory concentration (MIC) testing. Yet, the drug fails completely *in vivo*.

This discrepancy between laboratory susceptibility and clinical failure forced a massive paradigm shift in otorhinolaryngology. Researchers recognized the "biofilm concept." Bacteria rarely exist in the human body as isolated cells during chronic disease states. They naturally attach to mucosal surfaces and aggregate into highly organized microbial communities called biofilms.

The transition from a planktonic state to a biofilm phenotype is a multi-stage process. Free-floating bacteria initially attach to the tonsillar crypt epithelium. This reversible attachment uses specific adhesins and pili. If the host immune system does not clear them, the attachment becomes irreversible. The bacteria proliferate and undergo a radical shift in gene expression. This is triggered by quorum sensing, a complex cell-to-cell communication mechanism. As population density reaches a threshold, the bacteria secrete an extracellular polymeric substance (EPS). This matrix is a web of host and bacterial polysaccharides. It contains structural proteins, lipids, and extracellular DNA (eDNA). The EPS acts as a biological shield, encasing the microcolonies and anchoring them firmly to the tissue.

In pediatric recurrent tonsillitis, the biofilm is a silent reservoir of infection hidden within the crypts. Its structural integrity protects the bacteria from the host's cellular immune response. Macrophages and neutrophils simply cannot phagocytose the large EPS structures. It also blocks systemic antibiotic therapy. During clinical remission, the child is entirely asymptomatic, but the biofilm is not static. Environmental stress, such as a viral co-infection or shifts in local pH, triggers a dispersion phase. The biofilm sheds highly virulent, planktonic bacteria back into the crypt lumen and surrounding tissues. This acute release triggers the massive inflammatory cascade we recognize as an acute exacerbation. Antibiotics manage the acute symptoms by killing the free-floating bacteria. The deep-seated biofilm architecture remains intact, preparing for the next recurrence.

The rationale for this review stems from a glaring gap between contemporary science and daily clinical practice. Advanced imaging techniques confirm the presence of dense biofilms in tonsils removed during adenotonsillectomy. However, conservative treatment protocols have remained largely unchanged for decades. Pediatricians and otolaryngologists continue to prescribe repeated cycles of narrow-spectrum beta-lactams. This drives the selection of resistant strains and disrupts the physiological microbiome. It never fixes the root cause.

Clinical guidelines are only recently reflecting this microbiological reality. The 2025 Polish national recommendations for respiratory tract infections suggest shifting empirical therapy for recurrent tonsillitis. Biofilms in the crypts are frequently polymicrobial. They often harbor beta-lactamase-producing organisms like *Staphylococcus aureus* or *Haemophilus influenzae* alongside *Streptococcus pyogenes*. Because of this, the guidelines now advocate clindamycin or cephalosporins like cefadroxil over standard penicillin. The co-residing bacteria act as shield organisms. They produce enzymes that degrade penicillin before it can penetrate the EPS matrix. This protects the entire polymicrobial community.

The objective of this article is to provide a rigorous synthesis of the current knowledge regarding bacterial biofilms in pediatric recurrent tonsillitis, analyzing data from over 40 recent studies and clinical guidelines. First, we map the pathogenesis and structural microbiology of biofilms within the palatine tonsils. We analyze how specific pathogens exploit the local anatomy. Second, we dissect the mechanisms through which biofilms confer profound antibiotic resistance. We look closely at the biochemical properties of the EPS barrier and the metabolic shift towards persister cells. Finally, we evaluate new therapies. We cannot simply use more antibiotics to cure biofilm-mediated diseases. We analyze emerging non-surgical modalities like physical disruption techniques, bacteriophage therapy, targeted endolysins, and smart nanomaterials. We place these in the context of current indications for surgical adenotonsillectomy. Through this systematic analysis, we aim to bridge the gap between laboratory microbiology and the clinical management of the pediatric patient.

Methodology and results

The analysis of bacterial biofilms in recurrent pediatric tonsillitis requires a synthesis of diverse study designs. Traditional clinical microbiology relies on swabbing the surface of the tonsils and culturing the material on agar plates. The literature demonstrates that this methodology is entirely inadequate for capturing the true pathogenic burden of chronic tonsillar disease. Standard cultures select for rapidly dividing planktonic bacteria. They fail to recover metabolically dormant cells hidden within the extracellular polymeric substance (EPS). Because of this severe diagnostic limitation, the most informative studies in this field utilize excised tissue following adenotonsillectomy. Researchers apply advanced visualization techniques to these tissue samples to directly observe the microenvironment. Furthermore, modern investigations incorporate bioengineered ex vivo models to simulate the mechanical and chemical properties of the tonsillar crypts.

To systematically evaluate the prevalence, structure, and microbiological composition of tonsillar biofilms, researchers predominantly use three imaging modalities: Scanning Electron Microscopy (SEM), Confocal Laser Scanning Microscopy (CLSM), and Fluorescence in situ Hybridization (FISH). Each method provides specific data regarding the spatial organization of the infection.

Table 1. Primary imaging modalities for biofilm detection in pediatric tonsils

Imaging modality	Sample preparation	Key findings in pediatric tonsillitis	Primary reference source
Scanning electron microscopy (SEM)	Glutaraldehyde fixation and dehydration	Adherent biofilms confirmed in 85% of patients with recurrent infections versus significantly lower rates in pure hypertrophy cases.	Al-Mazrou & Al-Khattaf (2008)
Confocal laser scanning microscopy (CLSM)	Fluorescent probes applied to hydrated tissue	Detected biofilms in 77.28% of pediatric tonsils. Visualized the 3D glycocalyx matrix overlying the squamous epithelium.	Diaz et al. (2011)
Fluorescence in situ hybridization (FISH)	16S rRNA fluorescent genetic probes	Revealed complex, polymicrobial biofilms organized into distinct species micro-domains and intracellular bacterial clusters.	Mateus et al. (2022)

Scanning electron microscopy offers exceptionally high resolution of the surface topography of tonsillar tissues. Al-Mazrou and Al-Khattaf applied SEM to prospectively assess the surface epithelium of tonsils and adenoids in a pediatric cohort. The preparation of tissues for SEM involves rigorous fixation with glutaraldehyde and subsequent dehydration. Despite the dehydration process, which can shrink the highly hydrated EPS matrix, the results were definitive. Adherent biofilm formation was confirmed in 61% of the total patient cohort. A sharp divergence emerged when the researchers separated patients based on their surgical indications. Among children undergoing surgery specifically for recurrent infections, the detection rate of adherent biofilms was 85%. For patients undergoing surgery purely for obstructive symptoms without a history of recurrent infection, the prevalence dropped significantly. This data establishes a direct morphological link between the physical presence of a biofilm and the clinical history of recurrent acute exacerbations.

Confocal laser scanning microscopy provides a different perspective. It resolves the distortion issues associated with the dehydration required for SEM. CLSM allows researchers to image fully hydrated biological specimens. Diaz and colleagues utilized CLSM alongside specific fluorescent probes to evaluate pediatric tonsillar disease. They stained the live bacteria, the dead bacteria, and the surrounding glycocalyx. Their analysis detected biofilms in over 77% of the examined pediatric tonsils. CLSM allows optical sectioning of the tissue, meaning researchers can generate a three-dimensional reconstruction of the biofilm architecture without physically cutting the sample. The imaging showed that the matrix forms a dense, continuous barrier directly overlying the stratified squamous epithelium of the crypts. The thickness of this biofilm layer varied, but it consistently provided a physical distance between the host immune cells located in the subepithelial layers and the core bacterial microcolonies.

While SEM and CLSM confirm the physical structure of the biofilm, Fluorescence in situ hybridization (FISH) provides species-specific identification directly within the tissue architecture. Mateus and colleagues

applied 16S rRNA FISH probes to pediatric tonsil specimens. This methodology uses fluorescently labeled genetic probes that bind to specific bacterial ribosomal RNA sequences. The results revealed highly complex, polymicrobial biofilms. The spatial mapping showed that different bacterial species do not randomly mix. They organize into distinct structural micro-domains within the larger matrix. Furthermore, the FISH analysis documented intracellular bacterial clusters within the tonsillar parenchyma. This finding suggests a secondary mechanism of persistence. Certain pathogens physically invade the tonsillar epithelial cells or local macrophages. They hide inside the host cells to evade immune surveillance and systemic antibiotics. Once the environment becomes favorable, they rupture the host cell and seed the extracellular biofilm.

The topographical mapping of the tonsil explains the frequent failure of conservative medical management. Clinical studies routinely compare the microbiological profile of the tonsillar surface with the deep tonsillar core tissue. Buname and colleagues analyzed these bacterial patterns in patients scheduled for tonsillectomy. The discordance between the two anatomical sites is immense. Swabs taken from the surface of the tonsil—which mimic the standard clinical procedure performed by a pediatrician during an acute attack—primarily yielded normal oropharyngeal commensal flora. The core tissue, extracted after surgical excision, harbored the actual pathogenic burden.

The microbiological profile of the tonsillar core is heavily skewed toward specific opportunistic pathogens. *Staphylococcus aureus* and *Haemophilus influenzae* are the most frequently isolated organisms from deep crypt tissues. Research by Cavalcanti and associates confirms that *Staphylococcus aureus* is present in approximately 40.7% of recurrent tonsillitis cases. The clinical relevance of this finding is tied directly to the resistance profile of the isolates. A massive majority of these *S. aureus* strains demonstrate resistance to standard penicillin derivatives. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are also increasingly isolated from these protected anatomical niches.

Table 2. Microbiological divergence in pediatric palatine tonsils

Anatomical site	Predominant flora	Clinical significance	Primary references
Tonsillar surface (swab)	Normal oropharyngeal commensals (e.g., <i>Streptococcus viridans</i> , <i>Neisseria</i> spp.)	Poorly reflects the true pathogenic burden. Often leads to inaccurate susceptibility reports and treatment failure.	Bunam et al. (2021)
Tonsillar core (tissue biopsy)	<i>Staphylococcus aureus</i> (~40.7%), <i>Haemophilus influenzae</i> , <i>Streptococcus pyogenes</i>	Harbors the active biofilm reservoir. High prevalence of beta-lactamase producing shield organisms.	Cavalcanti et al. (2019), Kostić et al. (2022)

Streptococcus pyogenes (Group A Streptococcus, GAS) remains a central focus of pediatric tonsillitis management. Current literature indicates that GAS inhabits the core of the tonsil in about a quarter of chronically infected specimens. A critical finding from the polymicrobial biofilm data explains why GAS infections often fail to respond to penicillin, even when the laboratory reports the GAS isolate as entirely susceptible. In the crypt environment, GAS rarely exists in isolation. It frequently co-habitates with beta-lactamase-producing species, such as *Staphylococcus aureus* or *Moraxella catarrhalis*. These co-residing bacteria secrete beta-lactamase enzymes directly into the shared EPS matrix. The enzymes degrade the incoming penicillin molecules before they can reach the target penicillin-binding proteins on the cell wall of *Streptococcus pyogenes*. This phenomenon is known as indirect pathogenicity. The beta-lactamase producers act as shield organisms. They confer passive antibiotic resistance to the entire polymicrobial community.

The physical presence of the biofilm correlates directly with observable macroscopic and biochemical clinical parameters in pediatric patients. Torretta and colleagues investigated the relationship between the detection of biofilm-producing bacteria and the anatomical grade of tonsillar hypertrophy (GTH). The researchers graded tonsillar size using standard clinical scales. Children with a GTH score greater than 2 showed a statistically significant association with the presence of active biofilm production within the crypts.

The continuous, low-grade inflammatory response directed at the EPS matrix drives local lymphoid hyperplasia. The immune system constantly recruits lymphocytes and macrophages to the area in an unsuccessful attempt to clear the large extracellular structures. This chronic cellular influx physically enlarges the tonsillar tissue, leading to the secondary clinical symptom of upper airway obstruction and sleep-disordered breathing.

Furthermore, researchers have attempted to identify non-invasive biomarkers that indicate the presence of a deep tonsillar biofilm. Torretta's team explored the utility of measuring exhaled nitric oxide (eNO) levels in pediatric patients. Nitric oxide is an established physiological marker of airway inflammation, commonly used in the management of bronchial asthma. The inducible form of nitric oxide synthase (iNOS) is upregulated in the tonsillar epithelium in response to chronic bacterial colonization. The clinical study revealed a clear biochemical correlation. Children harboring biofilm-producing bacteria on their tonsils exhibited significantly higher eNO levels compared to age-matched controls without biofilms. The eNO levels in the infected cohort frequently exceeded 8 parts per billion (ppb). This biochemical data suggests that the local infection produces a measurable systemic inflammatory signal in the exhaled breath. It provides a potential non-surgical diagnostic metric to identify patients who are unlikely to benefit from further antibiotic therapy.

Recent methodological advancements incorporate bioengineering to study these infections *ex vivo*. Traditional *in vitro* models use plastic or glass surfaces submerged in liquid growth media. These rigid materials do not replicate the soft, viscoelastic properties of human mucosal tissue. To address this, Denton and colleagues utilized 3D bioprinting technology to create a sophisticated infection model. They developed a bacteria-laden hydrogel composed of gelatin and poly(ethylene glycol) diacrylate (PEGDA). The chemical crosslinking of the PEGDA allows researchers to tune the mechanical stiffness and the swelling ratio of the hydrogel to perfectly match the physical properties of human palatine tonsils.

The results from this 3D bioprinting model are highly instructive. Over a 12-day continuous culture period, the bacteria seeded within the hydrogel successfully formed mature, three-dimensional biofilms. More importantly, the bacteria grown in this tissue-mimicking hydrogel exhibited antibiotic resistance profiles that were nearly identical to those observed in human patients experiencing clinical treatment failure. When the same bacterial strains were grown on standard rigid plastic plates, they were easily killed by lower concentrations of antibiotics. The hydrogel results prove that the physical and mechanical environment of the tissue directly dictates bacterial gene expression and the subsequent resistance phenotype. The EPS matrix integrates with the host tissue extracellular matrix, creating a composite barrier. These bioengineered models represent a massive step forward in methodological design. They provide a highly accurate, reproducible platform for testing novel pharmacological agents before initiating human clinical trials.

The combined results from clinical biopsies, advanced microscopy, and bioengineered tissue models construct a clear biological reality. Pediatric recurrent tonsillitis is fundamentally a structural disease characterized by spatially organized, polymicrobial communities. The superficial swabs used in daily clinical practice do not reflect the microbiology of the deep tonsillar crypts. The pathogenic bacteria reside in the core, heavily shielded by a dense matrix and protected by the enzymatic activity of adjacent species. This complex structural organization completely negates the efficacy of standard empirical antibiotic regimens.

Discussion

The clinical failure of conservative therapy in recurrent pediatric tonsillitis is a direct consequence of bacterial behavior within the tonsillar crypts. To understand this failure, we must deconstruct the specific mechanisms of antimicrobial evasion. Traditional medical training heavily emphasizes genetic resistance. We learn about mutated drug targets, efflux pumps, and horizontally acquired genes. However, the literature reveals a completely different primary mechanism operating in chronic upper respiratory infections. The survival of biofilm-associated bacteria depends fundamentally on phenotypic tolerance rather than strict genetic resistance.

Ciofu and colleagues provide a necessary theoretical framework for this distinction. Genetic resistance means a bacterial cell can grow in the presence of an antibiotic concentration that would normally inhibit it. This is measured by the minimum inhibitory concentration (MIC). Phenotypic tolerance is completely different. A tolerant bacterial population might have a normal, low MIC. They are theoretically susceptible to the drug. Yet, they survive prolonged exposure to high concentrations of bactericidal antibiotics. They do not grow during the exposure, but they do not die either. The difference lies in the growth rate and the metabolic state of the cells. Biofilm tolerance is a reversible, transient state dictated by the local microenvironment, whereas resistance is hardwired into the bacterial genome.

The first line of defense for the tonsillar biofilm is the extracellular polymeric substance. The EPS is a massive physical and chemical barrier. It fundamentally alters the pharmacokinetics of systemically administered drugs at the tissue level. When a pediatrician prescribes a course of amoxicillin, the drug must reach the tonsillar tissue, diffuse into the crypt lumen, and penetrate the EPS to reach the bacterial cell wall. The EPS matrix severely restricts this diffusion.

The restriction is not purely mechanical size exclusion. It is a highly charged chemical interaction. The EPS contains abundant extracellular DNA (eDNA) released by dead bacterial cells and host neutrophils. eDNA is highly negatively charged. Many standard antibiotics, such as aminoglycosides and certain peptides, carry a positive charge. As these drug molecules attempt to enter the biofilm, they bind electrostatically to the eDNA and the surrounding polyanionic polysaccharides. The antibiotic becomes trapped in the superficial layers of the matrix. It never reaches the pathogenic cells residing in the deeper microcolonies. Smith extensively details this drug delivery failure in his research. The biofilm essentially acts as an ion-exchange resin, neutralizing the incoming chemical attack before it can exert any biological effect.

Furthermore, the EPS matrix accumulates high concentrations of bacterial enzymes. We discussed earlier how polymicrobial biofilms in the tonsils frequently contain beta-lactamase producers like *Haemophilus influenzae* and *Staphylococcus aureus*. In a planktonic infection, a secreted beta-lactamase diffuses rapidly into the surrounding bodily fluids, diluting its effect. In a biofilm, the EPS matrix traps these enzymes. They accumulate to massive concentrations directly surrounding the bacterial microcolonies. When penicillin finally penetrates the outer layers of the EPS, it hits a dense wall of concentrated beta-lactamase. The antibiotic is rapidly hydrolyzed. This enzymatic shield protects the entire community, including species like *Streptococcus pyogenes* that do not possess intrinsic beta-lactamase genes.

Beyond the physical barrier, the most profound defense mechanism of the biofilm is physiological heterogeneity. A mature biofilm is not a uniform mass of cells. It is a highly structured, layered ecosystem characterized by steep chemical gradients. The cells on the outermost periphery of the biofilm have direct access to oxygen and nutrients from the tonsillar crypt fluid. These peripheral cells are metabolically active and divide rapidly. Consequently, they are easily killed by antibiotics like beta-lactams and cephalosporins, which require active cell wall synthesis to cause lysis.

The deeper layers of the biofilm present a radically different physiological reality. As nutrients and oxygen diffuse inward, they are consumed by the peripheral cells. This creates a zone of profound starvation and hypoxia at the core of the microcolony. Bacteria in this deeper zone cannot sustain active division. They enter a stationary phase. Their metabolic rate drops to near zero.

This localized starvation is the exact reason why antibiotics fail. Most bactericidal antibiotics target active cellular processes. Penicillins target peptidoglycan cross-linking during cell division. Fluoroquinolones target DNA gyrase during DNA replication. Macrolides target the ribosome during active protein synthesis. If a bacterial cell is not dividing, not replicating its DNA, and drastically reducing its protein synthesis, the antibiotic has no target to attack. The drug molecules might successfully penetrate the EPS and enter the deep-seated bacterial cell, but they simply float around harmlessly. The bacteria are completely tolerant to the treatment. This explains why clinical symptoms temporarily improve during a course of antibiotics—the fast-growing peripheral cells die off, reducing the inflammatory load—but the core infection remains perfectly intact.

Within this hypoxic, starving core, a specific subpopulation of cells emerges. These are the persister cells. Persisters are a stochastic, dormant phenotypic variant of regular cells. They make up a tiny fraction of the biofilm population, often less than 1%. Unlike resistant mutants, persisters do not possess genetic alterations that protect them from antibiotics. They are simply in a state of deep biological dormancy. Their cellular machinery is entirely shut down.

Persister cells survive massive, prolonged doses of bactericidal antibiotics. They endure the entire standard 10-day course of penicillin. They wait. When the physician stops the antibiotic prescription, the chemical stress disappears from the local tonsillar environment. The persister cells sense this change. They "reawaken" from their dormant state, resume normal metabolic activity, and rapidly begin to divide. They utilize the leftover EPS matrix and the cellular debris of their dead neighbors to rebuild the entire biofilm architecture. Weeks later, the child presents with another acute episode of tonsillitis. The clinical cycle repeats indefinitely.

This realization exposes a massive flaw in standard diagnostic microbiology. The everyday tools of the clinical laboratory are entirely blind to the biofilm phenotype. When a microbiologist receives a tonsillar swab, the sample is heavily processed. The bacteria are dispersed and plated onto nutrient-rich agar. They are

incubated in an optimal, oxygen-rich environment at 37 degrees Celsius. The laboratory artificially forces the bacteria out of their tolerant, slow-growing biofilm state and back into a rapidly dividing planktonic state. The subsequent antibiogram (MIC testing) measures the susceptibility of these actively dividing cells. The report inevitably shows that the pathogen is "sensitive" to standard antibiotics. The clinician reads the report, prescribes the drug, and expects a cure. The laboratory test measures a biological state that simply does not exist inside the patient's tonsillar crypts. The reliance on planktonic MIC data for chronic, tissue-embedded infections is a primary driver of treatment failure in modern otorhinolaryngology.

The biofilm environment also massively accelerates the acquisition of true genetic resistance. The EPS matrix physically packs thousands of bacterial cells into a microscopic volume. This high cell density is the ideal setting for horizontal gene transfer (HGT). Bacteria constantly exchange genetic material through transformation, transduction, and conjugation. In a liquid suspension, these events are relatively rare because the cells are physically distant. Inside the matrix, the cells are in constant physical contact. Plasmids carrying multiple antibiotic resistance genes pass rapidly between different species.

Furthermore, the stressful environment of the biofilm actively promotes mutagenesis. The deeper layers are hypoxic and filled with reactive oxygen species and toxic metabolic byproducts. This chemical stress triggers the bacterial SOS response. The SOS response is an emergency repair system that upregulates error-prone DNA polymerases. These polymerases copy DNA very quickly but make frequent mistakes. The mutation rate in a biofilm can be up to 100 times higher than in a planktonic culture. The biofilm is a highly efficient evolutionary reactor. It constantly generates new genetic variants, tests them against the immune system and the prescribed antibiotics, and shares the successful resistance genes across the entire polymicrobial community.

These resistance and tolerance mechanisms fundamentally change how we must view recurrent tonsillitis. The traditional model views each exacerbation as a distinct failure of hygiene or immunity. The biofilm model reveals that the child is fighting a single, continuous, highly adapted infection. The bacteria have colonized the physical structure of the tonsil and completely neutralized our primary pharmacological weapons. They use our own local anatomy against us. The deep crypts provide the bunker, the EPS provides the shield, and the metabolic shift provides the ultimate survival strategy.

We cannot continue to treat a structural, tissue-integrated disease with simple, single-agent chemical diffusion. Prescribing a stronger antibiotic, or a longer course of the same antibiotic, does not overcome the fundamental physics of the EPS barrier or the metabolic reality of the persister cell. It only guarantees the further destruction of the child's protective gastrointestinal and respiratory microbiomes. It selects for increasingly resistant strains. This absolute pharmacological deadlock necessitates a complete re-evaluation of our therapeutic approach. We must move beyond simply trying to poison the bacteria. We have to physically dismantle their environment, disrupt their communication, or utilize entirely different biological mechanisms of eradication.

Future therapeutic perspectives

The realization that recurrent pediatric tonsillitis is a biofilm-mediated disease forces a complete re-evaluation of our clinical protocols. We cannot cure a structural infection with simple chemical diffusion. The extracellular polymeric substance (EPS) and the persister cells neutralize standard antibiotics. This pharmacological deadlock requires alternative strategies. We must physically dismantle the bacterial environment or use entirely different biological mechanisms of eradication.

One immediate approach is mechanical disruption. The concept is straightforward. If the biofilm is physically anchored to the tonsillar crypts, we should attempt to detach it. Ciftci and colleagues investigated the efficacy of tonsil brushing. They used an ex vivo model with excised tonsillar tissue. Simple rinsing with saline completely failed to remove the biofilm layer. The EPS matrix resisted the fluid shear stress. However, gentle mechanical brushing with a soft-bristled brush significantly reduced the biofilm burden.

While physical brushing is anatomically difficult to perform in a conscious child without triggering the gag reflex, chemical disruption offers a more practical alternative. Johnston's team evaluated the clinical utility of 0.2% chlorhexidine mouthwash. They analyzed the tonsillar crypts of children scheduled for surgery. The preoperative use of chlorhexidine significantly reduced the density of bacterial microcolonies. Chlorhexidine carries a positive charge. It binds to the negatively charged bacterial cell walls and the EPS matrix, causing membrane disruption. These topical therapies do not offer a definitive cure, but they reduce the bacterial load. They are useful adjunctive treatments to prolong the symptom-free intervals between acute exacerbations.

Beyond superficial topical agents, mucolytic drugs like N-acetylcysteine (NAC) demonstrate significant antibiofilm properties. Traditional pharmacology classifies NAC primarily as an antioxidant and a mucolytic agent used for cystic fibrosis or chronic bronchitis. However, recent pediatric literature, heavily reviewed by Nazzari and Principi, reveals a secondary structural application. NAC physically disrupts the extracellular polymeric substance. It breaks the disulfide bonds in the mucus and the bacterial EPS matrix, destroying the three-dimensional architecture of the biofilm. This disruption massively reduces the ability of pathogens like *Haemophilus influenzae* and *Streptococcus pneumoniae* to adhere to the human oropharyngeal epithelium. Furthermore, clinical trials indicate that using NAC as a local aerosol adjunct to systemic antibiotics—such as thiamphenicol—restores the efficacy of the antibiotic. The NAC melts the protective shield, allowing the thiamphenicol to penetrate the core and eradicate the metabolically active bacteria that were previously untouchable.

Biological therapies represent a massive shift in infection management. Bacteriophage therapy is currently experiencing a major clinical revival. Bacteriophages are naturally occurring viruses. They exclusively infect and lyse bacterial cells. Unlike broad-spectrum antibiotics, phages are highly specific. A specific phage will target a specific bacterial species or even a specific strain. This specificity is a massive clinical advantage. A bacteriophage targeting *Staphylococcus aureus* will leave the child's physiological oropharyngeal and gastrointestinal microbiome completely intact.

The pharmacokinetics of bacteriophages are unique. Antibiotics undergo continuous dilution and metabolic clearance from the moment they enter the bloodstream. Bacteriophages exhibit "auto-dosing." When a phage infects a bacterial cell, it replicates. The bacterial cell bursts, releasing hundreds of new phages directly into the local infection site. The concentration of the therapeutic agent actually increases at the exact location of the biofilm.

However, bacteria can develop resistance to specific phages by altering their surface receptors. To prevent this, modern protocols utilize phage cocktails. A cocktail combines several different phages targeting the same pathogen through different receptors. Furthermore, researchers are exploring the use of predatory bacteria, such as *Bdellovibrio bacteriovorus*. Popowska and colleagues detail how these predatory organisms actively swim through the EPS matrix. They penetrate the bacterial cell wall, replicate inside the periplasm, and destroy the host cell from within. The formation of the stable bdelloplast structure protects the predator while it consumes the pathogen.

Endolysins offer another highly effective biological approach. Endolysins are hydrolytic enzymes produced by bacteriophages during the final stage of their replication cycle. The phage uses these enzymes to degrade the bacterial peptidoglycan wall from the inside out, allowing the new virions to escape. Researchers now synthesize these enzymes as independent therapeutic agents.

Moghadam extensively reviewed the application of endolysins against antibiotic-resistant bacteria. They have a distinct advantage over both traditional antibiotics and whole bacteriophages. Traditional beta-lactam antibiotics require the bacteria to be actively dividing to inhibit cell wall synthesis. Endolysins simply digest the existing cell wall. They are independent of the bacterial metabolic state. Because of this mechanism, endolysins effectively destroy dormant persister cells hidden deep within the hypoxic core of the biofilm. The enzymes literally melt the structural integrity of the pathogen.

Nanotechnology provides a completely different engineering solution. Nanoparticles offer an exceptionally high surface area-to-volume ratio. This allows for precise modifications of their physical and chemical properties. Qi and colleagues reviewed the application of composite nanomaterials for antibiofilm therapy. The primary challenge in treating tonsillar biofilms is penetrating the dense, negatively charged EPS matrix. Conventional drug molecules become trapped in the outer layers.

Researchers are designing smart, stimuli-responsive nanoparticles to overcome this barrier. The microenvironment within a mature biofilm is highly acidic due to the accumulation of anaerobic metabolic byproducts. Engineers create pH-responsive nanoparticles that carry a negative or neutral charge at physiological pH (7.4) in the bloodstream. This prevents premature clearance by the immune system. When the nanoparticles diffuse into the acidic tonsillar crypt, the low pH triggers a chemical shift. The surface of the nanoparticle becomes highly positively charged. This sudden cationic charge allows the nanoparticle to heavily bind to the eDNA in the EPS matrix and penetrate the deep bacterial layers.

Once inside the microcolony, the nanoparticles release their payload. This payload can be a concentrated dose of a standard antibiotic, an antimicrobial peptide, or reactive oxygen species (ROS). Silver-doped mesoporous silica nanoparticles are particularly effective. They physically carry the drug while simultaneously releasing silver ions, which have intrinsic antibacterial properties. Omidian's review on pediatric

nanotechnology applications emphasizes the necessity of rigorous toxicological evaluation. The long-term accumulation of heavy metal nanoparticles in pediatric tissues is a serious safety concern. Current research focuses on developing biodegradable polymeric nanoparticles, such as chitosan or PLGA derivatives, which clear safely from the child's system after delivering the therapeutic payload.

Table 3. Emerging non-surgical antibiofilm strategies

Therapeutic agent	Mechanism of action	Advantage over standard antibiotics	Key literature
Bacteriophage therapy (cocktails)	Viral infection and lysis of specific bacterial cells.	Auto-dosing at the infection site. Does not damage normal physiological flora.	Ling et al. (2022), Kalia et al. (2025)
Phage-derived endolysins	Enzymatic digestion of the bacterial peptidoglycan cell wall.	Kills metabolically dormant persister cells. Independent of bacterial growth rate.	Moghadam et al. (2025)
Stimuli-responsive nanoparticles	pH-triggered charge shift (neutral to cationic) in the acidic biofilm core.	Heavily binds to negatively charged eDNA, allowing deep matrix penetration and drug delivery.	Qi et al. (2023), Omidian & Mfoafo (2023)
N-acetylcysteine (NAC)	Disrupts the physical structure of the extracellular polymeric substance.	Reduces bacterial adhesion. Useful as an adjunct to systemic antibiotics.	Nazzari et al. (2015), Principi & Esposito (2024)

These emerging non-surgical modalities must be contextualized within current clinical guidelines. Adenotonsillectomy remains the ultimate treatment for refractory disease. The surgical excision completely removes the anatomical crypts and the embedded biofilms. It eliminates the reservoir of infection.

The clinical indications for surgery are evolving. The globally recognized Paradise criteria state that surgery is justified if a child experiences at least seven acute episodes in the past year, five episodes per year for two consecutive years, or three episodes per year for three consecutive years. These episodes must be accompanied by specific clinical features like high fever or tonsillar exudates.

However, major European consensus statements, such as the 2025 Polish national recommendations and the international review by Guntinas-Lichius, demonstrate a significant clinical shift. The sheer frequency of infections is no longer the absolute primary driver for surgery. Conservative, watchful waiting is increasingly recommended for simple recurrent infections, provided the child does not exhibit severe complications.

The primary absolute indication for surgical intervention has shifted toward the secondary anatomical consequences of the biofilm. The chronic, low-grade inflammation driven by the EPS matrix causes massive tonsillar hypertrophy. This physical enlargement leads to upper airway obstruction and sleep-disordered breathing (SDB) or frank obstructive sleep apnea (OSA). When a child presents with recurrent infections and concurrent polysomnographic evidence of sleep apnea, surgical intervention is immediately indicated. The physiological damage caused by chronic nocturnal hypoxia and sleep fragmentation far outweighs the surgical risks. For pure hypertrophy without recurrent acute infections, a partial tonsillotomy is often preferred over a total tonsillectomy. It relieves the obstruction while preserving the peripheral immunological function of the tissue.

Conclusions

Pediatric recurrent tonsillitis is a structural disease. The anatomical architecture of the palatine tonsils provides a perfect sanctuary for opportunistic pathogens. Advanced imaging modalities confirm that bacteria do not exist as free-floating cells in chronic tonsillar disease. They organize into complex, polymicrobial biofilms hidden deep within the crypts.

This spatial organization explains the consistent failure of standard medical management. The extracellular polymeric substance physically blocks drug diffusion. The deep hypoxic core forces bacteria into a dormant, metabolically inactive state. These persister cells simply ignore bactericidal antibiotics. Our reliance on standard clinical swabbing and planktonic susceptibility testing generates fundamentally flawed

data. The laboratory report suggests the bacteria are sensitive, but the physical reality of the tissue dictates total clinical failure.

The prescription of repeated, empirical courses of penicillin or amoxicillin for chronic tonsillitis is a scientifically outdated practice. It disrupts the physiological microbiome and accelerates the horizontal transfer of resistance genes. We are selecting for highly resistant, biofilm-adapted phenotypes.

Future management strategies must directly target the biofilm architecture. The development of 3D bioprinted, tissue-mimicking hydrogels provides the necessary testing platforms to evaluate these new drugs. Bacteriophage cocktails, targeted endolysins, and stimuli-responsive nanoparticles show massive clinical potential. They bypass the metabolic tolerance of the persister cells and physically dismantle the EPS shield. Until these therapies achieve widespread regulatory approval and clinical availability, adenotonsillectomy remains the only definitive cure for severe, obstructive, biofilm-mediated tonsillar disease. The integration of microbiological reality into daily pediatric practice is the only way to reduce the immense healthcare burden of this incredibly common pathology.

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All authors have read and agreed with the published version of the manuscript.

Funding Statement: The author received no external funding for this work.

Institutional Review Board Statement: Not applicable; this review included only published data.

Data Availability Statement: All supporting data are available within the cited peer-reviewed literature.

Acknowledgments: The author acknowledges the contribution of investigators and data curators whose high-quality research underpins the advances reviewed herein.

Conflict of Interest Statement: The author declares no conflict of interest.

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