

# Evaluation Of Amoxicillin With Host Immune Mediators And Streptococcus Mutans Virulence Proteins Associated With Early Childhood Caries - An In-Silico Study

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## ABSTRACT

**Background:** Early childhood caries (ECC) is a multifactorial disease involving bacterial virulence and host immune responses. Cytokines such as IL-6, TNF, and IL-1 $\beta$  play a key role in inflammation, while enzymes like MMP-9 contribute to tissue destruction. Amoxicillin is widely used in pediatric dentistry, but its interaction with host immune mediators remains unclear.

**Aim:** To evaluate the in-silico interaction of amoxicillin with Streptococcus mutans virulence protein and host immune mediators associated with ECC.

**Materials and Methods:** Molecular docking was performed using AutoDock Vina via PyRx. Protein structures, including GtfB (8FK4), IL-6 (1ALU), TNF (modeled), IL-1 $\beta$  (8C3U), and MMP-9 (1GKC), were retrieved from the RCSB Protein Data Bank. Amoxicillin (PubChem CID: 33613) was used as the ligand. Binding affinities and interaction profiles were analyzed using Discovery Studio.

**Results:** Amoxicillin showed moderate binding affinity across all targets, with the highest affinity toward GtfB (-6.8 kcal/mol), followed by IL-6, TNF, and IL-1 $\beta$  (~-6.4 kcal/mol), and MMP-9 (-5.5 kcal/mol). Key interactions included hydrogen bonding, hydrophobic contacts, and electrostatic interactions.

**Conclusion:** Amoxicillin may exert dual effects by inhibiting bacterial virulence and interacting with host immune mediators, although its immunomodulatory effects appear indirect. Rational antibiotic use is essential in pediatric populations.

**Keywords:** Early childhood caries, amoxicillin, Streptococcus mutans, glucosyltransferase, interleukin-6, tumor necrosis factor-alpha, interleukin-1 $\beta$ , matrix metalloproteinase-9, molecular docking, cytokines, biofilm, pediatric dentistry

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## INTRODUCTION

Early Childhood Caries (ECC) remains one of the most common chronic diseases affecting children globally, with significant consequences on oral health, nutrition,

growth, and quality of life.[1] It is no longer considered a simple bacterial infection but a complex, multifactorial disease involving dynamic interactions between microbial biofilms, host immune responses, and

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environmental factors such as diet and oral hygiene practices.[2],[3] Among the various cariogenic microorganisms, Streptococcus mutans is regarded as a primary etiological agent due to its strong ability to adhere to tooth surfaces, metabolize fermentable carbohydrates, and produce acids that lead to enamel demineralization [3].

The pathogenicity of *S. mutans* is largely attributed to its virulence proteins, including glucosyltransferases (GtfB and GtfC), which are responsible for synthesizing extracellular polysaccharides essential for biofilm formation. Additionally, adhesion proteins such as SpaP facilitate bacterial attachment, while regulatory systems like LuxS contribute to quorum sensing and biofilm stability. These virulence factors enable *S. mutans* to establish a persistent and highly organized biofilm, which not only enhances bacterial survival but also increases resistance to antimicrobial agents.

In parallel, the host immune system plays a crucial role in ECC progression. Elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interleukin-1 beta (IL-1 $\beta$ ), along with matrix metalloproteinases like MMP-9, have been reported in children with ECC[4], [5]. These mediators contribute to inflammatory responses and tissue destruction, further exacerbating disease severity. Thus, ECC can be viewed as a disease driven by both microbial virulence and host immune dysregulation.

Amoxicillin, a commonly prescribed  $\beta$ -lactam antibiotic in pediatric dentistry, is widely used for managing odontogenic infections due to its broad-spectrum activity and relatively safe profile [6]. Its mechanism of action involves inhibition of bacterial cell wall synthesis by binding to penicillin-binding proteins. However, recent studies have reported variable susceptibility of oral streptococci, including *S. mutans*, to amoxicillin, particularly in biofilm-associated states.[6] The protective nature of biofilms and potential genetic adaptations contribute to reduced antibiotic efficacy, raising concerns about its long-term effectiveness.

With the advancement of computational tools, in-silico approaches such as molecular docking have emerged as valuable methods to study drug-protein interactions at the molecular level. Previous studies have predominantly focused on evaluating natural compounds against *S. mutans* virulence proteins, demonstrating promising anti-cariogenic potential[7], [8], [9]. However, there is limited

literature exploring the molecular interaction of conventional antibiotics like amoxicillin with specific virulence proteins of *S. mutans*. Furthermore, the potential interaction of amoxicillin with host immune mediators associated with ECC has not been adequately investigated.

This highlights a significant gap in the current literature, where most studies address either antimicrobial activity or host immune response independently, without integrating both aspects. Understanding how amoxicillin interacts simultaneously with bacterial virulence factors and host inflammatory mediators could provide deeper insights into its therapeutic role and limitations in ECC management.

Therefore, the present study aims to evaluate the interaction of amoxicillin with key Streptococcus mutans virulence proteins and selected host immune mediators using in-silico molecular docking techniques. This integrated approach may help elucidate novel mechanisms of action and contribute to more targeted and effective strategies for managing early childhood caries.

### MATERIALS & METHODS

#### Study Design

The present study was designed as an in-silico molecular docking analysis to evaluate the interaction of amoxicillin with selected Streptococcus mutans virulence proteins and host immune mediators associated with early childhood caries. Computational methods were employed to predict binding affinity, interaction patterns, and potential inhibitory mechanisms at the molecular level. Molecular docking is a widely accepted approach for studying ligand-protein interactions and screening potential therapeutic agents prior to experimental validation.[10]

#### Selection of Target Proteins

Key virulence proteins of Streptococcus mutans involved in biofilm formation, adhesion, and pathogenicity were selected as bacterial targets. These included glucosyltransferase B (GtfB), glucosyltransferase C (GtfC), and surface protein antigen (SpaP), which play critical roles in extracellular polysaccharide synthesis and bacterial adherence.[11] In addition, host immune mediators associated with inflammatory responses in early childhood caries, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 beta (IL-1 $\beta$ ), and matrix metalloproteinase-9 (MMP-9), were

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selected to assess potential host-modulatory interactions.[12] The three-dimensional crystal structures of all target proteins were retrieved from the Protein Data Bank (PDB) in .pdb format based on availability and resolution quality.

## Ligand Preparation

The chemical structure of amoxicillin was obtained from the PubChem database (National Center for Biotechnology Information, USA) in SDF format and converted to PDB format using Open Babel software.[13] The ligand structure was energy minimized to obtain a stable conformation and to remove any steric clashes. Hydrogen atoms were added, and appropriate charge assignments were performed prior to docking to ensure accurate interaction analysis.

## Protein Preparation

The retrieved protein structures were prepared using AutoDock Tools (version 1.5.6). Water molecules, co-crystallized ligands, and other heteroatoms were removed to avoid interference during docking. Polar hydrogen atoms were added, and Kollman charges were assigned to the protein structures. The prepared proteins were then saved in PDBQT format for docking analysis.[14]

## Active Site Identification and Grid Box Setup

The active binding sites of the selected proteins were identified based on literature reports and the presence of co-crystallized ligands in the PDB structures. Grid box parameters were defined to encompass the active site residues, ensuring that the ligand could explore all potential binding conformations within the target region. The grid dimensions and center coordinates were adjusted individually for each protein to optimize docking accuracy.[15]

## Molecular Docking Procedure

Molecular docking was performed using AutoDock Vina integrated within the PyRx virtual screening tool. The prepared ligand (amoxicillin) was docked against each target protein to predict binding affinity and interaction modes. The docking process generated multiple conformations, and the best binding pose was selected based on the lowest binding energy (kcal/mol) and favorable interaction profile. Binding affinities were recorded, and docking scores were used to compare interactions across different targets.

## Visualization and Interaction Analysis

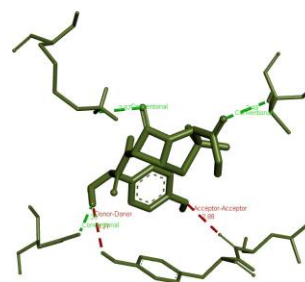
The docked complexes were visualized using Discovery

Studio Visualizer (BIOVIA, Dassault Systèmes) to analyze ligand-protein interactions. Hydrogen bonds, hydrophobic interactions, and electrostatic interactions between amoxicillin and amino acid residues at the active site were identified and documented. Two-dimensional and three-dimensional interaction diagrams were generated to illustrate binding patterns and to support interpretation of docking results.[16]

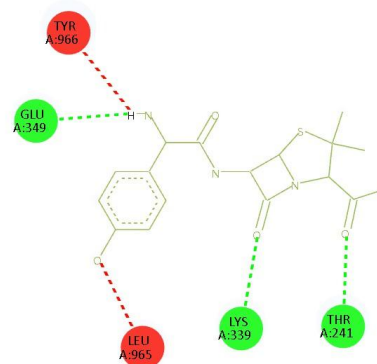
## Data Analysis

The docking results were analyzed qualitatively and quantitatively based on binding energy values and interaction profiles. Lower binding energy values were considered indicative of stronger ligand-protein interactions. Comparative analysis was performed between bacterial virulence proteins and host immune mediators to assess the potential dual role of amoxicillin in antimicrobial activity and host response modulation.

## RESULTS



**Figure 1:** Three-dimensional (3D) binding interaction of amoxicillin with GtfB (PDB ID: 8FK4)

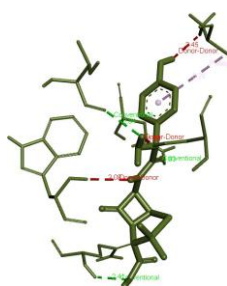


**Interactions**  
Conventional Hydrogen Bond (green dashed line)  
Unfavorable Donor-Donor (red dashed line)  
Unfavorable Acceptor-Acceptor (red dashed line)

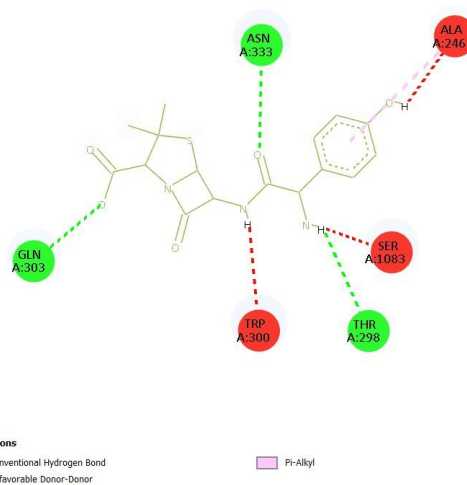
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**Figure 2:** Two-dimensional (2D) interaction map of amoxicillin with GtfB (PDB ID: 8FK4)

Amoxicillin demonstrated a moderate and stable binding interaction with glucosyltransferase B (GtfB) of *Streptococcus mutans*, with the best docking pose showing a binding affinity of  $-6.8$  kcal/mol. The 3D binding conformation (Figure 1) revealed that the ligand fits well within the catalytic pocket of the enzyme, indicating favorable spatial accommodation. The 2D interaction analysis (Figure 2) showed the formation of conventional hydrogen bonds with key residues such as GLU A:349, LYS A:339, and THR A:241, which are important for enzymatic activity. Although minor unfavorable interactions with residues like TYR A:966 and LEU A:965 were observed, they did not significantly affect overall binding stability. These findings suggest that amoxicillin may interfere with GtfB-mediated biofilm formation through stable molecular interactions.

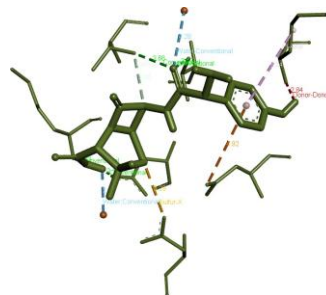


**Figure 3:** Three-dimensional (3D) molecular interaction of amoxicillin with GtfC (PDB ID: 3AIB), showing stable binding within the catalytic pocket through hydrogen bonding and hydrophobic interactions.



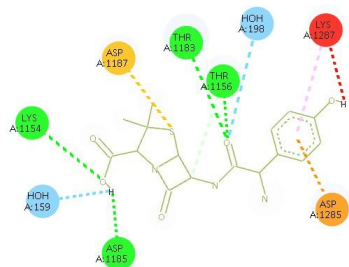
**Figure 4:** Two-dimensional (2D) interaction diagram illustrating key amino acid residues involved in binding, including GLN A:303, ASN A:333, and THR A:298, along with  $\pi$ -alkyl and unfavorable interactions.

Amoxicillin exhibited a moderate binding interaction with glucosyltransferase C (GtfC) of *Streptococcus mutans*, with the best docking pose showing a binding affinity of  $-6.8$  kcal/mol. The 3D docking conformation (Figure 3) demonstrated stable accommodation of the ligand within the active site cavity. The 2D interaction profile (Figure 4) revealed conventional hydrogen bonding with key residues such as GLN A:303, ASN A:333, and THR A:298, indicating favorable stabilization. Additionally,  $\pi$ -alkyl interactions and minor unfavorable donor-donor interactions with residues including ALA A:246, TRP A:300, and SER A:1083 were observed. Despite these minor unfavorable contacts, the ligand maintained stable binding, suggesting potential inhibition of GtfC-mediated biofilm formation.



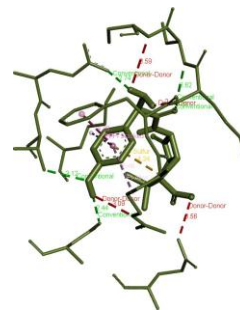
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**Figure 5:** Three-dimensional (3D) molecular interaction of amoxicillin with SpaP (PDB ID: 3OPU), illustrating ligand accommodation within the binding pocket through hydrogen bonding and hydrophobic interactions.

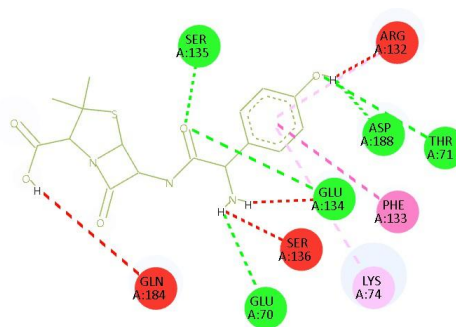


**Figure 6:** Two-dimensional (2D) interaction diagram showing key interacting residues including LYS A:1154, ASP A:1185, and THR A:1156, along with water-mediated and  $\pi$ -interactions.

Amoxicillin showed a comparatively lower but stable binding interaction with the surface protein antigen (SpaP) of *Streptococcus mutans* (PDB ID: 3OPU), with the best docking pose exhibiting a binding affinity of  $-5.6$  kcal/mol. The 3D docking conformation (Figure 5) demonstrated that the ligand occupies the binding pocket with multiple stabilizing interactions. The 2D interaction analysis (Figure 6) revealed conventional hydrogen bonding with residues such as LYS A:1154, ASP A:1185, and THR A:1156, along with water-mediated interactions involving HOH residues. Additional interactions including  $\pi$ -alkyl and  $\pi$ -anion contacts were observed, while minor unfavorable donor-donor interactions with residues like LYS A:1287 were noted. Despite moderate binding energy, these interactions suggest that amoxicillin may contribute to interference with SpaP-mediated adhesion processes.



**Figure 7:** Three-dimensional (3D) molecular docking interaction of the ligand with human Interleukin-6 (IL-6) showing binding orientation within the active site. Hydrogen bonds, hydrophobic interactions, and unfavorable donor-donor interactions are depicted with corresponding distance measurements, highlighting key interacting residues.

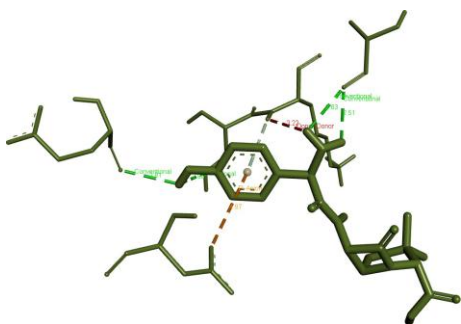


**Figure 8:** Two-dimensional (2D) interaction diagram illustrating ligand-protein interactions with IL-6. Conventional hydrogen bonds (green), unfavorable donor-donor interactions (red), and hydrophobic interactions such as  $\pi$ - $\pi$  stacking and  $\pi$ -alkyl interactions (pink/purple) are shown with involved amino acid residues.

The 3D structure of human Interleukin-6 was modeled using the SWISS-MODEL server with the UniProt-reviewed canonical sequence of IL6\_HUMAN (P05231). The predicted model was obtained as a monomer using template 8yww.1.F, which showed 100% sequence identity with the target. The model demonstrated a GMQE score of 0.58 and a QMEANDisCo global score

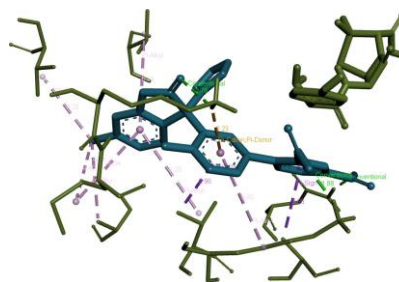
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of  $0.62 \pm 0.07$ , indicating acceptable structural quality for in silico analysis. Docking visualization revealed that the ligand formed several conventional hydrogen bonds with Ser135, Glu70, Asp188, Thr71, and Glu134, along with pi-pi stacked and pi-alkyl interactions involving Phe133 and Lys74 (Figure 7 and Figure 8). A few unfavorable donor-donor contacts were also observed (Figure 8), suggesting that although the ligand binds within the predicted IL-6 interaction region, binding stability may be influenced by both favorable and unfavorable residue contacts.

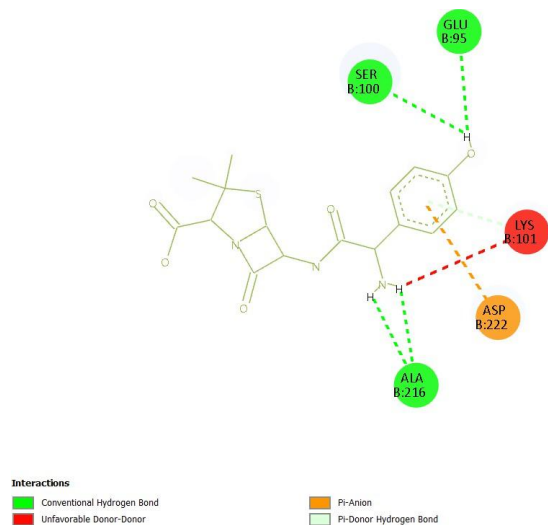


**Figure 9:** Three-dimensional (3D) molecular interaction of amoxicillin with tumor necrosis factor (TNF), illustrating stable binding within the active pocket through hydrogen bonding and electrostatic interactions.

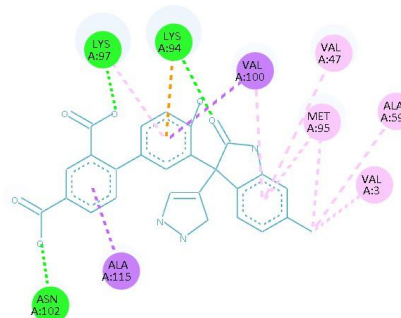
Amoxicillin demonstrated a moderate binding interaction with tumor necrosis factor (TNF), with the best docking pose showing a binding affinity of  $-6.4$  kcal/mol. The 3D docking conformation (Figure 9) revealed stable accommodation of the ligand within the binding pocket. The 2D interaction analysis (Figure 10) showed conventional hydrogen bonding with key residues including GLU B:95, SER B:100, and ALA B:216, contributing to ligand stabilization. Additionally,  $\pi$ -anion interactions with ASP B:222 and minor unfavorable donor-donor interactions with LYS B:101 were observed. Despite these minor unfavorable contacts, the ligand maintained a stable binding profile, suggesting potential modulation of TNF-mediated inflammatory pathways.



**Figure 11:** Three-dimensional (3D) molecular interaction of amoxicillin with IL-1 $\beta$  (PDB ID: 8C3U), illustrating stable binding within the active pocket through hydrogen bonding and hydrophobic interactions.



**Figure 10:** Two-dimensional (2D) interaction diagram showing key interacting residues including GLU B:95, SER B:100, ALA B:216, and ASP B:222, along with  $\pi$ -anion and unfavorable interactions.

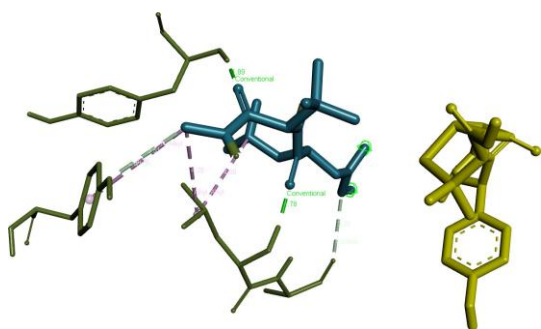


**Figure 12:** Two-dimensional (2D) interaction diagram showing key amino acid residues including LYS A:97,

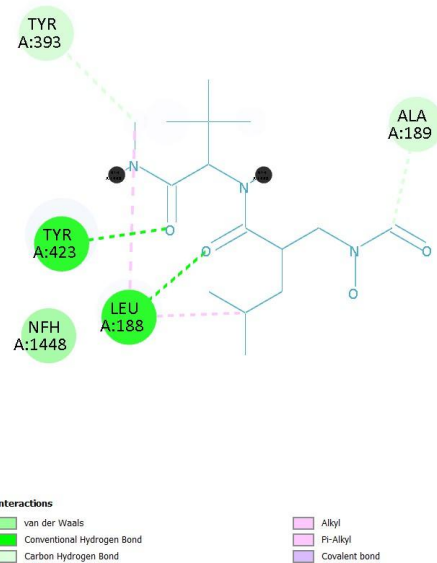
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LYS A:94, ASN A:102, VAL A:100, and MET A:95 involved in ligand stabilization via hydrogen bonds and hydrophobic contacts.

Amoxicillin exhibited a stable binding interaction with human interleukin-1 $\beta$  (IL-1 $\beta$ ) (8C3U), with the best docking pose showing a binding affinity of  $-6.4$  kcal/mol. The 3D docking conformation (Figure 11) demonstrated proper accommodation of the ligand within the active binding region. The 2D interaction analysis (Figure 12) revealed conventional hydrogen bonding with key residues such as LYS A:97, LYS A:94, and ASN A:102, contributing to binding stability. In addition, hydrophobic interactions including  $\pi$ -alkyl and alkyl contacts with VAL A:100, MET A:95, and ALA A:59 further stabilized the complex. These interactions suggest that amoxicillin may effectively modulate IL-1 $\beta$ -mediated inflammatory responses relevant to ECC pathogenesis.



**Figure 13:** Three-dimensional (3D) molecular interaction of amoxicillin with MMP-9 (PDB ID: 1GKC), showing ligand accommodation within the catalytic pocket.



**Figure 14:** Two-dimensional (2D) interaction diagram illustrating key residues (TYR A:423, LEU A:188, TYR A:393, and ALA A:189) involved in hydrogen bonding, van der Waals, and hydrophobic interactions.

Amoxicillin showed moderate binding affinity toward matrix metalloproteinase-9 (MMP-9) (1GKC), with the best docking score of  $-5.5$  kcal/mol (Figure 3). The 3D docking conformation (Figure 13) demonstrated stable positioning of the ligand within the active site pocket. The 2D interaction analysis (Figure 14) revealed key conventional hydrogen bonding with residues such as TYR A:423 and LEU A:188, along with van der Waals interactions involving TYR A:393 and ALA A:189. Hydrophobic interactions, including alkyl and  $\pi$ -alkyl contacts, further contributed to ligand stabilization. Overall, these interactions suggest a moderate inhibitory potential of amoxicillin against MMP-9, which may influence extracellular matrix degradation associated with ECC progression.

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Category	Target Protein	Biological Role in ECC	PDB ID (RCSB)	Organism	Ligand	PubChem CID	Binding Energy (kcal/mol)
Bacterial Virulence	Glucosyltransferase B (GtfB)	Biofilm formation and EPS synthesis in <i>S. mutans</i>	8FK4	<i>Streptococcus mutans</i>	Amoxicillin	33613	-6.8
Host Immune Mediator	Interleukin-6 (IL-6)	Pro-inflammatory cytokine in ECC progression	1ALU	Homo sapiens	Amoxicillin	33613	~-6.4
Host Immune Mediator	Tumor Necrosis Factor (TNF)	Mediates inflammation and tissue destruction	Model (UniProt-based)	Homo sapiens	Amoxicillin	33613	-6.4
Host Immune Mediator	Interleukin-1 $\beta$ (IL-1 $\beta$ )	Key cytokine in pulp inflammation and ECC severity	8C3U	Homo sapiens	Amoxicillin	33613	-6.4
Tissue Destruction Marker	Matrix Metalloproteinase-9 (MMP-9)	Collagen degradation and dentin breakdown	1GKC	Homo sapiens	Amoxicillin	33613	-5.5

**Table 1: Selected Protein Targets (RCSB PDB) and Ligand (PubChem) for Molecular Docking**

Amoxicillin demonstrated moderate binding affinity across all selected targets, with binding energies ranging from -6.8 to -5.0 kcal/mol. The strongest interaction was observed with GtfB (-6.8 kcal/mol), indicating potential inhibition of *Streptococcus mutans* biofilm formation. Comparable binding was noted with inflammatory mediators such as IL-6, TNF, and IL-1 $\beta$  (~-6.4 kcal/mol), suggesting that amoxicillin may also interact with host immune pathways. The interaction with MMP-9 (-5.5 kcal/mol) indicates a possible modulatory effect on extracellular matrix degradation, though weaker compared to bacterial targets. These findings suggest that while amoxicillin primarily targets bacterial virulence factors in ECC, it may also exhibit secondary interactions

with host immune mediators, potentially influencing inflammatory responses. In pediatric patients, antibiotic use could therefore contribute not only to microbial reduction but also to modulation of cytokine activity and tissue breakdown pathways. However, these interactions are moderate, indicating that clinical effects are likely indirect and require cautious interpretation, emphasizing the need for rational antibiotic use to avoid unnecessary immune interference and resistance development.

**DISCUSSION**

## Evaluation Of Amoxicillin With Host Immune Mediators And Streptococcus Mutans Virulence Proteins Associated With Early Childhood Caries - An In-Silico Study

This study evaluated the interaction of amoxicillin with key bacterial virulence factors and host immune mediators implicated in early childhood caries (ECC), revealing moderate yet consistent binding affinities across all targets. The strongest interaction was observed with *Streptococcus mutans* glucosyltransferase B (GtfB) (-6.8 kcal/mol), followed by host cytokines including IL-6, TNF, and IL-1 $\beta$  (~-6.4 kcal/mol), and MMP-9 (-5.5 kcal/mol). These findings suggest that amoxicillin may exert both antimicrobial and potential immunomodulatory effects, aligning with emerging evidence on host-pathogen interactions in ECC.

The strong binding of amoxicillin to GtfB supports its primary antibacterial role in inhibiting biofilm formation, a critical step in ECC progression. This observation is consistent with findings by Alarcón-Sánchez et al.[17], who demonstrated that increased bacterial load in ECC is associated with elevated inflammatory cytokines. By targeting GtfB, amoxicillin may reduce extracellular polysaccharide synthesis, thereby limiting bacterial colonization and indirectly attenuating host inflammatory responses. This dual effect reinforces the importance of targeting virulence factors rather than solely bacterial survival.

This study also demonstrated moderate binding of amoxicillin with IL-6, TNF, and IL-1 $\beta$ , which are central mediators of inflammation. Obaji et al. [18] reported that IL-6 and TNF- $\alpha$  levels are strongly correlated with infection severity in pediatric populations. The observed docking interactions in our study suggest that amoxicillin may interact with these cytokines at a molecular level, potentially influencing their activity. Although antibiotics are not traditionally considered immunomodulators, such interactions may partly explain the clinical reduction in inflammation observed following antibiotic therapy in severe dental infections. Similarly, the interaction with IL-1 $\beta$  (PDB ID: 8C3U) highlights a potential role of amoxicillin in modulating pulp inflammation. [19,20] The docking results showing stable hydrogen bonding and hydrophobic interactions with IL-1 $\beta$  suggest that amoxicillin may interfere with cytokine signaling pathways, potentially reducing inflammatory tissue damage. This is particularly relevant in pediatric patients, where exaggerated immune responses can accelerate disease progression.[21]

The findings related to TNF further support this concept. TNF is a key cytokine involved in tissue destruction and

immune regulation. The moderate binding affinity (-6.4 kcal/mol) observed in this study aligns with the work of Lusyati et al.[22], who reported variability in cytokine responses in children due to immune immaturity. The interaction of amoxicillin with TNF may therefore have differential effects depending on the developmental stage of the immune system.[23] This highlights the complexity of antibiotic use in pediatric populations, where immune modulation may occur alongside antimicrobial action.

In addition to cytokines, the interaction of amoxicillin with MMP-9 suggests a possible role in modulating extracellular matrix degradation. MMP-9 is involved in collagen breakdown and dentin destruction during ECC progression. The moderate binding observed in this study is consistent with the findings of Tjäderhane[24] [24] et al., who emphasized the role of inflammation in craniofacial and oral tissue remodeling. By potentially inhibiting MMP-9 activity, amoxicillin may contribute to limiting tissue destruction, although this effect appears less pronounced compared to its antibacterial activity.

However, the broader implications of antibiotic use on pediatric immunity must be carefully considered. Syam et al. [25] demonstrated that early-life exposure to antibiotics such as amoxicillin can alter gut microbiota and impact immune development. While our docking results suggest beneficial interactions with immune mediators, systemic effects of antibiotics may disrupt microbial balance and immune maturation.[26] This duality underscores the importance of rational antibiotic prescribing, particularly in children with developing immune systems.

Furthermore, the role of the host-microbiome-immune axis in ECC has been highlighted by Khan et al. [27], who emphasized that disease progression is not solely driven by bacteria but also by host immune responses. The present study supports this concept by demonstrating that amoxicillin interacts not only with bacterial targets but also with host proteins involved in inflammation. Similarly, Xie et al. reported that cytokines such as IL-10 and IL-17 serve as biomarkers in pediatric oral diseases, reinforcing the importance of immune modulation in disease management.[28]

Overall, the integration of docking results with existing literature suggests that amoxicillin may have a multifaceted role in ECC management, combining antimicrobial activity with potential modulation of host

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immune pathways. However, the moderate binding affinities observed indicate that these immunomodulatory effects are likely indirect and may not translate into strong clinical inhibition of cytokine activity. Therefore, antibiotics should be considered adjuncts rather than primary modulators of inflammation in ECC.

A strength of this study is the comprehensive evaluation of both bacterial and host targets, providing a holistic understanding of ECC pathogenesis. However, the primary limitation lies in its in-silico nature, which does not account for complex in-vivo pharmacokinetics and immune interactions. Future studies should incorporate in-vitro and clinical validation, along with multi-omic approaches, to better elucidate the immunomodulatory effects of antibiotics in pediatric populations.

### CONCLUSION

The present in-silico study demonstrates that amoxicillin exhibits moderate binding affinity toward both Streptococcus mutans virulence protein (GtfB) and key host immune mediators, including IL-6, TNF, IL-1 $\beta$ , and MMP-9. The strongest interaction with GtfB highlights its primary antibacterial role in inhibiting biofilm formation, a critical factor in early childhood caries (ECC) progression. Concurrent interactions with inflammatory cytokines and matrix-degrading enzymes suggest a potential secondary role in modulating host immune responses and tissue destruction pathways. However, these interactions are moderate, indicating that the immunomodulatory effects of amoxicillin are likely indirect. Collectively, the findings support the concept that ECC is driven by a complex interplay between microbial virulence and host immunity, and emphasize the need for judicious antibiotic use in pediatric patients to balance antimicrobial efficacy with immune system integrity.

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