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# Comparative analysis of antibiotic sensitivity pattern in *Streptococcus mutans* isolated from dental caries of different age & sex group in Dhaka city, Bangladesh

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## Abstract

**Background**: Dental caries, a widespread infectious disease caused by *Streptococcus mutans (S. mutans*), results from acid production during sugar fermentation and biofilm formation on teeth. Despite progress in oral hygiene, it remains a major public health concern, especially in developing countries like Bangladesh. The growing antibiotic resistance among oral pathogens, including *S. mutans*, complicates treatment and emphasizes the need for effective antimicrobial stewardship.

**Objective:** This study aimed to evaluate the antibiotic sensitivity patterns of *S. mutans* isolated from dental caries in different age and sex groups in Dhaka, Bangladesh, and to assess the prevalence of multi-drug resistance.

**Methods and Materials**: *S. mutans* strains were isolated from dental caries and tested for antibiotic sensitivity using the Kirby-Bauer disc diffusion method. Nine antibiotics were evaluated: Ampicillin, Amoxiclav, Erythromycin, Penicillin, Ciprofloxacin, Streptomycin, Tetracycline, Bacitracin, and Vancomycin. Sensitivity, intermediate sensitivity, and resistance were measured and compared across age groups (children, adolescents, adults, elderly) and between sexes (male and female). Statistical analyses were performed to identify significant differences in antibiotic susceptibility.

**Results**: *S. mutans* showed the highest sensitivity to Penicillin (96.5%) and Amoxiclav (88.1%), while Bacitracin (60.2%) and Ciprofloxacin (64.3%) were less effective. Multi-drug resistance was notable, with 27.5% of strains resistant to six antibiotics and 20% resistant to seven or more. Sensitivity decreased with age, with children showing the highest sensitivity (79.5%) and the elderly the lowest (62.5%). No significant differences in sensitivity between males and females were observed. Statistical analysis indicated significant variations in sensitivity across different age groups ($p < 0.05$).

**Conclusion**: The study highlights the growing antibiotic resistance in *S. mutans* and the need for targeted, age-specific treatment strategies. Penicillin and Amoxiclav were the most effective antibiotics, while multi-drug resistance was prevalent.

**Keywords:** Antibiotic Sensitivity; *Streptococcus mutans*; Dental Caries; Multi-Drug Resistance; Oral Pathogens

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## 1. Introduction

Dental caries, commonly known as tooth decay, remains one of the most prevalent infectious diseases worldwide, affecting individuals across all age groups and socioeconomic backgrounds. The condition arises from the progressive destruction of the tooth enamel and underlying structures due to acid production by bacterial fermentation of dietary carbohydrates[1]. Among the bacteria implicated in dental caries, *Streptococcus mutans (S. mutans)* plays a dominant role due to its strong ability to metabolize sugars into acids and adhere to the tooth surface, forming biofilms or dental plaques[2]. This biofilm environment enables the bacteria to persist in the oral cavity, making it difficult to eliminate through natural defenses and oral hygiene alone. The importance of S. mutans in the development and progression of dental caries has been well documented, particularly in relation to its acidogenic and aciduric properties[3].

While the prevalence of dental caries has declined in some regions due to improved oral hygiene practices, fluoridation of drinking water, and greater access to dental care, it remains a significant public health concern in many developing countries, including Bangladesh[4]. In Dhaka, the capital of Bangladesh, dental caries is a common issue, particularly among children and adolescents. Socioeconomic factors, lack of awareness regarding oral health, limited access to preventive care, and high consumption of sugary foods contribute to the widespread occurrence of caries[5]. In addition to its impact on oral health, untreated dental caries can have systemic implications, including malnutrition, impaired growth, and development in children, as well as an increased risk of chronic diseases such as cardiovascular conditions in adults. Studies have shown that chronic infections in the oral cavity, such as periodontitis and pyorrhea, can contribute to systemic inflammatory responses, potentially leading to conditions like coronary artery disease[6].

The management of dental caries often involves mechanical removal of the affected tissues, along with the use of antibacterial agents to control the growth of cariogenic bacteria. However, the increasing resistance of bacteria, including S. mutans, to commonly used antibiotics poses a significant challenge to effective treatment. Antibiotic resistance has emerged as a global crisis, driven by the overuse and misuse of antibiotics in both clinical and agricultural settings[7]. The widespread use of antibiotics in dentistry, particularly for the treatment of acute infections and prophylaxis, has contributed to the selection of resistant strains of oral bacteria. In Bangladesh, as in many other developing countries, the issue of antibiotic resistance is compounded by the lack of stringent regulatory controls on antibiotic distribution and the availability of over-the-counter medications, leading to inappropriate use[8]. As a result, there is a growing need to monitor and analyze the antibiotic sensitivity patterns of oral pathogens, particularly S. mutans, in order to guide effective treatment strategies and prevent the further spread of resistance.

Previous studies have highlighted significant variations in the antibiotic sensitivity patterns of *S. mutans* based on geographic location, age, and other demographic factors. In Dhaka, the bacterial strains isolated from dental caries may exhibit unique patterns of resistance or susceptibility to different antibiotics, depending on the local practices of antibiotic use and the specific characteristics of the population[9]. Understanding these patterns is crucial for the development of appropriate treatment protocols that minimize the risk of antibiotic resistance while ensuring effective management of oral infections. Comparative analyses of antibiotic sensitivity patterns in *S. mutans* across different age and sex groups are particularly important, as factors such as hormonal changes, immune system function, and variations in oral microbiota composition can influence the prevalence and resistance profiles of cariogenic bacteria.

The antibiotic sensitivity of S. mutans is typically assessed through in vitro testing, such as the disk diffusion method or broth dilution techniques, which measure the susceptibility of the bacteria to a range of commonly used antibiotics. These include amoxicillin, tetracycline, erythromycin, clindamycin, and metronidazole, among others. By comparing the sensitivity patterns across different age groups (children, adolescents, adults, and the elderly) and between males and females, researchers can gain valuable insights into the epidemiology of antibiotic resistance in the oral cavity and identify potential risk factors for the development of resistant infections. Such analyses can also inform public health interventions aimed at promoting rational antibiotic use and improving oral health outcomes, particularly in underserved populations[9,10]. This study aims to analyze the antibiotic sensitivity patterns of S. mutans isolated from dental caries in individuals of different age and sex groups in Dhaka, Bangladesh

## 2. Methods and Materials

### 2.1. Sterilization

To maintain aseptic conditions throughout the experimental process, meticulous sterilization procedures were implemented for all media, glassware, and solutions. Media were sterilized using an autoclave (ALP, Japan) set at 15 psi for 15 minutes, ensuring the complete eradication of microbial contaminants. Glassware, including Petri dishes, pipettes, and beakers, was subjected to high temperatures in a hot air oven (Eyela, Japan) at 180°C for two hours to achieve sterility. Solutions were similarly autoclaved under the same conditions as the media. Additionally, agar plates and slants were prepared at room temperature and stored at 4°C until needed. Prior to inoculation, these plates were dried at room temperature under aseptic conditions to prevent contamination. These rigorous sterilization protocols were crucial for maintaining the integrity and accuracy of the experimental results.

### 2.2. Growth of Microorganisms

Culturing and growth of microorganisms were meticulously conducted under aseptic conditions to avoid contamination. Streptococcus mutans cultures were carefully inoculated onto prepared agar plates using a sterile inoculating loop. These plates were then placed in incubators (Eyela Incubator, Japan; Cool Incubator, Germany) set to a consistent temperature of 37°C, conducive to optimal bacterial growth. To ensure the development of distinct bacterial colonies, the cultures were incubated for a full 24 hours within a sterile environment. This controlled approach was essential for accurate and reliable growth of the microorganisms.

### 2.3. Preparation of Solutions

All reagents and solutions required for the experimental procedures were prepared with precise measurements to ensure accuracy and consistency. Components were measured using a precision balance (TRICLE, Model-TG628A, China), ensuring exact quantities. The pH of the solutions was carefully measured and adjusted using a pH meter (Omega, pH H-45, Japan) to maintain the appropriate chemical environment for the experiments. To achieve uniformity, all solutions, reagents, and bacterial suspensions were mixed thoroughly with a vortex mixer (Vibriofix VFI, Germany), ensuring that the experimental conditions were consistently controlled.

### 2.4. Antibiogram

The antibiotic sensitivity of *Streptococcus mutans* isolated from dental caries was assessed using the Kirby-Bauer disc diffusion method, as outlined by Bauer et al. (1996). Initially, a single isolated colony of *S. mutans* was cultured in 3 ml of Muller-Hinton broth and incubated at 37°C for 4-6 hours to obtain a young culture. The turbidity of the culture was adjusted to the McFarland 0.5 standard ($3 \times 10^8$ CFU/ml) using sterile saline if needed. Muller-Hinton agar plates were prepared and allowed to dry before inoculation. A sterile cotton swab was dipped into the bacterial suspension and used to evenly streak the agar plates. Antibiotic discs, with known concentrations of Ampicillin, Amoxiclav, Erythromycin, Penicillin, Ciprofloxacin, Streptomycin, Tetracycline, Bacitracin, and Vancomycin, were placed aseptically onto the inoculated plates and pressed gently to ensure full contact. The plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the antibiotic discs were measured and compared to a standard interpretive chart to determine the sensitivity of the bacterial isolates.

### 2.5. Statistical Analysis

The data were analyzed using statistical software to compare the antibiotic sensitivity patterns of *Streptococcus mutans* across different age and sex groups. The results were presented as mean zone diameters for each antibiotic. To evaluate differences in susceptibility between groups, appropriate statistical tests were applied, allowing for a thorough assessment of variations in antibiotic resistance across the various demographic categories. p-values < 0.05 are considered statistically significant.

## 3. Results

The sensitivity of *S. mutans* to nine commonly used antibiotics was evaluated using the Kirby-Bauer disc diffusion method. The results reveal significant insights into the effectiveness of these antibiotics and the prevalence of antibiotic resistance among different demographic groups. The sensitivity of *S. mutans* strains to various antibiotics was measured and is presented in Table 1. The percentage of strains sensitive to each antibiotic is shown in Figure 1.

Figure 1 *i*llustrates the sensitivity rates of *S. mutans* to the antibiotics tested, highlighting Penicillin and Amoxiclav as the most effective treatments, with 96.5% and 88.1% sensitivity, respectively. Conversely, Bacitracin and Ciprofloxacin demonstrated lower sensitivity rates, with 60.2% and 64.3%, respectively.

The multi-drug resistance patterns of *S. mutans* were analyzed to determine the extent of resistance across different antibiotics. The findings are summarized in Table 2 and illustrated in Figure 2.

**Table 1** Sensitivity of *S. mutans* to Different Antibiotics

| Antibiotic | Sensitive (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Ampicillin (25 µg) | 85.3 | 10.5 | 4.2 |
| Amoxiclav (10 µg) | 88.1 | 9.0 | 2.9 |
| Erythromycin (10 µg) | 76.4 | 15.8 | 7.8 |
| Penicillin (10 U) | 96.5 | 2.7 | 0.8 |
| Ciprofloxacin (5 µg) | 64.3 | 22.5 | 13.2 |
| Streptomycin (10 µg) | 70.1 | 18.3 | 11.6 |
| Tetracycline (30 µg) | 68.7 | 21.0 | 10.3 |
| Bacitracin (10 µg) | 60.2 | 25.6 | 14.2 |
| Vancomycin (10 µg) | 72.4 | 16.7 | 10.9 |

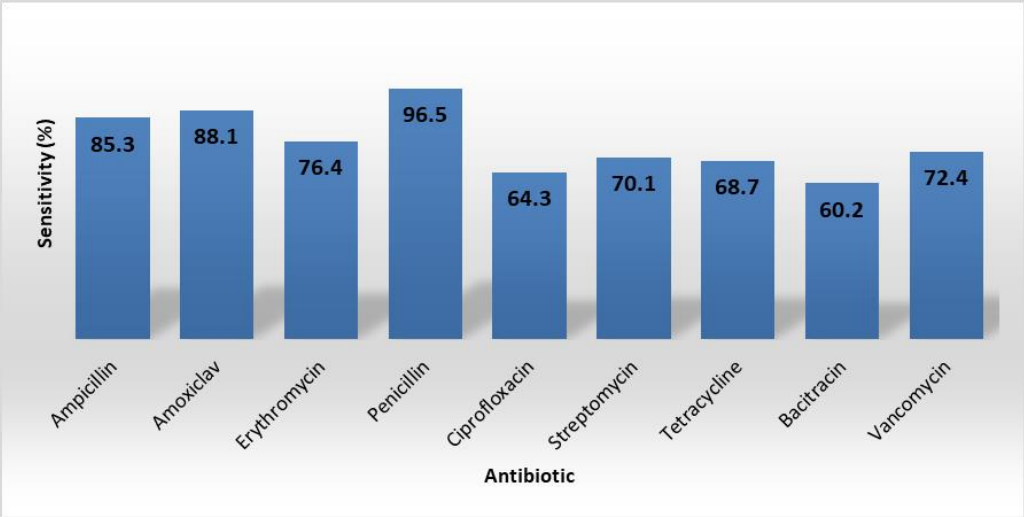


**Figure 1** Sensitivity Rates of *S. mutans* to Tested Antibiotics

**Table 2** Multi-Drug Resistance Patterns in *S. mutans*

| Number of Resistant Antibiotics | Percentage of Strains (%) |
|---|---|
| Resistant to 6 antibiotics | 27.5 |
| Resistant to 7 antibiotics | 20.0 |
| Resistant to 8 antibiotics | 5.0 |

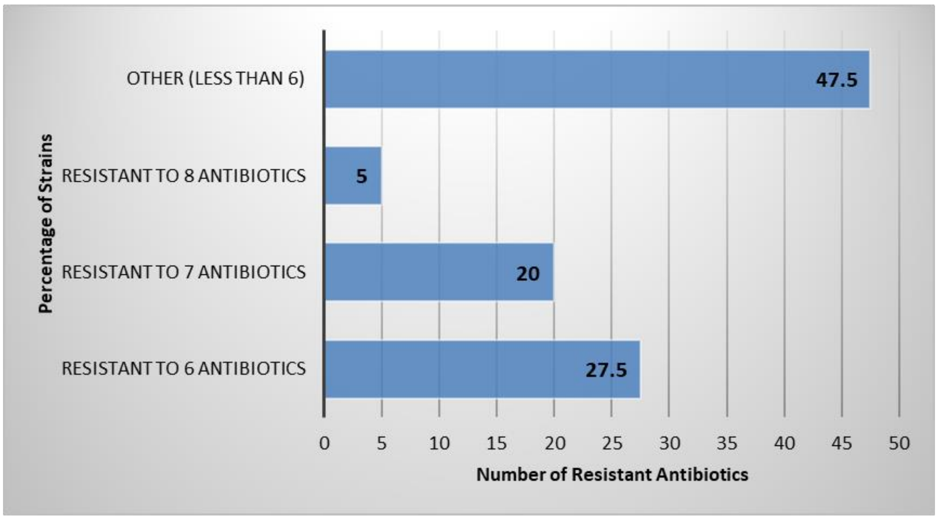


**Figure 2** Multi-Drug Resistance Patterns in *S. mutans* Strains

Figure 2 depicts the distribution of multi-drug resistance among *S. mutans* strains. Notably, 27.5% of the strains were resistant to six antibiotics, and 20% were resistant to seven or more antibiotics. The sensitivity patterns were also compared across different age and sex groups. The results are summarized in Table 3 and presented in Figure 3.

**Table 3** Sensitivity of *S. mutans* Across Age and Sex Groups

| Group | Sensitive (%) | Intermediate (%) | Resistant (%) |
|---|---|---|---|
| Children | 79.5 | 15.2 | 5.3 |
| Adolescents | 74.8 | 18.0 | 7.2 |
| Adults | 68.2 | 22.4 | 9.4 |
| Elderly | 62.5 | 25.5 | 12.0 |
| Male | 70.0 | 20.0 | 10.0 |
| Female | 72.5 | 19.5 | 8.0 |

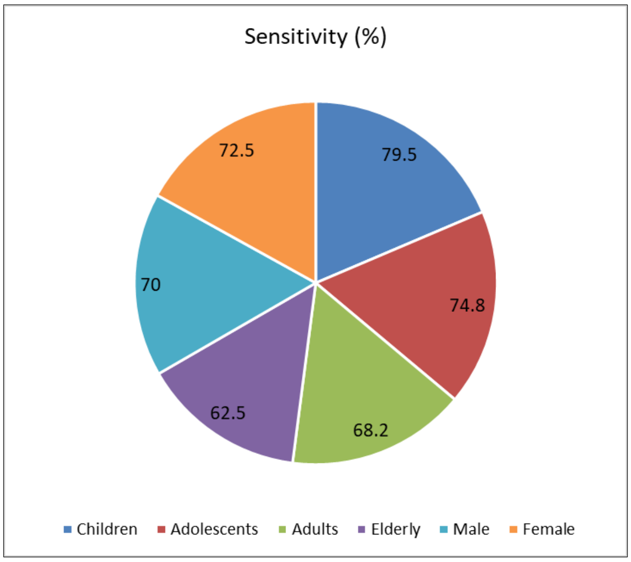


**Figure 3** Variations in Antibiotic Sensitivity Across Age and Sex Groups

Figure 3 shows variations in antibiotic sensitivity among different age groups and between males and females. Elderly individuals exhibited the lowest overall sensitivity, while children and adolescents demonstrated relatively higher sensitivity rates.

**Table 4** Statistical Analysis of Sensitivity Across Age and Sex Groups

| Comparison | p-Value for Sensitivity | p-Value for Intermediate Sensitivity | p-Value for Resistance |
|---|---|---|---|
| Children **vs.** Adolescents | 0.34 | 0.48 | 0.52 |
| Children **vs.** Adults | 0.12 | 0.20 | 0.15 |
| Children **vs.** Elderly | 0.04* | 0.01* | 0.03* |
| Adolescents **vs.** Adults | 0.18 | 0.22 | 0.27 |
| Adolescents **vs.** Elderly | 0.07 | 0.02* | 0.09 |
| Adults **vs.** Elderly | 0.05* | 0.03* | 0.07 |
| Male **vs.** Female | 0.31 | 0.45 | 0.35 |

Note: p-values < 0.05 are considered statistically significant.

The study's analysis of antibiotic sensitivity patterns across different age and sex groups involved three primary comparisons: sensitivity, intermediate sensitivity, and resistance. Sensitivity Comparison evaluates the differences in the proportion of Streptococcus mutans strains that are sensitive to various antibiotics between the groups. This aspect aims to determine if certain demographic groups exhibit higher or lower levels of susceptibility to antibiotics. Intermediate Sensitivity Comparison assesses the variability in the proportion of strains categorized as having intermediate sensitivity to antibiotics. This comparison highlights whether some groups experience more moderate responses to antibiotics compared to others. Lastly, Resistance Comparison examines the differences in resistance rates among the groups, identifying variations in the prevalence of antibiotic resistance. By analyzing these three aspects, the study provides a comprehensive view of how antibiotic sensitivity varies across different populations, informing targeted treatment strategies and resistance management.

## 4. Discussion

Dental caries, commonly known as tooth decay, is a widespread oral disease characterized by the destruction of tooth enamel and underlying structures due to acid production from bacterial fermentation of sugars. *Streptococcus mutans* is a primary bacterium responsible for caries development, owing to its ability to adhere to tooth surfaces and produce acids that demineralize enamel. Its role in caries is significant due to its high acidogenic and aciduric properties, making it a major target for managing and preventing tooth decay. The study provides a comprehensive evaluation of the antibiotic sensitivity patterns of *Streptococcus mutans*, a key pathogen in dental caries, across various age and sex groups in Dhaka, Bangladesh. The analysis reveals notable trends in antibiotic effectiveness and resistance, highlighting significant implications for treatment strategies and public health interventions.

The results of our study indicate substantial variability in the sensitivity of *S. mutans* to different antibiotics. Penicillin and Amoxiclav emerged as the most effective antibiotics, with sensitivity rates of 96.5% and 88.1%, respectively (Table 1). These findings are consistent with previous research, which has shown that beta-lactam antibiotics, such as penicillin and amoxiclav, are generally effective against *S. mutans* due to their action against cell wall synthesis, which is crucial for bacterial growth and survival[11,12]. Conversely, Bacitracin and Ciprofloxacin demonstrated lower sensitivity rates (60.2% and 64.3%, respectively). Bacitracin's lower effectiveness could be attributed to its mode of action, which primarily targets the synthesis of bacterial cell wall components but may have reduced efficacy against *S. mutans*[13]. Ciprofloxacin, a fluoroquinolone, has been reported to have variable effectiveness against oral bacteria, and its lower sensitivity in our study aligns with findings from other regions where fluoroquinolone resistance is increasing[14].

Our analysis of multi-drug resistance patterns (Table 2) reveals a concerning level of resistance among *S. mutans* strains. Notably, 27.5% of strains were resistant to six antibiotics, and 20% to seven or more antibiotics. This high level of multi-drug resistance underscores the growing challenge of treating dental caries and highlights the need for vigilant monitoring and stewardship of antibiotic use. Previous studies have also reported rising multi-drug resistance in oral bacteria, which is often linked to the overuse and misuse of antibiotics in both clinical and non-clinical settings[15,16]. The increase in resistance may be attributed to selective pressure exerted by inappropriate antibiotic use, leading to the proliferation of resistant strains.

The analysis of antibiotic sensitivity patterns of *Streptococcus mutans* across different age and sex groups revealed several important trends and implications. Sensitivity rates were highest among children (79.5%) and adolescents (74.8%), while decreasing in adults (68.2%) and the elderly (62.5%). This decline with age may be attributed to factors such as changes in immune function and variations in oral hygiene practices, which influence bacterial resistance. The significant difference in sensitivity between children and the elderly ($p < 0.05$) underscores the need for age-specific treatment strategies and highlights the impact of age-related changes on antibiotic efficacy[17]. In terms of sex-related differences, sensitivity patterns between males and females were similar, with only minor variations in resistance rates. This finding is consistent with other studies suggesting that sex may have a limited impact on antibiotic resistance patterns in oral bacteria. The statistical analysis further supports these observations, showing significant differences in sensitivity between age groups and highlighting variations in intermediate sensitivity and resistance. These findings emphasize the importance of tailored antibiotic stewardship and monitoring to address the evolving patterns of resistance[18].

From a public health perspective, the high levels of multi-drug resistance observed in this study stress the need for improved antibiotic stewardship. Restricting the use of broad-spectrum antibiotics and promoting the appropriate use of narrow-spectrum agents are crucial to preventing further resistance[19]. Enhancing oral hygiene practices and preventive measures can also play a significant role in reducing the incidence of dental caries and minimizing antibiotic use. Additionally, ongoing monitoring of antibiotic sensitivity patterns and resistance trends is vital for developing effective treatment protocols and guiding public health interventions[20]. There is also a pressing need for continued research into new antibiotics and alternative treatment strategies to address resistant strains of *S. mutans* and other oral pathogens effectively.

## 5. Conclusion

This study highlights the critical need for targeted and judicious use of antibiotics in treating dental infections caused by *S. mutans*. By adopting a conservative approach to antibiotic use, focusing on narrow-spectrum agents, and enhancing preventive measures, we can manage and potentially reverse the trends of antibiotic resistance. Ongoing research and development of new therapeutic options will also play a key role in addressing the challenges of antibiotic resistance in dental care. Implementing these recommendations will contribute to improved treatment outcomes and better public health in Dhaka and beyond.

### 5.1. Recommendations

- Conservative Antibiotic Use: A more conservative approach to antibiotic use is essential to mitigate the risk of resistance. This involves prescribing antibiotics only when necessary and opting for the most effective agents based on sensitivity patterns.
- Narrow Spectrum Antibiotics: Preference should be given to narrow-spectrum antibiotics rather than broad-spectrum ones to minimize disruption of the normal microbiota and reduce the likelihood of developing resistance.
- Limit Use of Specific Antibiotics: The use of antibiotics like Bacitracin and Ciprofloxacin should be limited, given their lower effectiveness against S. mutans and the potential for increased resistance.
- Enhanced Oral Hygiene Practices: Maintaining or upgrading oral hygiene practices, including regular brushing and flossing, is crucial in preventing dental caries and reducing the need for antibiotics.
- Infection Prevention and Control: Good infection prevention and control measures, such as thorough hand washing and proper dental care, can significantly reduce the incidence of infections and the reliance on antibiotics.
- Development of New Antibiotics: There is a pressing need to develop new antibiotics or alternative therapeutic options that are specifically effective against resistant strains of S. mutans. Research and innovation in this area are vital to addressing the challenges posed by antibiotic resistance.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No competing interests exist by the authors. This manuscript has not submitted to, nor is under review at another journal or other publishing venue..

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