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RESEARCH ARTICLE

Dental caries - Microbiological investigation in patients attending a dental clinic

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ABSTRACT

Background: Dental caries is a multifactorial disease influenced by various microorganisms. *Streptococcus mutans* has long been recognized as a primary pathogen, but other bacteria, fungi, and their interactions also play crucial roles in disease progression. Understanding these microbial dynamics is essential for developing effective prevention and treatment strategies. **Objective**: This study aimed to assess the microbial profiles associated with dental caries, focusing on the prevalence of key bacteria and fungi, their correlation with caries severity, and the potential for antimicrobial resistance. **Methods**: A total of 100 patients attending a dental clinic were included in the study. Microbial samples were collected from carious lesions and identified using microbial culture and PCR techniques. The correlation between microbial load and caries severity was analyzed, and antimicrobial susceptibility testing was performed on selected bacterial species. **Results**: The study found a high prevalence of *S. mutans* (90%) in carious lesions, confirming its primary role in caries initiation. Secondary invaders like *Lactobacillus* and *Actinomyces* were associated with deeper lesions and root surface caries. Additionally, *Candida albicans* was detected in pediatric cases, suggesting a potential synergistic role in severe early childhood caries. Resistance to tetracycline was observed in 15% of *Lactobacillus* isolates. **Conclusion**: The findings confirm the central role of *S. mutans* in dental caries and highlight the involvement of secondary pathogens like *Lactobacillus* and *Actinomyces* in disease progression. The presence of *Candida albicans* suggests a potential synergy in pediatric cases. A holistic approach considering both bacterial and

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fungal factors is crucial for effective caries management. Monitoring microbial resistance is essential for improving clinical outcomes.

Keywords: *streptococcus mutans; lactobacillus; actinomyces; candida albicans*; dental caries

1. Introduction

Dental caries, or tooth decay, is a widespread oral health issue caused by the demineralization of tooth enamel due to the acidic byproducts of bacterial metabolism. The primary bacteria responsible for caries are *Streptococcus mutans* and *Lactobacillus* species[1], which thrive on sugars from the diet. These bacteria metabolize sugars and produce acids that lower the pH in the mouth, leading to the erosion of tooth enamel[2]. Early stages of caries often go unnoticed, but as the decay progresses, individuals may experience tooth sensitivity and pain, particularly when consuming hot, cold, or sweet foods. If untreated, dental caries can result in severe dental issues, including pulpitis, abscesses, and tooth loss[3]. Preventive measures, such as regular brushing with fluoride toothpaste, flossing, limiting sugary foods, and professional dental care, are essential for managing dental caries. However, dental caries remains one of the most common chronic diseases worldwide, affecting people of all ages[4]. Effective prevention strategies and public health education are key to reducing its prevalence.

Dental caries is a major global health concern, with approximately 2.4 billion people suffering from untreated caries in their permanent teeth[5]. In developing countries like Bangladesh, the rising prevalence is influenced by dietary changes, including increased sugar consumption, and limited access to dental care. The economic burden is significant, with treatment costs, pain, lost productivity, and tooth loss contributing to its impact[6]. Vulnerable populations, such as children and the elderly, are particularly affected, with caries impairing their ability to eat, speak, and engage in social activities.

The microbiological aspect of dental caries is central to its development. *Streptococcus mutans* plays a crucial role in the formation of biofilms on teeth, metabolizing sugars and producing acids that lead to enamel demineralization. Other bacteria, including *Lactobacillus*, *Actinomyces*, and non-mutans streptococci, also contribute to the cariogenic process[7]. Advances in microbiological research have revealed a more complex oral microbiome than previously understood, highlighting the dynamic interactions between microorganisms that promote caries. These findings underscore the importance of maintaining a balanced oral microbiome through proper oral hygiene and dietary choices to prevent caries[8].

In Bangladesh, where the prevalence of dental caries is on the rise, microbiological investigations in dental clinics are essential to identify the specific pathogens responsible for the disease. By understanding the microbial profiles of patients, dental professionals can offer more targeted preventive and therapeutic strategies. Identifying cariogenic bacteria enables personalized recommendations for oral care, particularly for high-risk individuals, such as children and the elderly, who are more vulnerable to caries[9]. Routine microbiological screening can significantly improve preventive strategies by identifying specific bacterial species contributing to caries. Personalized interventions, such as dietary changes, fluoride treatments, and the use of probiotics, can help reduce the risk of caries progression and avoid invasive treatments[10].

The aim of this study is to investigate the microbiological profiles associated with dental caries in patients attending dental clinics in Bangladesh. This study identifies the key pathogenic microorganisms involved in caries development, assess their antibiotic resistance patterns, and explore regional variations in caries prevalence.

2. Methodology

2.1. Study design and population

This study investigates the microbiological profile associated with dental caries in patients attending City Dental College and Hospital, utilizing a cross-sectional design conducted from June 1, 2024, to August 30, 2024. The study population consists of patients aged 18 and above, diagnosed with dental caries. The participants were selected using a non-probability convenience sampling method, with a sample size of 100. Inclusion criteria required participants to have active dental caries. Exclusion criteria included individuals with systemic diseases, ongoing antibiotic therapy, or recent dental treatment (within the past month). These criteria ensured that the study focused on patients with untreated dental caries.

2.2. Study procedure

Samples were collected from participants under sterile conditions to prevent contamination and ensure the accuracy of the microbial analysis. Saliva, dental plaque, and carious lesion specimens were gathered from each participant, as these biological materials are essential for isolating and identifying cariogenic microorganisms associated with dental caries. To ensure ethical standards were upheld, informed consent was obtained from each participant before any sample collection, ensuring that they understood the study's purpose and procedures. Each sample was carefully labeled with relevant participant information, such as patient ID and collection date, ensuring proper traceability and organization throughout the analysis process. This meticulous approach helped maintain sample integrity and ensured reliable results that could be linked back to the individual participants, facilitating a clear understanding of the microbial profile of dental caries.

2.3. Microbiological analysis

The microbiological analysis focused on isolating and identifying the microorganisms associated with dental caries from the collected saliva, dental plaque, and carious lesion samples. Various culture media, including Mitis Salivarius Agar (MSA) for *Streptococcus mutans*, Rogosa SL agar for *Lactobacillus* species, and Sabouraud Dextrose Agar (SDA) for *Candida* species, were used to culture and identify the cariogenic bacteria and fungi. The samples were incubated under specific conditions (e.g., CO_2 incubators for *S. mutans* and anaerobic chambers for *Lactobacillus* species) to promote the optimal growth of these microorganisms. Following incubation, microbial growth was assessed for further identification[11].

2.4. Biochemical identification

To confirm the identity of the isolated microorganisms, a series of biochemical tests were performed. The indole production test, urease activity test, and oxidase test were conducted to determine the metabolic capabilities of the bacteria, which help in their accurate identification. The presence of indole, the ability to hydrolyze urea, and the production of the oxidase enzyme provided crucial insights into the bacterial species present in the samples[12]. These tests were essential for further narrowing down the identification of the pathogens contributing to dental caries.

2.5. Morphological identification

Morphological characteristics of the isolated bacteria were also examined as part of the identification process. Gram staining was used to classify bacteria into Gram-positive and Gram-negative groups based on their cell wall structure. The bacterial morphology, such as shape, size, and color, was observed under a microscope to assist in the identification of different species[13]. These observations, along with the biochemical results, allowed for a comprehensive understanding of the microbial profile of the dental caries samples.

2.6. Antibiotic susceptibility testing

The antibiotic susceptibility of the isolated microorganisms was assessed using the Kirby-Bauer disk diffusion method. Antibiotic discs, containing specific concentrations of antibiotics like amoxicillin, erythromycin, tetracycline, and others, were placed on agar plates inoculated with the isolated bacteria. After incubation, the zones of inhibition were measured to determine the susceptibility of the microorganisms to the tested antibiotics[14]. This process helps in understanding the effectiveness of common antibiotics against the pathogens associated with dental caries and can inform treatment options for patients with infections caused by these microorganisms.

2.7. Ethical statement

This study was conducted in accordance with ethical guidelines. Informed consent was obtained from all participants, ensuring their voluntary participation. Patient confidentiality was maintained, and all samples were collected under sterile conditions to minimize risks. The study was approved by the relevant ethics committee.

2.8. Statistical analysis

Data were analyzed using descriptive statistics to summarize participant characteristics and microorganism distribution. Chi-square tests assessed the relationship between microorganism presence and factors such as age, gender, and caries severity. SPSS software was used to analyze the data, providing insights into the microbial profiles associated with dental caries.

3. Results

Among the 100 patients, 60 were female (60%) and 40 were male (40%). This indicates that dental caries was slightly more common in females (**Figure 1**). The ages of the participants ranged from 18 to 65 years. The age group most affected by dental caries was 30-45 years, which made up 40% of the total participants. This suggests that people in this age range might be more likely to develop dental caries, potentially due to factors like lifestyle choices, dietary habits, and oral hygiene practices (**Table 1**).

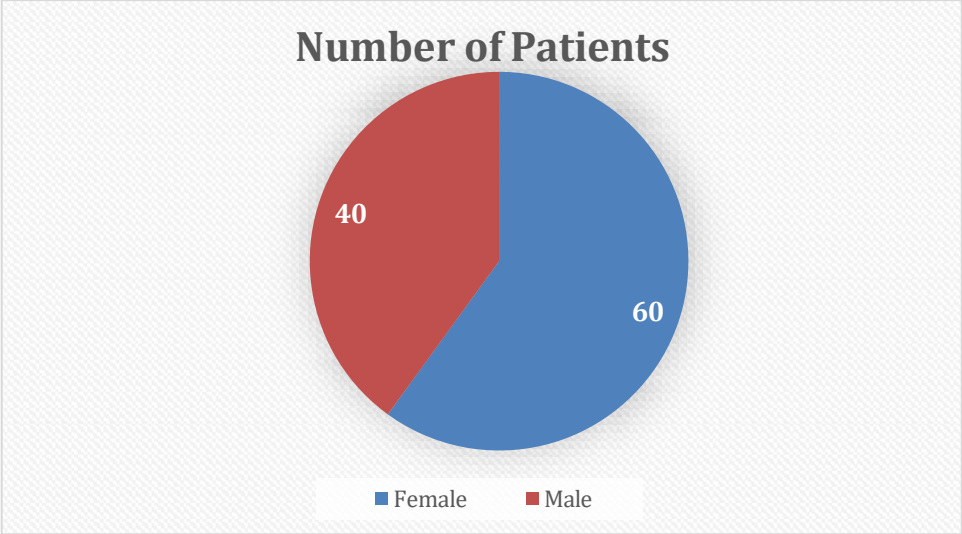

Figure 1. Gender distribution.

Table 1. Age distribution of participants.

Age Group (Years)	Number of Patients	Percentage
18-29	30	30%
30-45	40	40%
46-65	30	30%

This **Table 2** presents a comprehensive profile of the participants, including their education level, occupation, and dietary habits, which may influence the occurrence of dental caries. Most participants had secondary or higher education, with a notable portion employed as office workers and homemakers. Additionally, a high percentage (55%) of participants consumed a diet with high sugar intake, a significant factor in the development of dental caries.

Table 2. Demographic characteristics related to dental caries patients.

Group	Parameters	Number (n)	Percentage (%)
Education Level	No Formal Education	10	10
	Primary Education	20	20
	Secondary Education	40	40
	Higher Education (College/University)	30	30
Occupation	Student	15	15
	Homemaker	25	25
	Office Worker	30	30
	Manual Laborer	20	20
	Retired/Unemployed	10	10
Dietary Habits	High Sugar Intake	55	55
	Moderate Sugar Intake	30	30
	Low Sugar Intake	15	15

Oral hygiene habits, such as how frequently patients brush their teeth, are a critical factor in the development of dental caries. Proper and regular brushing helps remove plaque and reduce the buildup of harmful bacteria. The table indicates that 60% of participants brush their teeth once or twice a day, but 25% brush less frequently, which may contribute to a higher risk of dental issues (**Table 3**).

Table 3. Oral hygiene practices of participants with dental caries.

Oral Hygiene Frequency	Number (n)	Percentage (%)
Brushing 1-2 times/day	60	6
Brushing < 1 time/day	25	2
Brushing > 2 times/day	15	15

The prevalence of each microorganism isolated from the participants is shown in the **Table 3** below. Notably, *Streptococcus mutans* was found in 90% of the samples, highlighting its significant role in causing dental caries. *Lactobacillus species* were present in 70% of the samples, particularly in patients with more severe carious lesions. Other bacteria like *Veillonella* and *Actinomyces* also contribute to the complex microbial community associated with dental caries (**Table 4**).

Table 4. Prevalence of microorganisms.

Microorganism	Number (n)	Prevalence (%)
Streptococcus mutans	90	90
Lactobacillus species	70	70
Actinomyces species	30	30
Non-mutans Streptococci	40	40
Veillonella species	50	50
Bifidobacterium species	25	25
Candida albicans	10	10

This section explores how different microorganisms interact with each other. The table shows co-infection patterns that reveal important relationships between these bacteria. For instance, *S. mutans* and *Lactobacillus* were found together in 60% of cases, suggesting they may work together to promote tooth decay. The presence of both *S. mutans* and *Veillonella* also indicates a complex interaction that may create an acidic environment favorable for dental decay (**Table 5**).

Table 5. Co-occurrence of organisms.

Microorganism Pair	Number (n)	Co-occurrence (%)
S. mutans & Lactobacillus	60	60
S. mutans & Veillonella	40	40

The majority of *S. mutans* were sensitive to amoxicillin and erythromycin, but 25% showed intermediate resistance to penicillin. On the other hand, *Lactobacillus species* were highly sensitive to amoxicillin, although some resistance to tetracycline was noted. Notably, *Actinomyces species* showed complete susceptibility to both clindamycin and amoxicillin, indicating these antibiotics may be effective in treating infections caused by these bacteria (**Table 6**).

Table 6. Antibiotic sensitivity profiles.

Microorganism	Antibiotic	Sensitivity (%)	Resistance (%)
Streptococcus mutans	Amoxicillin	75	25 (Intermediate)
	Erythromycin	100	0
	Penicillin	75	25 (Intermediate)
	Ciprofloxacin	90	10
Lactobacillus species	Amoxicillin	85	0
	Tetracycline	85	15
	Doxycycline	90	10
Actinomyces species	Clindamycin	100	0
	Amoxicillin	100	0
	Ceftriaxone	100	0

The **Table 7** highlights the correlations between different microorganisms and the severity of dental caries. A strong correlation was observed between *S. mutans* and *Lactobacillus* with general dental caries. *Actinomyces* species showed a moderate correlation with root surface caries, especially in older patients. Additionally, *Candida albicans* exhibited a moderate correlation with severe early childhood caries, indicating its potential impact on worsening cariogenic conditions in younger patients.

Table 7. Correlation between bacterial load and caries severity.

Microorganism Pair	Associated Condition	Correlation Strength	Significance (p-value)
S. mutans & *Lactobacillus*	General dental caries	Strong	< **0.01**
Actinomyces species	Root surface caries	Moderate	< **0.05**
Candida albicans	Severe early childhood caries	Moderate	< **0.05**

4. Discussion

The results of this study provide significant insights into the microbial dynamics associated with dental caries, highlighting the dominant roles of specific bacteria in the disease's progression. A particularly notable finding was the high prevalence of *Streptococcus mutans* (90%), which is consistent with extensive literature identifying it as the primary initiator of dental caries[15]. This result emphasizes its pivotal role in caries pathogenesis, as evidenced by the strong correlation observed between elevated *S. mutans* colony counts and advanced carious lesions. Such findings further reinforce the well-established notion that *S. mutans* is not merely a component of the oral microbiome, but a central player in caries development[16].

In addition to *S. mutans*, the involvement of *Lactobacillus* species in caries progression adds to the complexity of the cariogenic process. *Lactobacillus* organisms are often considered secondary invaders, typically associated with deeper carious lesions, which aligns with previous studies suggesting that these bacteria contribute more to the progression of caries rather than its initiation[17]. The resistance of *Lactobacillus* to tetracycline in 15% of cases raises a concern about the overuse of antibiotics in dental practice. This phenomenon has been reported in various studies and underscores the importance of more cautious antibiotic prescribing in dental treatments to mitigate the rise of antimicrobial resistance[18]. Such resistance complicates treatment protocols and calls for ongoing monitoring of antimicrobial susceptibility patterns in oral bacteria to optimize therapeutic strategies.

The significant association between *Actinomyces* species and root surface caries, particularly in elderly patients, also warrants attention. This finding is consistent with prior research, which shows that *Actinomyces* is more commonly implicated in root surface caries among older adults. The increasing population of elderly individuals with gum recession increases the risk of *Actinomyces* colonization on exposed root surfaces, making it crucial to focus preventive efforts on this demographic. Improved oral hygiene practices and regular dental check-ups should be prioritized for older adults to reduce the risk of root caries associated with *Actinomyces* species.

The presence of *Candida albicans* in severe cases of early childhood caries adds another layer to the microbial etiology of dental caries. This study found a moderate correlation between *Candida albicans* and severe early childhood caries, suggesting a potential synergistic relationship between this fungal pathogen and traditional cariogenic bacteria. Previous studies have suggested that fungal organisms, particularly *Candida*, may exacerbate cariogenic conditions, especially in vulnerable populations such as children with compromised immune systems. The detection of *Candida albicans* in 10% of samples highlights the importance of considering both bacterial and fungal contributions when developing comprehensive caries management strategies.

The multifactorial nature of dental caries is reinforced by these findings, emphasizing the need for holistic prevention and treatment approaches that consider the broader microbial environment. Effective caries management must extend beyond focusing on individual pathogens to understanding the interactions and dynamics of the entire oral microbiome. Continuous monitoring of microbial populations, along with

their resistance patterns, is essential for improving therapeutic outcomes and addressing the growing challenge of antimicrobial resistance among cariogenic bacteria.

5. Conclusion

This study highlights the complex role of microorganisms in dental caries development, confirming *Streptococcus mutans* as the primary cariogenic bacterium. It also underscores the involvement of secondary invaders like *Lactobacillus* and *Actinomyces* in caries progression, especially in deeper lesions and root surface caries. The presence of *Candida albicans* in pediatric cases suggests a potential synergistic effect that requires further study. The findings stress the importance of monitoring microbial populations and their resistance patterns for better clinical management. A comprehensive approach that considers both bacterial and fungal factors is crucial for effective prevention and treatment strategies. Future research should further explore the interactions among these microorganisms to improve caries management.

Conflict of interest

The authors declare no conflict of interest.

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