International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Antibiotic Susceptibility Patterns of Periodontal Bacteria in Diabetic vs Non - Diabetic Individuals

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Abstract: ***Introduction: Periodontitis is a chronic inflammatory disease that compromises the supporting structures of teeth, often exacerbated in individuals with diabetes mellitus, a metabolic disorder marked by chronic hyperglycemia. This study aimed to analyse the susceptibility patterns of periodontitis - causing microorganisms in diabetic and non - diabetic patients, with a focus on antibiotic resistance. Methodology: Dental plaque specimens were collected from 100 patients with varying dental health statuses, including those with diabetes and dental caries. Bacterial isolates from the plaque samples were cultured on different media, identified based on morphological and biochemical tests, and subjected to antibiotic susceptibility testing using the Kirby - Bauer method. Statistical analysis: The study utilized descriptive statistics to determine the prevalence of bacterial isolates and their resistance patterns to antibiotics. The frequency of resistance among bacterial species was compared between diabetic and non - diabetic groups, and potential factors influencing these patterns were analysed. Results: Out of the 100 dental plaque samples, 70 exhibited bacterial growth. Gram - positive bacteria, particularly Porphyromonas gingivalis and Tannerella forsythia, were predominant. The antibiotic susceptibility test revealed high resistance rates to amoxicillin and cefotaxime among Gram - positive isolates. The findings highlight significant antibiotic resistance among periodontitis - causing bacteria, particularly in diabetic patients, underscoring the need for targeted treatment strategies and further research to explore factors influencing microbial susceptibility.***

Keywords: Susceptibility, Hyperglycemia, Antibiotic resistance, Diabetes mellitus

1. Introduction

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the teeth, leading to progressive attachment loss and bone destruction. It is caused by a complex interplay of microbial infection and the host's immune response. Various microorganisms, particularly Gram - negative anaerobes such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, have been implicated in the pathogenesis of periodontitis. The presence and virulence of these microorganisms can significantly influence the progression and severity of the disease (1).

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. It is well - established that diabetes increases the risk of developing periodontitis and exacerbates its severity. This bidirectional relationship is thought to be mediated through several mechanisms, including altered immune response, increased inflammatory mediators, and changes in the subgingival microbiota. These patients experience dysregulation in their immune responses, which compromises their ability to fend off bacterial infections. Neutrophils and macrophages, essential components of the immune defense against periodontal pathogens, are less effective in diabetics (2). This results in higher susceptibility to infections and greater colonization by pathogenic bacteria. Chronic hyperglycemia in diabetic patients leads to a heightened inflammatory state, characterized by increased levels of pro - inflammatory cytokines such as IL - 1β and TNF - α. These inflammatory mediators contribute to the destruction of periodontal tissues and can affect the balance of microbial communities, fostering an environment where pathogenic bacteria thrive. The oral microbiome of diabetic individuals is often more pathogenic compared to that of non - diabetics (3). The increased prevalence of key periodontal pathogens, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, in diabetics suggests a microbial environment predisposed to more aggressive periodontal disease.

The subgingival microbiota in diabetic patients is distinct from that in non - diabetic individuals, with a higher prevalence of certain pathogenic species and a greater microbial load. This altered microbial environment may contribute to the increased susceptibility and severity of periodontitis observed in diabetic patients. Additionally, diabetes is associated with impaired wound healing and reduced response to periodontal therapy, further complicating disease management (4)

Diabetes increases the risk and severity of periodontitis, while periodontitis can exacerbate the metabolic control of diabetes. This interplay is of significant concern in understanding the susceptibility and resistance patterns of periodontitis - causing microorganisms in diabetic versus non - diabetic patients (5).

1.1 Pathogenesis of Periodontitis

The host immune system responds to the bacterial challenge by recruiting inflammatory cells, such as neutrophils, macrophages, and lymphocytes, to the site of infection. While this response aims to eliminate the pathogens, it also results in the release of pro - inflammatory cytokines (e. g., IL - 1β, TNF - α, IL - 6) and enzymes (e. g., matrix

metalloproteinases) that degrade the extracellular matrix and bone (6).

Understanding the susceptibility patterns of periodontitis - causing microorganisms is crucial for effective treatment and management of the disease. Antibiotic therapy is often employed as an adjunct to mechanical debridement in the treatment of periodontitis, particularly in severe or refractory cases. However, the emergence of antibiotic - resistant strains poses a significant challenge to successful treatment outcomes. Diabetic patients, due to their altered immune status and unique microbial profile, may exhibit different susceptibility patterns compared to non - diabetic patients (7).

2. Materials and methods

In the current study, dental samples were collected from patients suffering from diabetes. Hundred (100) dental plaque specimens were obtained from patients with varying dental health statuses and suffering from dental caries. Medical histories were gathered from all participants after obtaining their oral consent using a questionnaire, which included information on smoking status, antibiotic use in the past two weeks, and the use of toothpaste and dental floss (8).

2.1 Culturing and identification of isolated periodontitis strains

All dental plaque swabs were cultured to detect aerobic bacterial growth by inoculating them in culture media, including MacConkey agar, blood agar, nutrient agar, and mannitol salt agar, and incubated at 37°C for 24 - 48 hours aerobically. The plates were examined for growth after 24 hours of incubation. The obtained bacterial colonies were identified according to standard microbiology laboratory protocols, based on morphological characteristics and biochemical tests (9).

2.2 Antibiotic susceptibility

The antibiotic sensitivity test was performed using the Kirby - Bauer method as outlined by Claus and Berkeley in 1984. Five milliliters of brain heart infusion broth were poured into sterile tubes and inoculated with a full loop of bacterial isolate. The inoculated tubes were then incubated at 37°C for 24 hours. After incubation, the turbidity of the inoculated broth was compared to a standard, and the density of the test suspension was adjusted to match the 0.5 McFarland standard (10).

The bacterial suspension was then transferred using a sterile cotton swab and streaked onto the surface of Mueller - Hinton agar plates. The inoculated plates were allowed to dry for about a minute at room temperature. Selected antibiotic disks, including amoxicillin/clavulanic acid, cefotaxime, tigecycline, erythromycin, and cefepime, were placed on the plates using sterile forceps. The plates were incubated in an inverted position at 37°C for 24 hours. After overnight incubation, the diameters of the inhibition zones were measured in millimetres using a ruler. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (11).

3. Results

A total of 100 swabs were obtained from dental plaque during the current study. Out of these, only 70 samples showed bacterial growth, while 30 samples showed no growth.40 % of the patients were smokers, and 60% did not use toothpaste and dental floss, respectively. Environmental, socioeconomic, and geographical factors affect the rates of dental caries worldwide. Despite advancements, many children continue to experience dental caries, which is a widespread condition and a risk factor for tooth loss in both children and adults. It is one of the most common diseases in humans, influenced by various microbes. The standard etiology of caries involves four key factors: the host, oral environment, time, and oral microorganisms. Excessive consumption of carbohydrates leads to an increase in acid - resistant and acid - producing oral microbes (12)

3.1 Identification of Bacterial Isolates.

Out of 100 samples obtained, all showed growth on culture media. Most of the bacterial isolates (69.0%) were Gram - positive, represented by *Porphyromonas gingivalis* (11 isolates, 26.1%) and *Tannerella forsythia* (18 isolates, 42.8%). Only 13 (30.9%) of the isolates were Gram - negative, represented by *Treponema denticola* (9 isolates, 21.3%) and *Aggregatibacter actinomycetemcomitans* (4 isolates, 9.5%), as shown in Figure 2. Figure 3 illustrates the shape and color of some bacterial colonies cultured on different media (13).

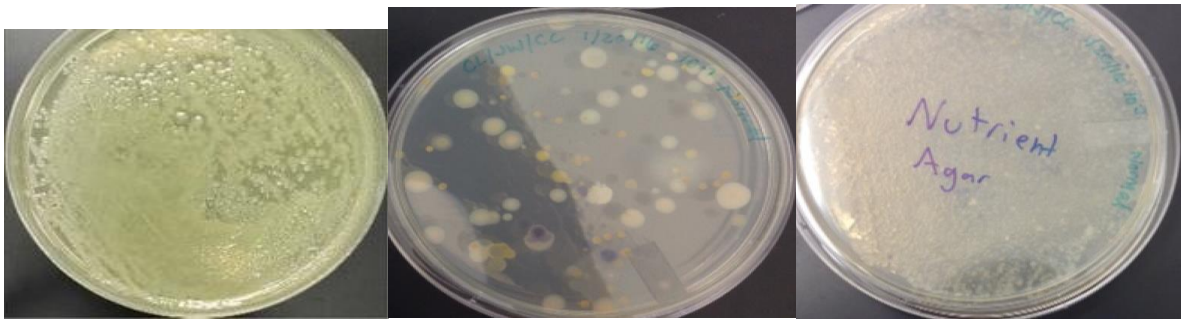


Figure 1: Different bacterial types on different culture media

3.2 Antibiotic susceptibility profile

An antimicrobial susceptibility test was performed for all obtained bacterial isolates against five antibiotics to determine the most effective treatment, as shown in Figure 4. According to Table 1, the results indicated that most Gram - positive and Gram - negative species exhibited resistance to amoxicillin, cefotaxime, and erythromycin, while showing less resistance to tigecycline. *Porphyromonas gingivalis* isolates showed high resistance to amoxicillin and cefotaxime, with a resistance rate of 90.9% for both (14). These findings are consistent with a local study that found most *Porphyromonas gingivalis* isolates were resistant to amoxicillin and erythromycin, with resistance rates of 90% and 70%, respectively. The most effective antibiotic against *Porphyromonas gingivalis* was cefotaxime, with a sensitivity rate of 88%.

The current study reported that *Porphyromonas gingivalis* isolates showed 72.7% sensitivity to cefotaxime. Most Gram - positive and Gram - negative bacteria causing dental plaque were susceptible to ceftriaxone and ciprofloxacin. *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* showed 100% sensitivity to ceftriaxone antibiotics (15). Generally, the variation in results may be attributed to factors such as patient age, sample size, sample collection period, and the frequency of antibiotic use in the population.

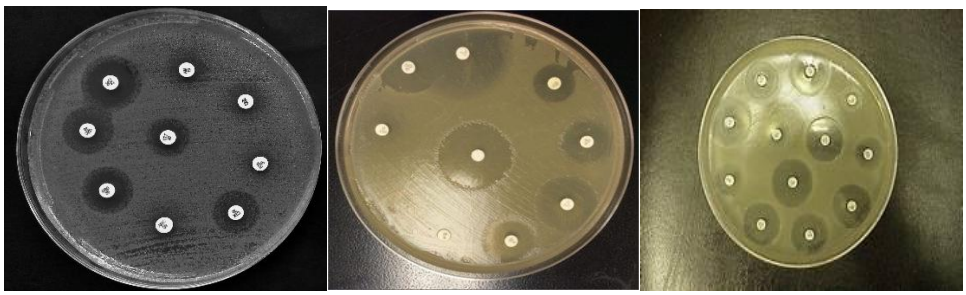
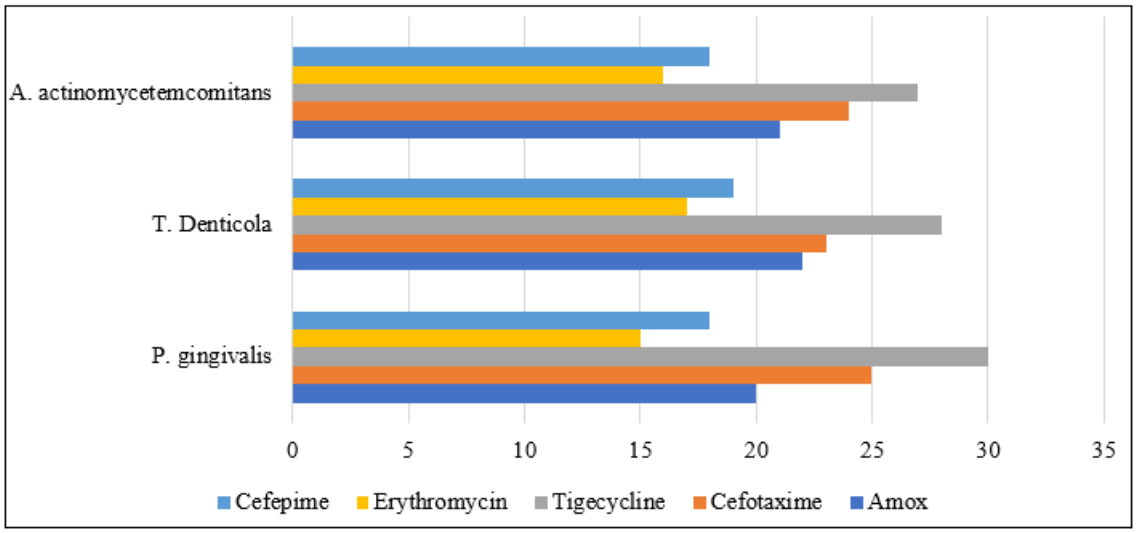


Figure 2: Results of antibiotic susceptibility test shows the inhibition zones of used antibiotics

Bacteria	Amoxicillin (mm)	Cefotaxime (mm)	Tigecycline (mm)	Erythromycin (mm)	Cefepime (mm)
Porphyromonas gingivalis	20	25	30	15	18
Treponema denticola	22	23	28	17	19
Aggregatibacter actinomycetemcomitans	21	24	27	16	18

Table 1: Bacterial species resistance against different antibiotics



Graph 1: Showing Bacterial species resistance against different antibiotics

4. Conclusion

Dental diseases are a significant public health issue and among the most prevalent conditions worldwide. Periodontitis - causing bacteria, such as Porphyromonas gingivalis and Treponema denticola, are commonly isolated from patients with tooth caries across different age groups. This is largely due to factors like inadequate teeth brushing, daily smoking, and diabetes. The incidence of caries tends to increase in individuals who do not brush their teeth regularly with fluoride toothpaste. Tooth brushing habits, smoking, the type of toothbrush used, and overall oral hygiene are all associated with the prevalence of dental caries. Therefore, it is crucial to promote health education focused on improving dental and oral health. Further studies are needed to understand additional factors contributing to the occurrence of dental caries across different age groups (16).

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