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Biofilm Formation on Dental Prostheses and Its Impact on Oral Microbial Load and Antibiotic Resistance

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تكوّن الغشاء الحيوي على التعويضات السنية وتأثيره في الحمل الميكروبي الفموي ومقاومته للمضادات الحيوية

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Abstract:
This study investigated biofilm formation on dental prostheses and its relationship with microbial colonization and antibiotic resistance. A cross-sectional study was conducted on 60 patients wearing dental prostheses in Libya. The results showed that 28.3% of participants had plaque accumulation. Streptococcus species were the most common isolates (70%), followed by Staphylococcus aureus (30%). A significant association was found between prosthetic material and biofilm formation, with zirconia prostheses showing higher susceptibility. Smoking, shorter duration of prosthesis use (<6 months), and increased number of prostheses were identified as independent risk factors. Antibiotic resistance analysis revealed significant resistance differences, particularly for penicillin, levofloxacin, norfloxacin, and imipenem, highlighting the impact of biofilm on antimicrobial tolerance. Biofilm formation is mainly influenced by prosthetic material and patient behavior rather than demographic factors. Proper material selection, early monitoring, and patient education, especially smoking cessation, are essential to reduce biofilm-related complications and antibiotic resistance.

Keywords: Biofilm formation on dental prostheses, Dental prostheses, Antibiotic resistance, Microbial colonization, Prosthetic material, Risk factors, Dental plaque.

الملخص:
هدفت هذه الدراسة إلى تقييم تكوّن الغشاء الحيوي على التركيبات السنية وعلاقته بالاستعمار البكتيري ومقاومة المضادات الحيوية. شملت الدراسة 60 مريضًا في ليبيا يستخدمون تركيبات سنية. أظهرت النتائج أن 28.3% من المشاركين لديهم تراكم لويحة جرثومية. وكانت بكتيريا Streptococcus الأكثر شيوعًا (70%)، تلتها Staphylococcus aureus (30%). وُجد ارتباط معنوي بين نوع مادة التركيبة وتكوّن الغشاء الحيوي، حيث أظهرت تركيبات الزركون أعلى قابلية للتراكم. كما تبين أن التدخين، وقصر مدة الاستخدام، وزيادة عدد التركيبات من أهم عوامل الخطورة. كما أظهرت النتائج وجود مقاومة ملحوظة لبعض المضادات الحيوية مثل البنسلين والليفوفلوكساسين والنورفلوكساسين والإيميبينيم. يتأثر تكوّن الغشاء الحيوي بشكل أساسي بنوع مادة التركيبة والعوامل السلوكية للمريض، مما يستدعي اختيار مواد مناسبة، والمتابعة المبكرة، وتعزيز التوعية الصحية لتقليل المضاعفات.

الكلمات المفتاحية: تكوّن الغشاء الحيوي على التركيبات السنية، التركيبات السنية، مقاومة المضادات الحيوية، الاستعمار الميكروبي، مادة التركيبة السنية، عوامل الخطورة، اللويحة الجرثومية.

Introduction:

The oral cavity harbors a complex microbial ecosystem influenced by environmental factors such as pH, temperature, and oxygen availability, all of which play critical roles in biofilm formation [1,2]. Dental prostheses are widely used to restore oral function and aesthetics; however, they introduce non-shedding surfaces that facilitate microbial adhesion and biofilm development [3,4]. Biofilm formation on prosthetic surfaces follows a structured, multi-stage process involving pellicle formation, microbial adhesion, co-aggregation, and maturation into complex multispecies communities (Figure 1) [5].

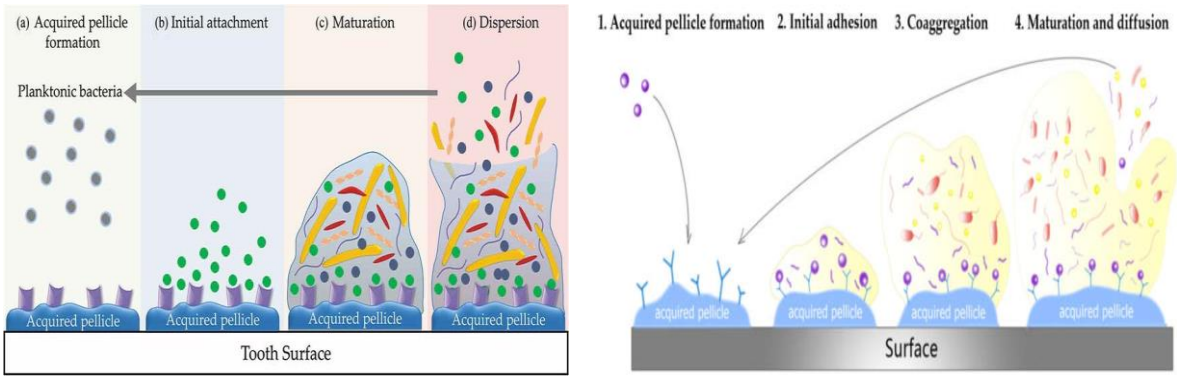

Figure (1): Schematic representation of the stages of dental biofilm formation on oral surfaces.

Early colonizers, particularly *Streptococcus* and *Actinomyces* species, play a fundamental role in initiating biofilm formation and promoting microbial succession [2,6]. A major clinical concern is the increased antimicrobial resistance exhibited by biofilm-associated bacteria, driven by mechanisms such as limited antibiotic penetration, metabolic adaptation, and persister cell formation [7,8]. This issue represents a significant global challenge, as antimicrobial resistance has been recognized as a major public health threat requiring urgent action [9].

Oral health is essential for overall well-being and quality of life, with periodontal diseases being highly prevalent worldwide [10]. Advanced conditions such as periodontitis may lead to tooth loss, necessitating prosthetic rehabilitation. However, poorly designed or maintained prostheses may increase plaque accumulation and exacerbate oral conditions [3,11]. Dental plaque is a structured microbial biofilm that readily forms on prosthetic surfaces, particularly in rough or difficult-to-clean areas, and demonstrates increased resistance to antimicrobial agents [5,8,12].

Despite extensive research on oral biofilms, limited attention has been directed toward dental prostheses as distinct sites for biofilm formation and antimicrobial resistance [3,11]. Moreover, the combined influence of prosthetic material characteristics on biofilm composition and antimicrobial resistance, as well as patient-related factors, remains insufficiently explored in clinical settings [4,13]. In Libya, there is a notable lack of region-specific data addressing these aspects.

Therefore, this study aims to investigate biofilm formation on dental prostheses and its association with microbial colonization and antibiotic resistance, with particular emphasis on prosthetic materials and patient-related factors [4,14]. The study further evaluates antibiotic resistance patterns of biofilm-associated oral bacteria and examines the impact of factors such as oral hygiene, smoking habits, and duration of prosthesis use.

This study is significant because bacteria within biofilms exhibit markedly higher resistance to antimicrobial agents compared to their planktonic counterparts due to protective mechanisms such as limited antibiotic penetration, altered gene expression, and metabolic adaptations [7,8]. This resistance may be up to 1000–1500 times greater [9], posing a substantial challenge in the management of chronic oral infections. Additionally, conventional susceptibility testing based on planktonic models may not accurately reflect biofilm behavior, thereby limiting treatment effectiveness [7].

The scope of this study is confined to adult patients using dental prostheses in selected dental clinics in Libya. It includes 60 participants over a three-month period and focuses on evaluating the relationship between prosthetic materials and patient-related factors, including smoking and oral hygiene practices, in relation to biofilm formation and bacterial colonization. Microbiological analysis was performed using conventional culture and antibiotic susceptibility testing methods, without the use of advanced molecular techniques.

Literature Review:

Biofilm formation is a highly organized and dynamic microbial process in which microorganisms adhere to biotic or abiotic surfaces and become embedded within a self-produced extracellular polymeric substance (EPS) matrix. This matrix provides structural stability, facilitates nutrient exchange,

and enhances microbial protection against antimicrobial agents and host immune responses [5]. In the oral environment, biofilm development begins with the formation of an acquired pellicle composed of salivary proteins, which promotes initial microbial adhesion and colonization [2].

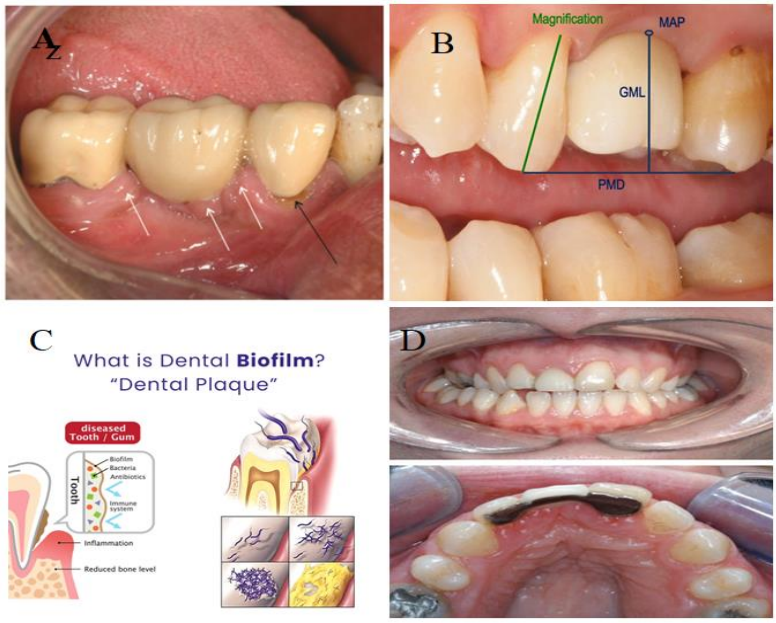

Figure (2): Dental biofilm formation and clinical manifestations associated with plaque accumulation and prosthetic surfaces: (A) Gingival inflammation around teeth (B) Periodontal measurements (C) Dental biofilm (plaque) structure (D) Plaque and deposits on teeth and prostheses

Dental prostheses provide favorable conditions for biofilm accumulation due to their surface properties and design, which significantly influence microbial adhesion and antibiotic resistance patterns [3,4]. The microbial composition of these biofilms develops progressively, starting with early colonizers such as *Streptococcus* and *Actinomyces*, followed by more complex communities that include anaerobic periodontal pathogens and, in some cases, fungi like *Candida albicans* [2].

Clinically, biofilm buildup on prostheses is associated with gingival inflammation and periodontal complications that may affect both oral health and prosthesis longevity [11,15]. Peri-implant mucositis is a common inflammatory complication affecting prosthetic and implant-supported restorations [15].
A key concern is the increased resistance of biofilm-associated microorganisms to antimicrobial agents, driven by protective mechanisms such as limited drug penetration and altered microbial activity [7,8].

Despite existing research, the role of dental prostheses in biofilm formation and antimicrobial resistance remains insufficiently explored, particularly regarding the effects of prosthetic materials and patient-related factors [4,13]. This highlights the need for further studies to improve prevention and management strategies in prosthodontic care.

Materials and Methods:

This descriptive cross-sectional study was conducted to evaluate bacterial colonization associated with biofilm formation on dental prostheses and to identify factors influencing plaque accumulation and antimicrobial resistance. The study was carried out over a three-month period in selected private and governmental dental clinics in Libya, with microbiological analysis performed at Al-Yasfeen Laboratory (Esbi'a, Libya).

The study included 60 adult participants (≥18 years) wearing dental prostheses for at least three months. Participants were recruited using a convenience sampling method. Individuals with active oral infections, recent antibiotic use (within 2–4 weeks), or immunocompromising conditions were excluded. Data were collected through a structured interviewer-administered questionnaire covering demographic, clinical, and behavioral variables, including oral hygiene practices, smoking, prosthesis characteristics, and oral symptoms. Clinical examination and sample collection were performed using sterile swabs from prosthetic surfaces and adjacent gingival areas, with plaque and gingival status assessed visually (Figure 3.1).

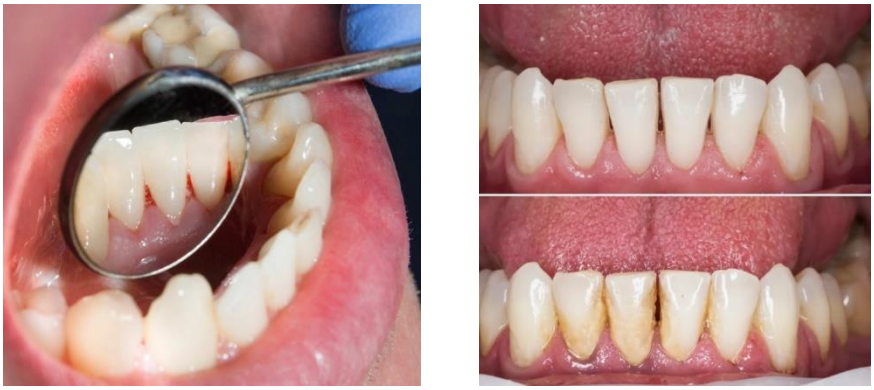

Figure (3): Clinical examination using a dental mirror to assess plaque accumulation and gingival condition around teeth and dental prostheses

Microbiological analysis involved bacterial culture on appropriate media, followed by identification using standard techniques such as colony morphology, Gram staining, and biochemical testing. Antibiotic susceptibility testing was conducted using conventional methods to assess resistance patterns of isolated bacteria.

Due to resource limitations, biofilm formation was assessed indirectly through clinical plaque evaluation, bacterial isolation, and antimicrobial resistance profiles rather than direct quantitative measurement.

Data were recorded using standardized forms and analyzed using SPSS. Descriptive statistics were applied, and associations between variables were tested using the Chi-square test, with statistical significance set at $p < 0.05$.

Ethical approval was obtained, and written informed consent was secured from all participants. Confidentiality was maintained, and standard infection control measures were followed throughout the study.

4. Results:

A total of 60 participants were included in this study, with ages ranging from 24 to 74 years (mean = 43.93 ± 10.49), indicating a demographically diverse sample suitable for evaluating biofilm formation across adult age groups (Table 4.1). Females constituted the majority of participants (66.7%), compared to 33.3% males (Table 4.2, Figure 4.1).

Table (1): Descriptive Statistics of Participants' Age in the Study on Biofilm Formation and Antibiotic Resistance

Total	60
Mean	43.9333
Std. Deviation	10.49595
Minimum	24.00
Maximum	74.00

Table (2): Gender Distribution in the Study Sample

Gender	Frequency	Percent
Male	20	33.3
Female	40	66.7

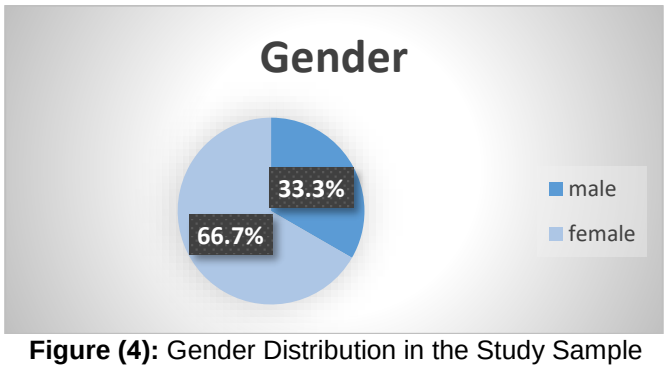

Figure (4): Gender Distribution in the Study Sample

Most participants were generally healthy, 83.3% reporting no chronic diseases and a similar proportion not taking regular medications (Tables 4.3–4.4, Figures 4.2–4.3). Allergies were uncommon (6.7%) (Table 4.5, Figure 4.4). Regarding lifestyle habits, the majority were non-smokers (83.3%), while 16.7% were smokers (Table 4.6, Figure 4.5).

Table (3): Prevalence of chronic diseases among study participants.

Chronic Diseases	Frequency	Percent
Yes	10	16.7%
No	50	83.3%

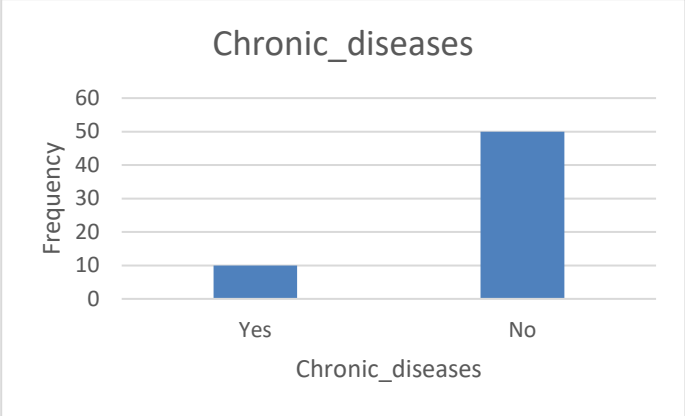

Figure (5): Prevalence of chronic diseases among study participants

Table (4): Regular medication use among study participants

Regular Medications	Frequency	Percent
Yes	10	16.7%
No	50	83.3%

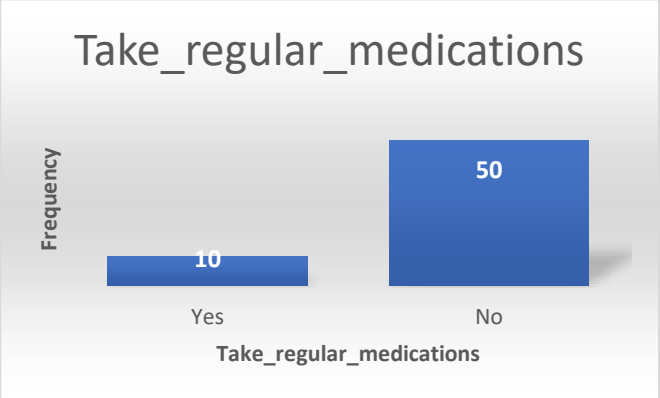

Figure (6): Regular medication use among study participants

Table (5): Prevalence of Allergies to Medical Substances and Drugs among Study Participants

Allergies	Frequency	Percent
Yes	4	6.7%
No	56	93.3%

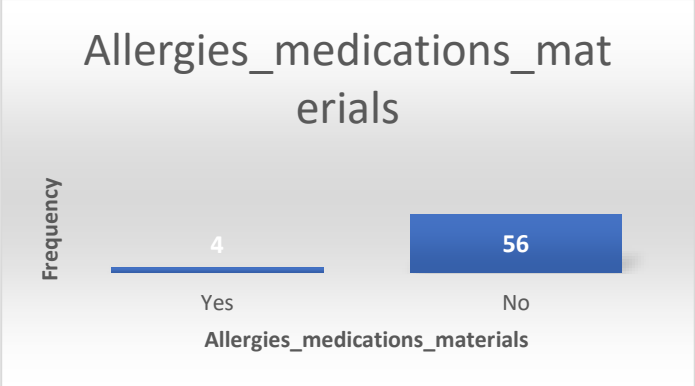

Figure (7): Prevalence of Allergies to Medical Substances and Drugs among Study Participants

Table (6): Smoking Habits among Study Participants

Smoking	Frequency	Percent
Yes	10	16.7%
No	50	83.3%

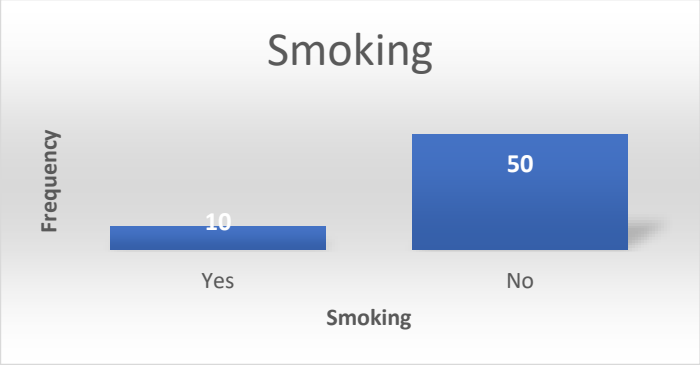

Figure (8): Smoking Habits among Study Participants

Fixed prostheses (crowns and bridges) were the most prevalent type (80%), followed by removable, implant-supported, and mixed types (each 6.7%) (Table 4.7, Figure 4.6). Most participants had used their prostheses for more than one year (86.7%) (Table 4.8, Figure 4.7). Zirconia was the most commonly used material (63.3%), followed by metal–ceramic (13.3%), while other materials were less frequent (Table 4.9, Figure 4.8).

Table (7): Distribution of Dental Prosthesis Types among Study Participants

Type of Prosthesis	Frequency	Percent
Fixed prosthesis (crowns/bridges)	48	80.0%
Removable denture (partial/full)	4	6.7%
Implant-supported prosthesis	4	6.7%
More than one type	4	6.7%

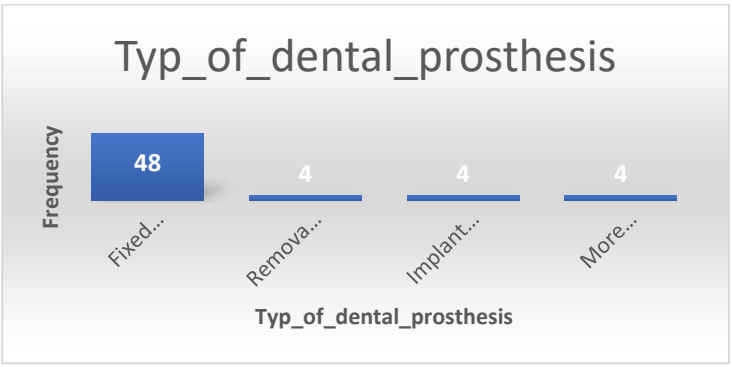

Figure (9): Distribution of Dental Prosthesis Types among Study Participants

Table (8): Duration of Dental Prosthesis Use among Study Participants

Duration of Use	Frequency	Percent
Less than 6 months	6	10.0%
6–12 months	2	3.3%
More than 1 year	52	86.7%

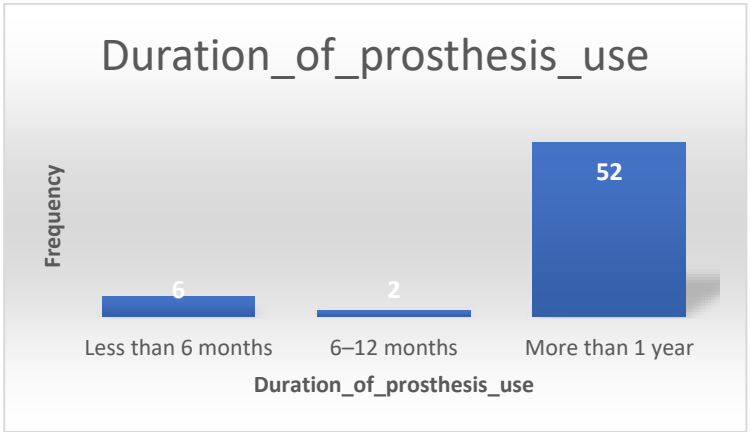

Figure (10): Duration of Dental Prosthesis Use among Study Participants

Table (9): Distribution of Prosthesis Materials among Study Participants

Material of Prosthesis	Frequency	Percent
Metal–ceramic	8	13.3%
Full ceramic	2	3.3%
Acrylic/Resin (removable)	2	3.3%
Zirconia	38	63.3%
Other	10	16.7%

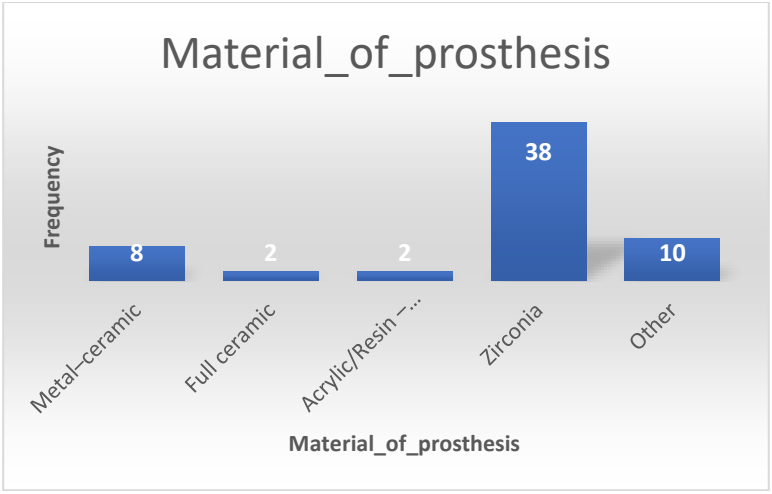

Figure (11): Distribution of Prosthesis Materials among Study Participants

In terms of oral health behavior, 73.3% reported maintaining oral hygiene, although dental visits were largely irregular, with 53.3% visiting only when needed and 16.7% never attending (Tables 4.10–4.11, Figures 4.9–4.10). Sugar and soft drink consumption was common, with 90% reporting either occasional or daily intake (Table 4.12, Figure 4.11).

Table (10): Oral Hygiene Maintenance among Study Participants

Maintain Oral Hygiene	Frequency	Percent
Yes	44	73.3%
No	16	26.7%

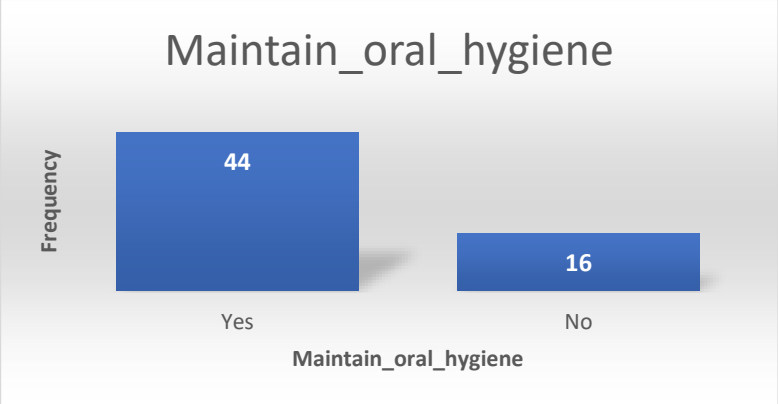

Figure (12): Oral Hygiene Maintenance among Study Participants

Table (11): Frequency of Dental Visits among Study Participants

Dental Visit Frequency	Frequency	Percent
Every 6 months	16	26.7%
Annually	2	3.3%
When needed	32	53.3%
Never	10	16.7%

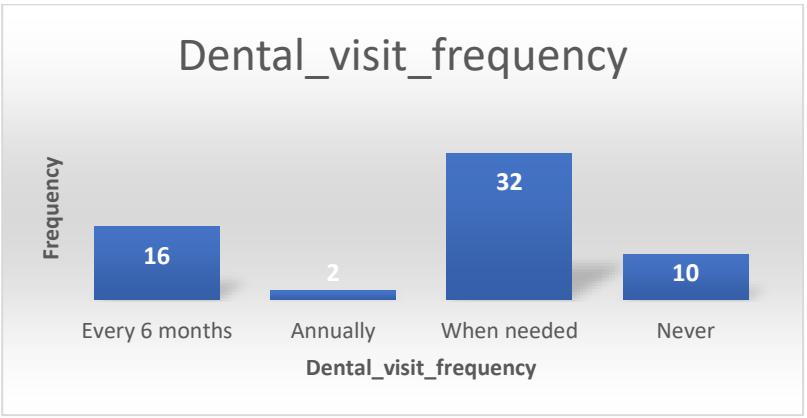

Figure (13): Frequency of Dental Visits among Study Participants

Table (12): Frequency of Sugars and Soft Drinks Consumption among Study Participants

Consumption Frequency	Frequency	Percent
Rarely	6	10.0%
Sometimes	34	56.7%
Daily	20	33.3%
Total	60	100.0%

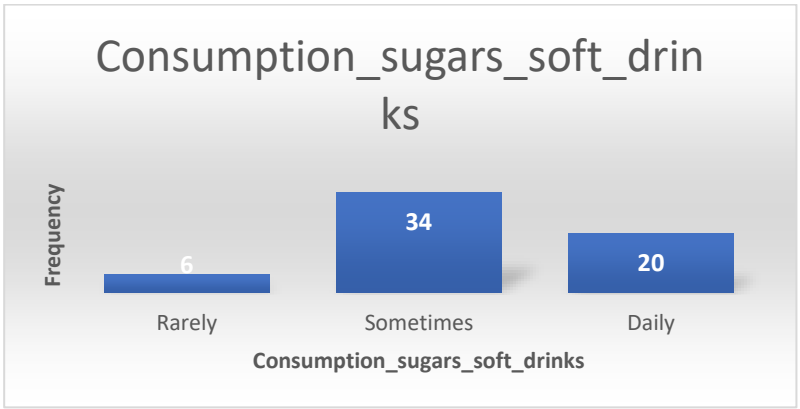

Figure (14): Frequency of Sugars and Soft Drinks Consumption among Study Participants

Clinically, plaque accumulation on prostheses was observed in 28.3% of participants (Table 4.13, Figure 4.12). Associated oral symptoms included bleeding during brushing (26.7%), bad breath (20.0%), and tooth sensitivity (16.7%) (Tables 4.14–4.16, Figures 4.13–4.15).

Table (13): Prevalence of Plaque Buildup on Dental Prostheses among Study Participants

Plaque Buildup	Frequency	Percent
Yes	17	28.3%
No	43	71.7%

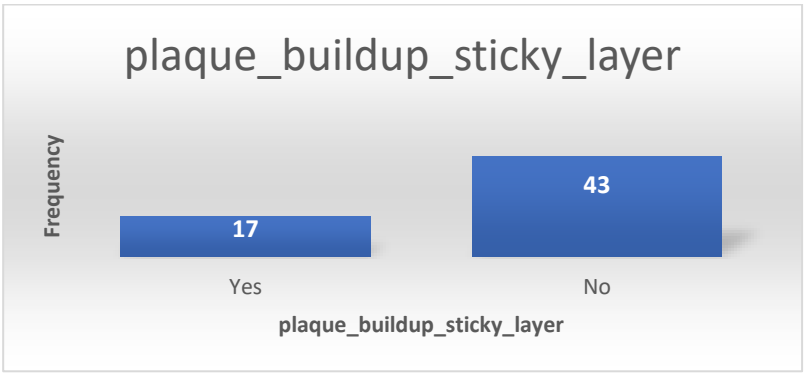

Figure (15): Prevalence of Plaque Buildup on Dental Prostheses

Table (14): Prevalence of Bad Breath among Study Participants

Bad Breath	Frequency	Percent
Yes	12	20.0%
No	48	80.0%

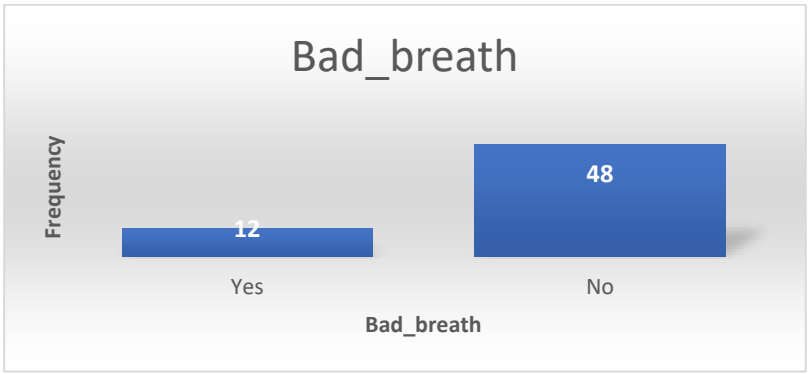

Figure (16): Prevalence of Bad Breath among Study Participants

Table (15): Prevalence of Bleeding during Brushing around Prostheses among Study Participants

Bleeding when brushing	Frequency	Percent
Yes	16	26.7%
No	44	73.3%

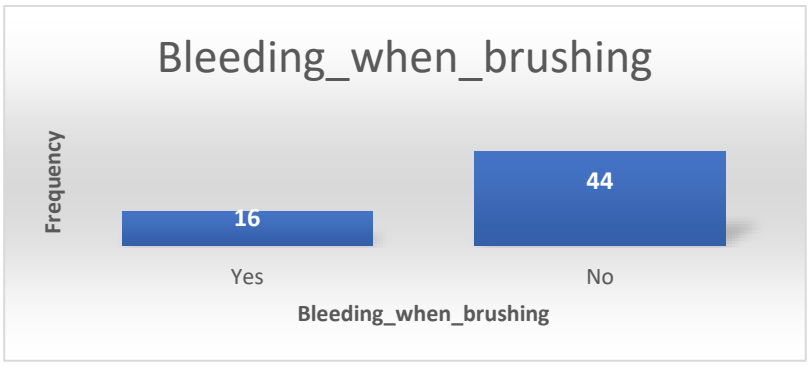

Figure (17): Prevalence of Bleeding during Brushing around Prostheses among Study Participants

Table (16): Prevalence of Tooth Sensitivity among Study Participants

Tooth Sensitivity	Frequency	Percent
Yes	10	16.7%
No	50	83.3%

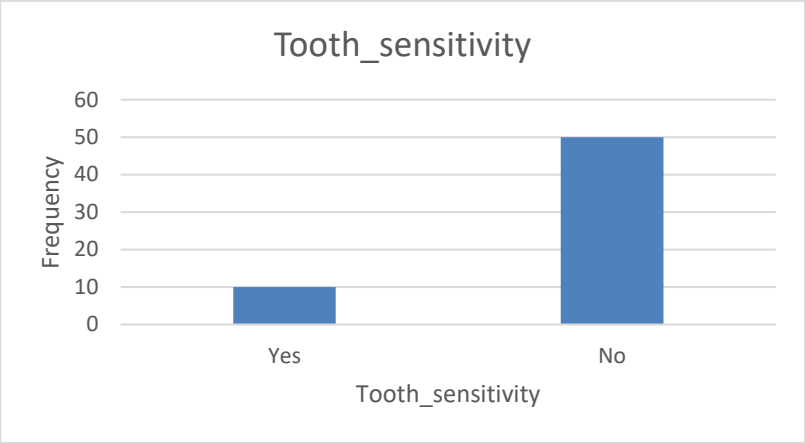

Figure (18): Prevalence of Tooth Sensitivity among Study Participants

Microbiological analysis revealed that Streptococcus species were the predominant isolates (70%), followed by Staphylococcus aureus (30%). *Streptococcus pyogenes* demonstrated β-hemolysis on blood agar, while *Streptococcus mutans* showed typical colony morphology, confirming their role in biofilm formation (Figures 4.16–4.17).

Figure (19): Streptococcus pyogenes showing beta-hemolysis on blood agar

Bacterial distribution and plaque formation varied according to prosthetic material. Zirconia showed the highest plaque prevalence (31.6%), while no plaque was detected in full ceramic prostheses (Table 4.17, Figures 4.18–4.20). Statistical analysis demonstrated a significant association between prosthetic material and plaque buildup ($\chi^2 = 9.842$, $p = 0.043$) (Table 4.18).

Table (17): Distribution of Bacteria and Plaque Buildup by Prosthetic Material (n=60)

Prosthetic Material	Staphylococcus aureus	Streptococcus	Plaque Buildup (Yes)
Metal–ceramic	25%	75%	25%
Full ceramic	0%	100%	0%
Acrylic/Resin	100%	0%	50%
Zirconia	36.8%	63.2%	31.6%
Other	0%	100%	20%

Table (18): Chi-Square Test of Association between Prosthetic Material and Plaque Buildup

Test	Value	Df	p-value (2-sided)	Interpretation
Pearson Chi-Square	9.842	4	0.043	Significant association
Likelihood Ratio	11.276	4	0.024	Significant
Linear-by-Linear Association	0.317	1	0.573	Not significant

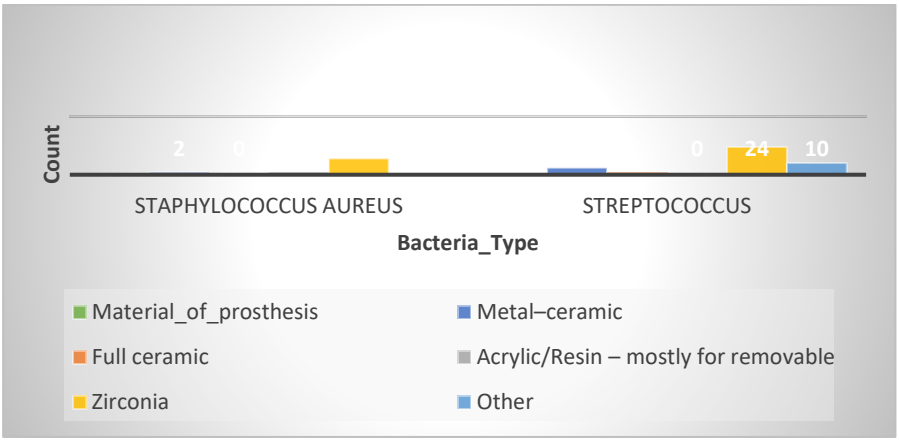

Figure (20): Distribution of bacterial isolates *(Streptococcus** vs *Staphylococcus aureus*) across prosthetic materials

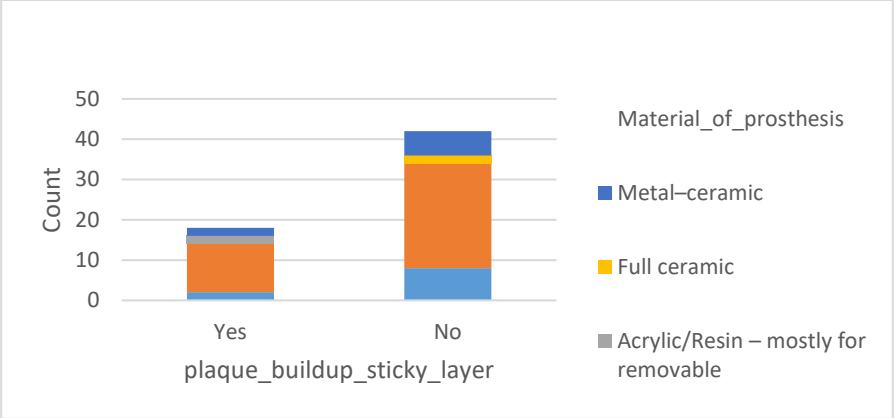

Figure (21): Plaque buildup (biofilm formation) in relation to prosthetic material type.

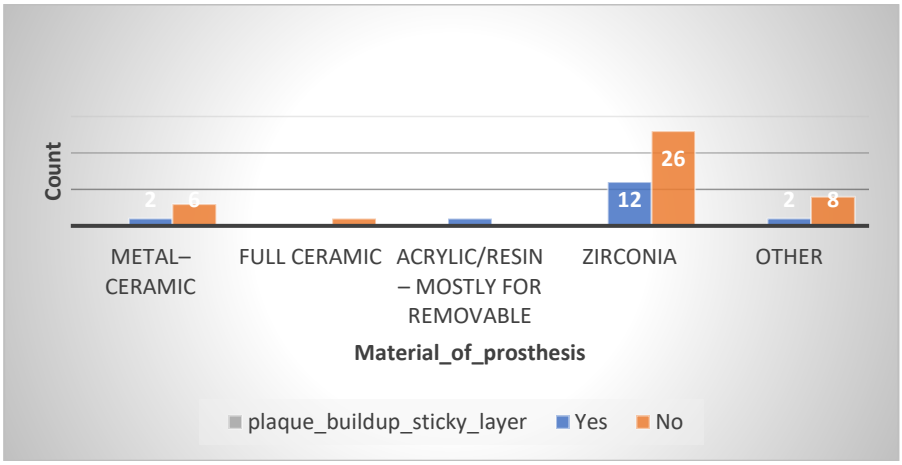

Figure (22): Combined distribution of plaque buildup across prosthetic materials

Patient-related factors showed variable influence. Smoking was significantly associated with plaque formation in Staphylococcus aureus isolates (p = 0.043), as was shorter prosthesis duration (<6 months) (p = 0.043). In contrast, oral hygiene showed no statistically significant association (Table 4.19, Figures 4.21–4.22).

Table (19): Association of Patient-related Factors with Plaque Buildup and Bacterial Type

Factor	Bacteria Type	Plaque Buildup (Yes)	p value	Interpretation
Oral hygiene	Staphylococcus aureus	62.5% (with hygiene)	0.094	Not significant
	Streptococcus	21.4% (with hygiene)	0.578	Not significant
Smoking	Staphylococcus aureus	100% (smokers)	0.043	Significant association
	Streptococcus	33.3% (smokers)	0.336	Not significant
Duration of use*	Staphylococcus aureus	100% (<6 months) vs 42.9% (>1 year)	0.043	Significant association
	Streptococcus	21.1% (>1 year)	0.594	Not significant

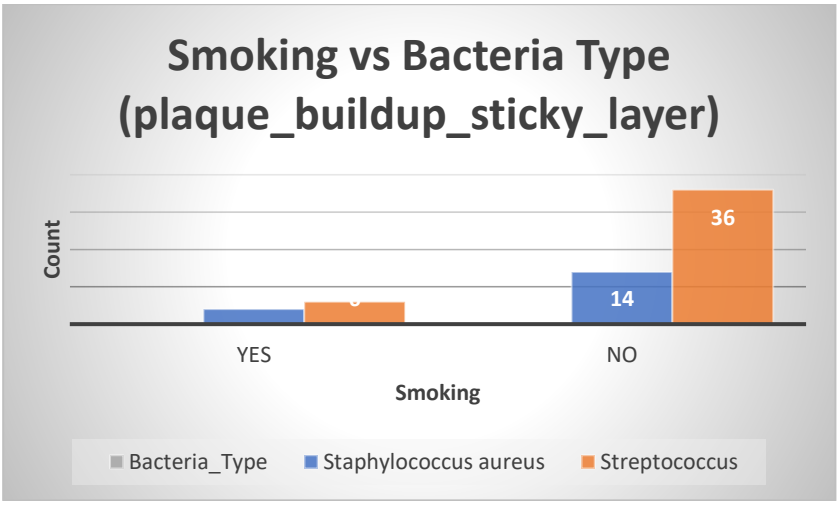

Figure (23): Impact of smoking on biofilm formation in prostheses colonized by different bacteria

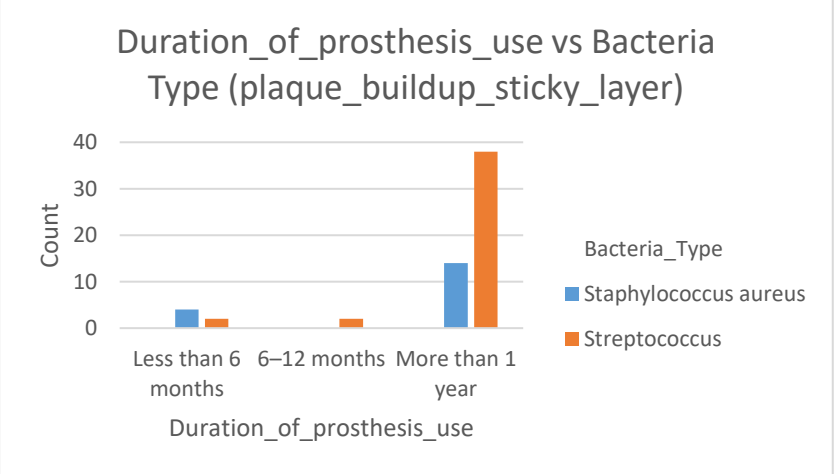

Figure (24): Correlation between prosthesis duration and bacterial plaque buildup across Staphylococcus aureus and Streptococcus specie

Regarding antibiotic resistance, most antibiotics showed no significant differences between bacterial species. However, significant differences were observed for levofloxacin ($p = 0.037$), norfloxacin ($p = 0.047$), penicillin ($p = 0.028$), and imipenem ($p = 0.023$). *Streptococcus* isolates exhibited higher resistance to fluoroquinolones, while *Staphylococcus aureus* showed higher resistance to penicillin (Table 4.20).

Table (20): Association between bacterial species and antibiotic resistance patterns in biofilm-forming isolates from dental prostheses

Antibiotic	Staphylococcus aureus (Resistant %)	Streptococcus (Resistant %)	χ^2 (df=1)	p-value	Interpretation
Amoxicillin–Clavulanic	11.1%	14.3%	0.110	0.740	NS (not significant)
Cefoxitin	22.2%	23.8%	0.018	0.894	NS
Cefotaxime	0.0%	9.5%	1.837	0.175	NS
Ceftriaxone	22.2%	19.0%	0.079	0.778	NS
Levofloxacin	11.1%	38.1%	4.369	0.037	Significant
Tetracycline	77.8%	81.0%	0.079	0.778	NS
Cefixime	66.7%	42.9%	2.857	0.091	NS (trend)
Norfloxacin	44.4%	71.4%	3.951	0.047	Significant
Penicillin	88.9%	100%	4.828	0.028	Significant
Kanamycin	100%	95.2%	0.887	0.346	NS
Imipenem	0.0%	23.8%	5.143	0.023	Significant

Overall, these findings highlight the multifactorial nature of biofilm formation on dental prostheses, with prosthetic material, smoking, and bacterial species playing key roles in plaque accumulation and antibiotic resistance patterns.

Discussion:

Biofilm formation on dental prostheses is influenced by both material-related properties and patient-associated behavioral factors [3,4,13]. The predominance of *Streptococcus* species reflects their role as primary colonizers that initiate biofilm development and facilitate microbial succession [2,6], while the presence of *Staphylococcus aureus* suggests progression toward more pathogenic communities.

Prosthetic material characteristics play a critical role in microbial adhesion and the subsequent development of biofilm-associated antimicrobial resistance, as differences in material composition have been shown to significantly influence bacterial colonization patterns [4,13]. Surface roughness, material composition, and clinical adjustments may significantly affect biofilm accumulation, particularly in zirconia-based restorations where surface alterations can enhance bacterial retention [4,13]. These findings support growing evidence that material-dependent properties influence not only biofilm formation but also microbial behavior.

Patient-related factors, particularly smoking, contribute to biofilm development by altering immune response and oral microbial balance, thereby promoting dysbiosis [10,15]. In addition, the early post-placement period appears to be a key phase for microbial colonization and biofilm establishment [2].

Biofilm-associated microorganisms demonstrate increased antimicrobial tolerance, which is further influenced by the type of prosthetic material, as demonstrated in previous studies evaluating material-dependent resistance patterns [13]. This highlights the limitations of conventional susceptibility testing and the need for biofilm-specific approaches.

Clinically, optimizing prosthetic material selection is recommended based on material-dependent effects on microbial adhesion and antibiotic resistance, as reported in recent studies on dental prosthetic materials [4,13]. Further research using molecular methods and quantitative biofilm analysis is recommended to better understand prosthesis-associated microbial dynamics and resistance patterns [8,14].

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