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Isolation and diagnosis of bacteria causing gingivitis in patients with type 2 diabetes

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Abstract
The aim of this research is Isolating the bacterial species that cause mouth infections from a sample of patients with type 2 diabetes and comparing them to the control group, and diagnosing the type of bacteria causing the contamination inside the mouth.
The cases were divided into two groups. The first group included 30 cases with type 2 diabetes and suffering from gingivitis, while the second group included 30 cases without diabetes (control sample) inside the dental clinic at Sabratha Teaching Hospital, where swabs were taken with a Swab for both groups for a period of time. Between 18/4/2023 and 22/8/2023, the samples were then transferred to the laboratory for analysis and testing. Volunteers in the age group 30-70 with type II Diabetes and controls that were healthy individuals without diabetes, male and female, denture wearer or non-denture wearer, with or without oral lesion were included in the study. Those volunteers with steroid or antibiotic use in the last 4 weeks of study were excluded.
The highest age group in Group A was > 60 years, and the percentage reached 43.3%. As for the second group, the highest percentage was for the age group 50-60 years, and the percentage reached 36.7%. The highest percentage of isolated samples in group A was Streptococcus mutans bacteria, which reached 33.3%, followed by Streptococcus anginosus, 20.4%. The highest percentage of isolated samples in group B (control sample) was Proteusus merapilis bacteria, which reached 47%, followed by Pseudomonas aeruginosa bacteria, which reached 35.4%.

Keywords: Isolation; Diagnosis; Bacteria; Gingivitis; Type 2 Diabetes.

المستخلص
تهدف هذه الدراسة إلى عزل الأنواع البكتيرية المسببة لالتهابات الفم من عينة من مرضى السكري من النوع الثاني ومقارنتها بالمجموعة الضابطة، وتشخيص نوع البكتيريا المسببة للتلوث داخل الفم. تم تقسيم الحالات إلى مجموعتين ، ضمت المجموعة الأولى (التجريبية) 30 حالة مصابة بمرض السكري من النوع الثاني وتعاني من التهاب اللثة، بينما ضمت المجموعة الثانية (الضابطة) 30 حالة غير مصابة بالسكري داخل عيادة الأسنان بمستشفى صبراتة التعليمي، حيث تم أخذ المسحات مع المسحة لكلا المجموعتين لمدة من الوقت، وفي الفترة ما بين 2023/4/18 ولغاية 2023/8/22، تم نقل العينات إلى المعمل للتحليل والاختبار. تم تضمين المتطوعين في الفئة العمرية 30-70 الذين يعانون من مرض السكري من النوع الثاني والضوابط التي كانت أفرادًا أصحاء بدون مرض السكري، ذكورًا وإناثًا، أو يرتدون أطقم أسنان أو لا يرتدون أطقم أسنان، مع أو بدون آفة في الفم، تم استبعاد هؤلاء المتطوعين الذين استخدموا الستيرويد أو المضادات الحيوية في الأسابيع الأربعة الأخيرة من الدراسة.
أعلى فئة عمرية في المجموعة (التجريبية) كانت أكبر من 60 سنة، وبلغت النسبة 43.3%. أما المجموعة الثانية (الضابطة) فكانت أعلى نسبة للفئة العمرية 50-60 سنة، وبلغت النسبة 36.7%، وكانت أعلى نسبة للعينات المعزولة في المجموعة (التجريبية) هي بكتيريا *Streptococcus mutans* والتي بلغت 33.3%، يليها *Streptococcus anginosus*، 20.4% وكانت أعلى نسبة للعينات المعزولة في المجموعة (الضابطة) هي بكتيريا *Proteusus merapilis* والتي بلغت 47% تليها بكتيريا *Pseudomonas aeruginosa* والتي بلغت 35.4%.
الكلمات المفتاحية: عزل ; تشخيص البكتيريا ;التهاب اللثة ; السكري النوع 2 .

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Introduction

The oral cavity is the same lineage of the upper intestine, lower intestine, and rectum. Pathogens that remain in the oral cavity may transmit easily to other parts of the intestine and related organs. Varieties of microorganisms inhabit the oral cavity that provides a unique niche with the source of water and nutrients at a moderate temperature [1]. There are distinct microenvironments in the oral cavity, notably, on the hard and sticky surfaces on the teeth, in saliva fluid, and in subgingival to gingival crevicular fluid (GCF). The complex oral microbiota contains several hundred to thousand diverse species, as evidenced by both culture-based and culture-independent molecular approaches [2]. Microorganisms form multispecies communities on tooth surfaces in a matrix of extracellular polymeric substances. The oral microbial community in healthy humans is distinct from that of the disease-associated oral microbiota [3].

Most oral microorganisms are non-pathogenic, but opportunistic commensals keep oral health condition stable and protect against pathogenic microorganisms [4]. The alteration of typical oral bacterial species to pathogenic members results in dysbiosis and periodontal diseases (PD), such as periodontitis and gingivitis [5]. Periodontal diseases are customarily separated into infections affecting the underlying tooth-supporting tissues of the periodontium, including the periodontal ligament, and the alveolar bone is known as periodontitis. PD is probably the most common chronic inflammatory disorder in adults and may lead to tooth loss in the absence of appropriate treatments. Infections particular to the gingival mucosa are known as gingivitis caused by a nonspecific inflammatory reaction in response to an increased mass of bacteria around the gingival crevice. Although oral diseases remain a significant public health burden worldwide with significant socio-economic impacts, yet these are frequently neglected in public health policy, particularly in developing countries. Further, PD are closely interlinked to non-communicable diseases such as pancreatic cancer, diabetes, atherosclerotic circulatory disease, osteoporosis, rheumatoid arthritis, pulmonary disorders, chronic renal disease, obesity and Alzheimer's disease. Therefore, there has been a continuing interest in assessing the composition of the dental microbiota associated with health and disease [6].

The World Health Organization estimates 422 million adults in the world (8.5% of the population) had diabetes in 2014. The figure is expected to rise to 642 million people living with diabetes worldwide by 2040 [7]. Moreover, 75% of people with diabetes live in low- and middle-income countries. The countries with the most people who have diabetes in upcoming years are predicted to be in Asian countries. The risk of periodontal diseases is increased by approximately threefold in diabetic subjects in comparison to non-diabetic individuals [8]. The diabetic status has been unequivocally reported as a significant risk factor for oral diseases. Very few reports are available to elucidate PD of the Bangladeshi population [9]. To our knowledge, no study has reported yet about dental microbiota associated with type 2 diabetes mellitus (T2DM) humans .

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia which results from defects in insulin secretion and/or insulin resistance over a prolonged period of time. Generally, there are two main types of DM: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) [10]. T1DM is due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency, including latent autoimmune diabetes of adulthood [11]. T2DM is due to a progressive loss of adequate β-cell insulin secretion and insulin resistance. According to the World Health Organization, DM currently affects approximately 422 million people globally and 1.6 million deaths are directly attributed to DM each year. In 2045, the number of diabetic patients is expected to increase to 629 million [12]. Of all the diagnosed diabetes cases, T2DM accounts for 90%-95% and affects more than 380 million people worldwide, representing 8.8% of individuals aged 20–79 years [13]. Moreover, T2DM may cause long-term complications including retinopathy, nephropathy, peripheral neuropathy and atherosclerotic cardiovascular, peripheral arterial and cerebrovascular

diseases [14]. Also, periodontal diseases are highly likely to occur and aggravate in individuals with DM especially in poorly controlled diabetics [15]. Likewise, since periodontal diseases may contribute to the body's overall inflammatory burden, individuals with periodontitis are more potentially to develop DM [16]. Thus, a 'two-way' relationship between the two diseases is established [17]. The impact of DM on periodontal diseases through hyperglycemia and inflammatory pathways is well described, while the effects of DM on oral microbiome remains controversial. Previous studies failed to reach a consensus on that DM affects the oral microbiome [18].

Objectives of study

1. Isolating the bacterial species that cause mouth infections from a sample of patients with type 2 diabetes and comparing them to the control group.
2. Diagnosing the type of bacteria causing the contamination inside the mouth.
3. Identify the most common bacterial type in the mouth.

Materials and methods

The cases were divided into two groups. The first group included 30 cases with type 2 diabetes and suffering from gingivitis, while the second group included 30 cases without diabetes (control sample) inside the dental clinic at Sabratha Teaching Hospital, where swabs were taken with a Swab for both groups for a period of time. Between 18/4/2023 and 22/8/2023, the samples were then transferred to the laboratory for analysis and testing. Volunteers in the age group 30-70 with type II Diabetes and controls that were healthy individuals without diabetes, male and female, denture wearer or non-denture wearer, with or without oral lesion were included in the study. Those volunteers with steroid or antibiotic use in the last 4 weeks of study were excluded. Volunteers were asked to complete a questionnaire bearing information on demographics (age, gender) and medical history. An informed consent was obtained from them after explaining the nature and purpose of study. Samples were collected from diabetic and non-diabetic individuals after oral examination and when these subjects were fasting.

Collection of Sample

The buccal swabs were collected aseptically and swabbed onto Nutrient Agar plate and incubated for 24 hours at 37ºC. By repeated streaking onto Nutrient Agar, pure cultures of each bacterium were obtained.

Isolation and Identification of bacterial isolates:

The isolated bacteria were identified based on colony morphology, gram staining, motility.

Results

Fig (1). clear that the largest percentage of the sample was males, 63.4%, while the percentage of females was 36.6%.

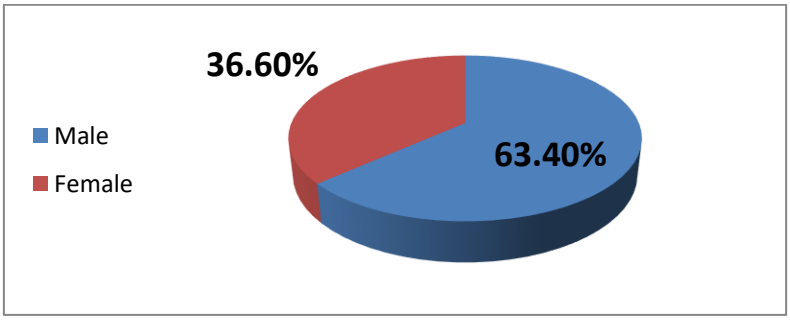

Figure 1. Sample distribution by gender

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Fig (2). it is clear that the most affected age group in Group A was > 60 with a percentage of 43.3%, followed by the age group of 50-60 years with a percentage of 33.3%, while the lowest percentage was for the age group of 20-30 years with a percentage of 3.3%. As for group B, the uninfected (control sample), the highest percentage was for the age group 50-60 years, with a percentage of 36.7%, while the percentage for the age group > 60 was 26.7%, and the lowest percentage was for the age group 30-40 years, with a percentage of 13.3%.

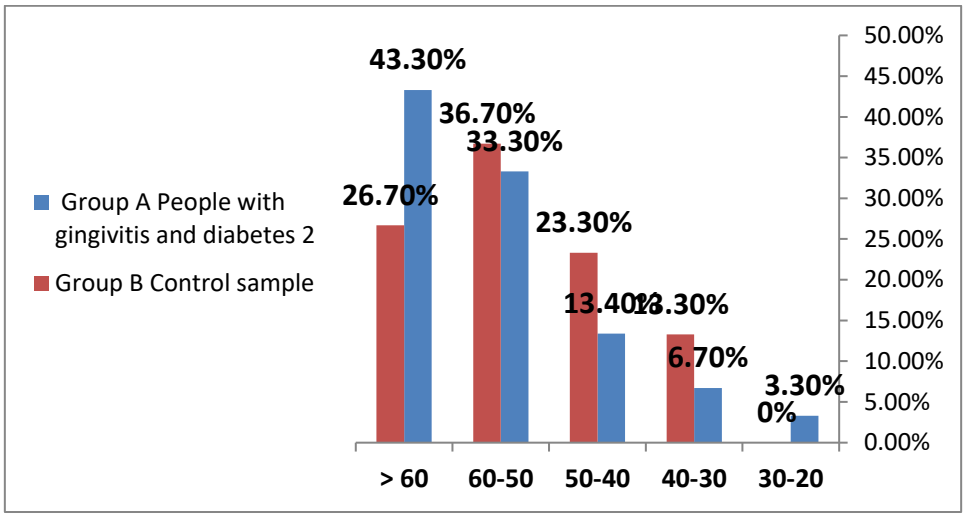

Figure 2. distribution of the sample according to age groups

Figure 3. it is clear that the highest percentage is *Streptococcus mutans* at 33.3%, followed by *Streptococcus anginosus* at 20.4%, then *Enterococcus faecalis* at 13%, and the lowest percentage is *Staphylococcus epidermidis* at 5.5%.

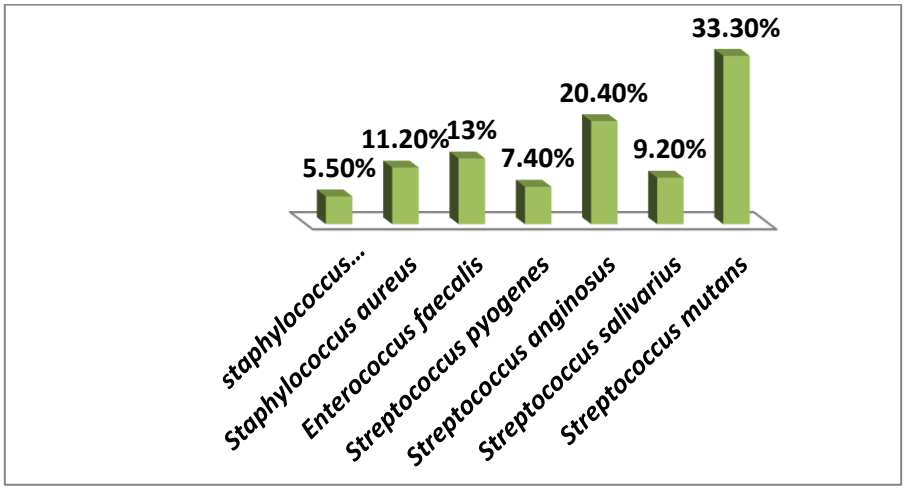

Figure 3. shows the bacterial species isolated from the gums of group A

Fig (4) it is clear that the highest percentage of bacteria isolated from the gums of group B (control sample) was for the two bacterial species: Proteusus merapilis (47%) and Escherichia coli (17.6%).

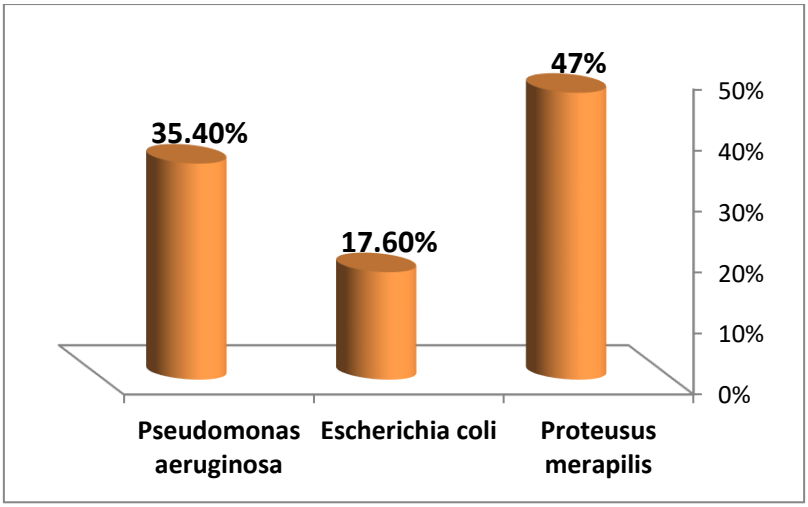

Figure 4. shows the bacterial species isolated from the gums of group B (control sample)

Figure 5. which shows the distribution of bacteria samples according to age for group A with gingivitis and type 2 diabetes, it is clear that the highest percentage of infection was for the age group 50-60 years, and bacterial infections were concentrated on Proteusus merapilis bacteria, and the percentage reached 54.5%, followed by Escherichia coli bacteria, 27.3%. The lowest percentage was for the bacteria type Pseudomonas aeruginosa, which was 18.2%. The next age group was > 60, and the highest percentage was for the bacteria type Pseudomonas aeruginosa, 50%, followed by Escherichia coli bacteria, 37.5%.

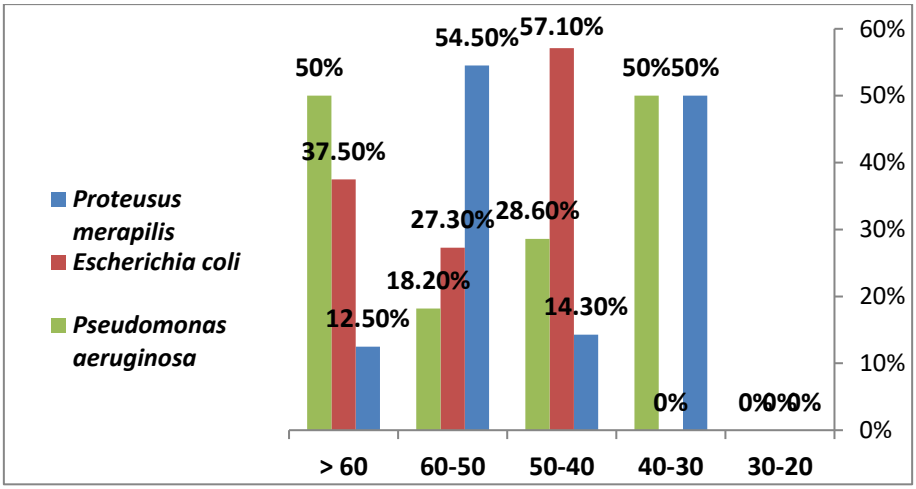

Figure 5. sample distribution of bacterial species according to age groups for samples isolated from the gums of group A

Figure 6. which shows the distribution of bacteria samples according to age for group B, the control sample, it is clear that the highest percentage of infection was for the age group > 60 years. Bacterial infections were concentrated on Streptococcus pyogene bacteria, and the percentage reached 38.5%, followed by Staphylococcus aureus bacteria, 27.3%, and the lowest percentage was for Streptococcus bacteria. mutans, and the percentage was 7.6%. The next age group was 50-60 years, and the highest percentage was for the Staphylococcus aureus bacteria, 30.7%, followed by Enterococcus faecalis, 20%.

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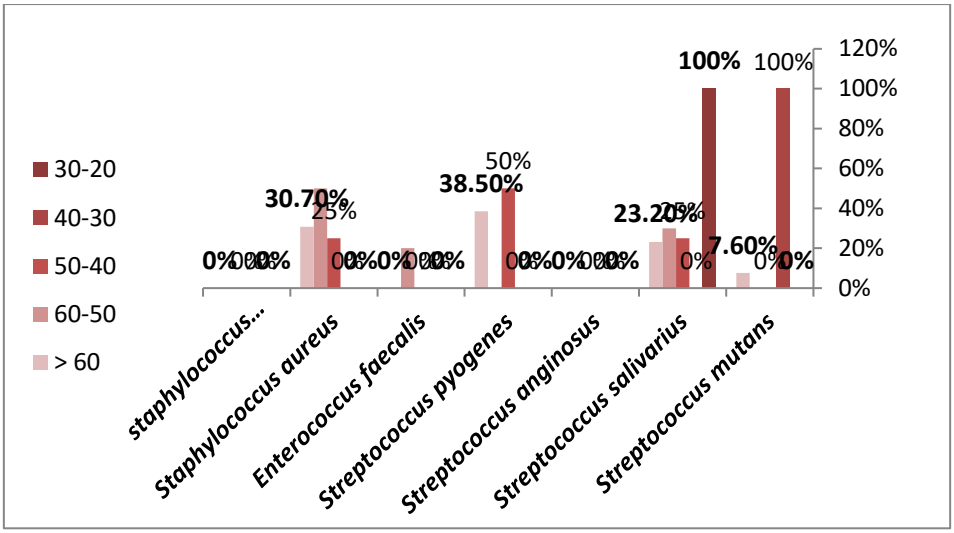

Figure 6. sample distribution of bacterial species according to age groups for samples isolated from the gums of group B (control sample)

Discussion

The present study investigated the distribution of demographic characteristics and bacterial isolates among patients with gingivitis associated with type 2 diabetes mellitus (Group A) compared with a control group without infection (Group B). The findings revealed clear differences in gender distribution, age groups, and bacterial profiles between the two groups, reflecting the influence of systemic and local factors on oral microbial ecology.

Regarding gender distribution (Table 1), males constituted the majority of the study sample (63.4%), while females represented 36.6%. This predominance of males is consistent with several previous studies reporting higher prevalence and severity of periodontal diseases among males, which may be attributed to poorer oral hygiene practices, higher smoking rates, and lower utilization of dental services compared with females [19.20]. Additionally, hormonal factors and greater health awareness among females may contribute to their lower prevalence of gingival inflammation.

Age distribution analysis (Table 2) showed that in Group A (patients with gingivitis and type 2 diabetes), the most affected age group was those older than 60 years (43.3%), followed by individuals aged 50–60 years (33.3%), whereas the youngest age group (20–30 years) showed the lowest prevalence (3.3%). This finding aligns with previous research indicating that the risk of periodontal disease increases with age due to cumulative plaque exposure, age-related immune dysfunction, and long-standing metabolic disorders such as diabetes mellitus [21.22]. In contrast, Group B (control group) showed the highest percentage in the 50–60 years age group, which may reflect age-related changes in oral flora even in the absence of overt gingival disease.

The microbiological findings revealed significant variations in bacterial species isolated from gingival samples. In Group A, Streptococcus mutans was the most frequently isolated bacterium (33.3%), followed by Streptococcus anginosus (20.4%) and Enterococcus faecalis (13%), while Staphylococcus epidermidis showed the lowest prevalence (5.5%) (Table 3). Streptococcus mutans is well recognized as a primary cariogenic organism due to its ability to ferment carbohydrates, produce acids, and form biofilms on tooth surfaces, thereby contributing to gingival inflammation and periodontal disease [23]. These findings are consistent with previous studies reporting Streptococcus mutans as one of the dominant organisms in gingivitis and dental caries, particularly among diabetic patients.

In Group B (Table 4), the highest percentage of bacterial isolates from gingival samples was Proteus mirabilis (47%), followed by Escherichia coli (17.6%). The presence of these Gram-negative bacteria may be related to transient colonization originating from the gastrointestinal tract, especially in older individuals or those with compromised immunity. Similar findings have been reported by several studies indicating that oral colonization by enteric bacteria can occur under conditions of poor oral hygiene or systemic illness [22].

Age-related distribution of bacterial isolates in Group A (Table 5) showed that individuals aged 50–60 years exhibited the highest infection rates, with Proteus mirabilis being the predominant organism (54.5%), followed by Escherichia coli (27.3%). In participants older than 60 years, Pseudomonas aeruginosa was the most prevalent isolate (50%), followed by Escherichia coli (37.5%). These findings suggest that advanced age combined with diabetes mellitus may favor colonization by opportunistic Gram-negative bacteria due to reduced immune defense mechanisms and altered oral environmental conditions. Similar trends have been observed in previous studies highlighting the role of diabetes in increasing susceptibility to opportunistic infections [21].

In Group B (Table 6), the highest infection rate was observed in individuals older than 60 years, with Streptococcus pyogenes being the most frequently isolated organism (38.5%), followed by Staphylococcus aureus (27.3%). In the 50–60 years age group, Staphylococcus aureus was the dominant isolate (30.7%). Staphylococci are known opportunistic pathogens commonly found on the skin, nasal mucosa, and oral cavity, and their ability to cause infection is enhanced by virulence factors such as surface adhesins, enzymes, and resistance-conferring plasmids. Their presence in oral infections has been documented in several clinical studies, particularly among hospitalized or immunocompromised individuals.

Overall, a total of 60 bacterial isolates were identified, with 30 isolates obtained from Group A. The predominance of Streptococcus species supports their central role in the initiation and progression of gingivitis. Moreover, the isolation rates of different bacteria may be influenced by dietary habits, particularly high carbohydrate intake, which promotes acidogenic bacterial growth and increases the risk of dental caries and gingival inflammation. This observation is supported by previous studies emphasizing the impact of diet on oral microbial composition [23].

Additionally, variations in bacterial isolation rates may be attributed to differences in oral hygiene practices, health awareness, cultural habits, and access to dental care. While laboratory factors such as culture media and isolation techniques play a role, they are generally considered less influential compared to host-related and behavioral factors. These findings are consistent with previous reports indicating that oral health behaviors and systemic health conditions significantly shape oral microbial diversity.

Conclusion

Through presenting and discussing the results, the following was concluded:

1. The highest percentage was for isolated samples of the male sex, and the percentage was 63.4%, while the percentage of the female sex was 36.6%.
2. The highest age group in Group A was > 60 years, and the percentage reached 43.3%. As for the second group, the highest percentage was for the age group 50-60 years, and the percentage reached 36.7%.
3. The highest percentage of isolated samples in group A was *Streptococcus mutans* bacteria, which reached 33.3%, followed by *Streptococcus anginosus*, 20.4%.
4. The highest percentage of isolated samples in group B (control sample) was *Proteusus merapilis* bacteria, which reached 47%, followed by *Pseudomonas aeruginosa* bacteria, which reached 35.4%.

5. According to the sample distribution for group A, the highest infection rate was for the age group 50-60 years, where the infection was concentrated in the *Proteusus merapilis* bacteria, and the percentage reached 54.5%.
6. According to the sample distribution for group B (control sample), the highest infection rate was for the age group > 60 years, where infection was concentrated in the *Proteusus merapilis* bacteria, and the percentage reached 38.5%.

Recommendation

Through the conclusions, the researchers recommend the following:
1. Conduct periodic examinations to detect infections affecting the gums and teeth.
2. Maintain the constant use of a toothbrush and replace it with another one every once in a while.
3. Follow the correct method of using the toothbrush and avoid random brushing that causes damage and tearing of the gums.
4. Quit smoking because of its great harm to the gums and teeth.
5. Continue and complete the research by taking larger surveys and expanding the research sample.
6. Conduct similar and complementary studies to this research to find out other types of bacteria that cause infections of the gums and teeth.

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