



Journal of Applied Science

Biannual Peer Reviewed Journal Issued by Research
and Consultation Center , Sabratha University

Issue (12)
April 2024





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Biannual Peer Reviewed Journal Issued by Research and Consultation Center,
Sabratha University

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Editorial

We start this pioneering work, which do not seek perfection as much as aiming to provide a scientific window that opens a wide area for all the distinctive pens, both in the University of Sabratha or in other universities and research centers. This emerging scientific journal seeks to be a strong link to publish and disseminate the contributions of researchers and specialists in the fields of applied science from the results of their scientific research, to find their way to every interested reader, to share ideas, and to refine the hidden scientific talent, which is rich in educational institutions. No wonder that science is found only to be disseminated, to be heard, to be understood clearly in every time and place, and to extend the benefits of its applications to all, which is the main role of the University and its scholars and specialists. In this regard, the idea of issuing this scientific journal was the publication of the results of scientific research in the fields of applied science from medicine, engineering and basic sciences, and to be another building block of Sabratha University, which is distinguished among its peers from the old universities.

As the first issue of this journal, which is marked by the Journal of Applied Science, the editorial board considered it to be distinguished in content, format, text and appearance, in a manner worthy of all the level of its distinguished authors and readers.

In conclusion, we would like to thank all those who contributed to bring out this effort to the public. Those who lit a candle in the way of science which is paved by humans since the dawn of creation with their ambitions, sacrifices and struggle in order to reach the truth transmitted by God in the universe. Hence, no other means for the humankind to reach any goals except through research, inquiry, reasoning and comparison.

Editorial Committee

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Journal Address:

Center for Research and Consultations, Sabratha University

Website: <https://jas.sabu.edu.ly/index.php/asjsu>

Email: jas@sabu.edu.ly

Local Registration No. (435/2018)

ISSN  2708-7301

ISSN  2708-7298

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- Keywords, max. 5 words.
- Introduction.
- Methodology.
- Results and Discussion.
- Conclusion.
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The Editorial Committee invites all researchers "Lectures, Students, Engineers at Industrial Fields" to submit their research work to be published in the Journal. The main fields targeted by the Journal are:

- Basic Science.
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CONTENTS

[1]	STUDY OF THE ACTIVE INGREDIENT OF FOUR DIFFERENT BRANDS OF COMMERCIAL DICLOFENAC SODIUM OF SELECTED PHARMACIES IN THE WESTERN REGION OF LIBYA.....	1
[2]	COMPARING SOLVING LINEAR PROGRAMMING PROBLEMS WITH APPLICATIONS OF THE MOORE-PENROSE GENERALIZED INVERSE TO LINEAR SYSTEMS OF ALGEBRAIC EQUATIONS	9
[3]	NITRATES IN MAN-MADE RIVER COMPARED TO GROUNDWATER WELLS IN EL-KUFRA AREA.....	19
[4]	DEGRADATION OF REACTIVE BLACK 5 DYE IN WATER FALLING FILM DIELECTRIC BARRIER DISCHARGE REACTOR (DBD).....	32
[5]	ANTIMICROBIAL RESISTANCE IN UROPATHOGEN ISOLATES FROM PATIENTS WITH URINARY TRACT INFECTIONS	44
[6]	SMOOTHING EFFECTS FOR A MODEL OF QUASI GEOSTROPHIC EQUATION.....	53
[7]	CLASSIFICATION OF BREAST CANCER USING MACHINE LEARNING ALGORITHMS.....	60
[8]	ENERGY-EFFICIENT INTRUSION DETECTION IN WSN: LEVERAGING IK-ECC AND SA-BILSTM	72
[9]	THE EFFECT OF ADDING POLYMER COMPOUNDS TO METALS ON ITS MECHANICAL PROPERTIES.....	89
[10]	EXPLORING DEADLOCK DETECTION ALGORITHMS IN CONCURRENT PROGRAMMING: A COMPARATIVE ANALYSIS AND EVALUATION	106
[11]	ISOLATION AND DETECTION OF BACTERIAL SPECIES CAUSING GINGIVITIS IN PATIENTS WITH TYPE 2 DIABETES.....	118

ISOLATION AND DETECTION OF BACTERIAL SPECIES CAUSING GINGIVITIS IN PATIENTS WITH TYPE 2 DIABETES

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Abstract

Background: People with diabetes mellitus type 2 (T2DM) tend to develop a variety of infections, including gum disease. Oral infections can make it difficult to control diabetes and cause many complications since the bacteria from severe gum disease may increase both blood sugar levels. Over the years, numerous studies have confirmed that oral dysbiosis plays a critical role in the pathogenesis and development of the most common oral diseases.

Materials and Methods: A survey study conducted at Sabratha Teaching Hospital (STH) aimed to detect the pathogenic bacterial species isolated from the oral cavity of diabetes mellitus patients type 2 (males and females) from different age groups as compared with those isolated from the oral cavity of healthy individuals. The first group (group A) included 30 cases of T2DM suffering from gingivitis, whereas the second group (group B) included 30 healthy participants as a control group who attended a dental clinic.

Results: The most common bacterial species present was *Streptococcus mutans*, with a frequency of 18 and a percent of 33.3%, followed by *Streptococcus anginosus*, 11 (20%), *Enterococcus faecalis*, 7 (13%), *Staphylococcus aureus*, 6 (11.2%), *Streptococcus salivarius*, 5 (9.2%), *Streptococcus pyogenes*, 4 (7.4%), and finally *Staphylococcus epidermidis*, 3 (5.5%). The most affected age group in Group A was > 60 years old, with a percentage of 43.3%, followed by the age group of 50–60 years old, 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 control group), 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%.

Conclusion: The most important observation of the current study was that T2DM increases the presence of some special bacterial species that may develop into pathogenic bacteria, which lead to infections under special conditions. Naturally, further studies are required to understand more details about this problem and to explore different complications related to this subject of research.

Introduction

Bacterial species in the human body are defined as a set of diverse microorganisms that inhabit specific niches of the human body, such as the gut, the oral cavity, the skin, and the genitourinary tract, and play a key role in the balance between health and disease (Blum, 2017). With regard to the current study object, the oral cavity houses one of the most diverse microbiomes in the human body, harboring hundreds of species (Deo et al., 2019; Zhang et al., 2018). Numerous studies have confirmed over the years that oral dysbiosis plays a crucial role in the pathogenesis and development of the most common oral diseases, such as dental caries, periodontal disease, and candidiasis, due to the presence of pathogenic and/or opportunistic microorganisms (e.g., *Streptococcus mutans*, *Staphylococcus aureus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Candida* sp.) (Hegde et al., 2006, Bagg et al., 2006). Additionally, due to the inherent risk of spreading to the various organs and tissues of the human body, these microorganisms can cause different systemic diseases, such as diabetes, obesity, or cardiovascular disease (Zhanget al., 2018; Kim et al., 2013).

Naturally, most oral microorganisms are non-pathogenic, but opportunistic commensals keep oral health conditions stable and protect against pathogenic microorganisms (Negrini et al., 2021). The alteration of typical oral bacterial species to pathogenic members results in dysbiosis and periodontal diseases (PD), such as periodontitis and gingivitis (Hegde et al., 1998; Negrini et al., 2021). Periodontal diseases are customarily separated into infections affecting the underlying tooth-supporting tissues of the periodontium, including the periodontal ligament, and the alveolar bone, 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 (Negrini et al., 2021). Although oral diseases remain a significant public health burden worldwide with significant socioeconomic impacts, they are frequently neglected in public health policy, particularly in developing countries (American Diabetes Association, 2019).

Diabetes mellitus has numerous complications in the oral cavity, represented by the increased pathogenic activity of the oral microbiota, which can cause diseases such as dental caries, PD, and oral candidiasis or cause physiological disorders such as dry mouth (xerostomia and decreased salivary flow rate), lesions in the oral mucosa, altered taste, geographic tongue, oral cancer, burning mouth syndrome, and temporomandibular disorders (Verhulst et al., 2019; Rohani, 2019; Reddy et al., 2019). Generally, diabetes frequently increases the number of oral microbiota, which leads to its diversification (Shillitoe, et al., 2012; Hsaine et al., 2018; Graves et al., 2019). Common bacterial species found in patients with DM compared to patients without DM or healthy people include hemolytic Streptococci, *Staphylococcus* spp., *Prevotella*

spp., *Leptotrichia* spp., and *Veillonella* spp. (Kulshrestha et al., 2011). On the other hand, the *Proteobacteria* species is not found in patients with DM, whereas it is clearly observed in healthy people (Sun et al., 2020; Matsha et al., 2020). Other species may not be different in either DM patients or non-diabetic patients, such as *Prevotella copri*, *Alloprevotella rava*, and *Ralstonia pickettii* (Sun et al., 2020).

The oral cavity's highly vascularized and innervated sites make it more susceptible to DM effects (Verhulst et al., 2019). Also, oral complications of DM play a significant role in increasing the severity of DM by increasing blood sugar levels (Rohani, 2019). According to previous studies, strong evidence was provided to support the positive effects of DM on the initiation or progression of different bacterial and fungal infections (Rohani, 2019). Moreover, hyperglycemia in the DM patient induces an increase in salivary glucose levels, which becomes a good nutrient for cariogenic bacteria in the dental biofilm (Vrhulst et al., 2019). A subsequent correlational analysis of the differential bacteria and clinical characteristics demonstrated that the oral microbiomes were related to drug treatment for DM and certain pathological changes (Yang et al., 2020), but the treatment did not lead to microbial recovery (Yang et al., 2020).

Dependently, the current study aimed to detect the pathogenic bacterial species isolated from the oral cavity of diabetes mellitus patients (males and females) from different age groups as compared with those isolated from the oral cavity of healthy individuals.

Materials and Methods

Study design and population: A survey study was conducted at Sabratha Teaching Hospital (STH). Participants have been divided into two groups. The first group (group A) included 30 cases of type 2 diabetes mellitus (T2DM) suffering from gingivitis, whereas the second group (group B) included 30 healthy participants as a control group who attended a dental clinic Table (1), Figure (1).

Table (1): Sample Distribution According to Gender.

Gender	Frequency	Percent %
Male	38	63.4%
Female	22	36.6%
Total	60	100%

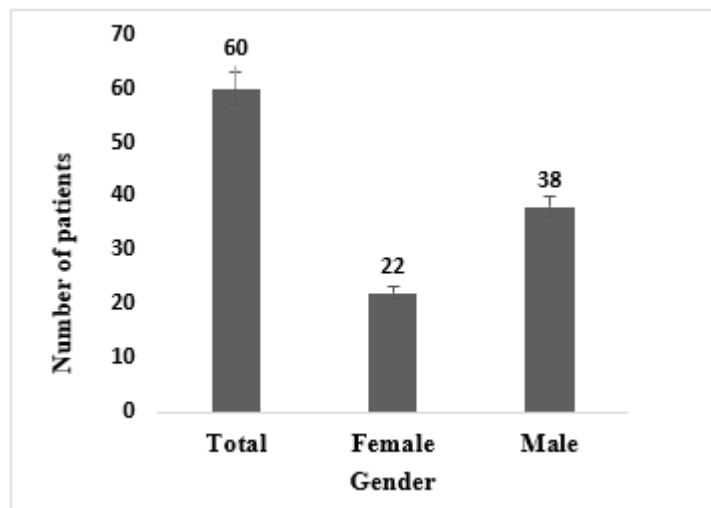


Figure (1): Sample Distribution According to Gender.

Swabs were taken periodically from both groups during the period of April 18th, 2023, to August 22nd, 2023. Samples are then transported immediately to the laboratory department (microbiology unit) for cultivation and detection. Both groups of participants ranged between 30 to 70 years in age. To facilitate the screening process, participants were divided into a number of age groups, as shown in. Denture wearers with or without oral lesions were included in this study. Participants with steroids or antibiotic use during the last 4 weeks have been excluded. Participants were asked to fill out a questionnaire form bearing information on demographics (age, sex) and medical history Table (2), Figure (2). Informed consent was obtained from each participant after explaining the nature and purpose of the study. All samples were collected after an oral examination.

Table (2): Distribution of the Sample According to Age.

Age (years)	Group A People with gingivitis/T2DM		Group B Control group	
	Frequency	Percent %	Frequency	Percent %
21-30	1	3.3%	0	0%
31-40	2	6.7%	4	13.3%
41-50	4	13.4%	7	23.3%
51-60	10	33.3%	11	36.7%
>60	13	43.3%	8	26.7%
Total	30	100%	30	100%

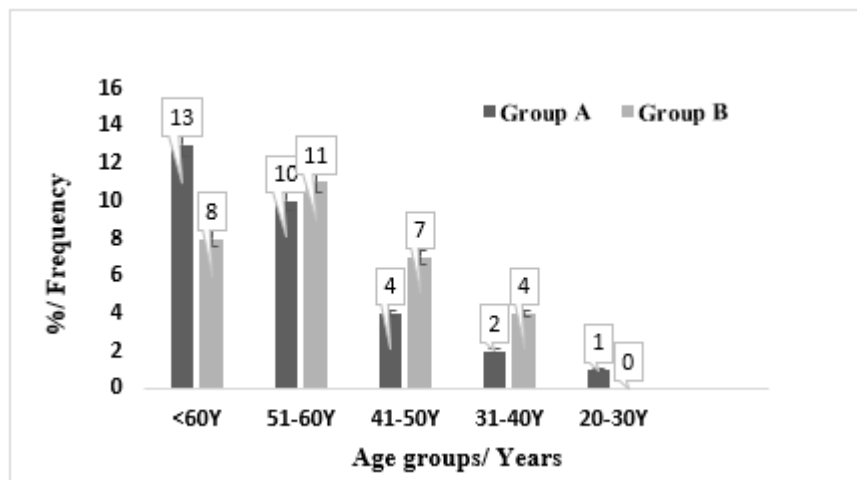


Figure (2): Distribution of The Sample According to Age.

Samples collection and cultivation: The buccal swabs were collected aseptically and inoculated into Petri dishes containing nutrient agar, then incubated for 24 hours at 37°C by repeated streaking onto the nutrient agar plate. Pure bacterial cultures were obtained. All quality assurance components were applied in the course of this study.

Statistical analysis: All data are arranged and entered into an Excel file (Microsoft Office version 2013) and then transferred into SPSS version 23 for statistical analysis. Different statistical measurements have been applied to the data, such as the mean value, the standard deviation P value, and the correlation between some important parameters. All results have been represented in tables and figures to clarify the different measurements done.

Results

Out of the 30 diabetic patients who participated in this study, the cultivation of samples indicated different species of bacteria available in their oral cavity. As shown in Table (3) and Figure (3), the most common bacterial species present was *Streptococcus mutans*, with a frequency number of 18 and a percent of (33.3%), followed by *Streptococcus anginosus*, 11 (20%), *Enterococcus faecalis*, 7 (13%), *Staphylococcus aureus*, 6 (11.2%), *Streptococcus salivarius*, 5 (9.2%), *Streptococcus pyogenes*, 4 (7.4%), and finally *Staphylococcus epidermidis*, 3 (5.5%).

Table (3): Bacterial Species Isolated from the Gums of the Diabetic Patients (Group A).

<i>Isolated bacterial species</i>	Frequency	Percent %
<i>Streptococcus mutans</i>	18	33.3%
<i>Streptococcus salivarius</i>	5	9.2%

<i>Streptococcus anginosus</i>	11	20.4%
<i>Streptococcus pyogenes</i>	4	7.4%
<i>Enterococcus faecalis</i>	7	13%
<i>Staphylococcus aureus</i>	6	11.2%
<i>Staphylococcus epidermides</i>	3	5.5%

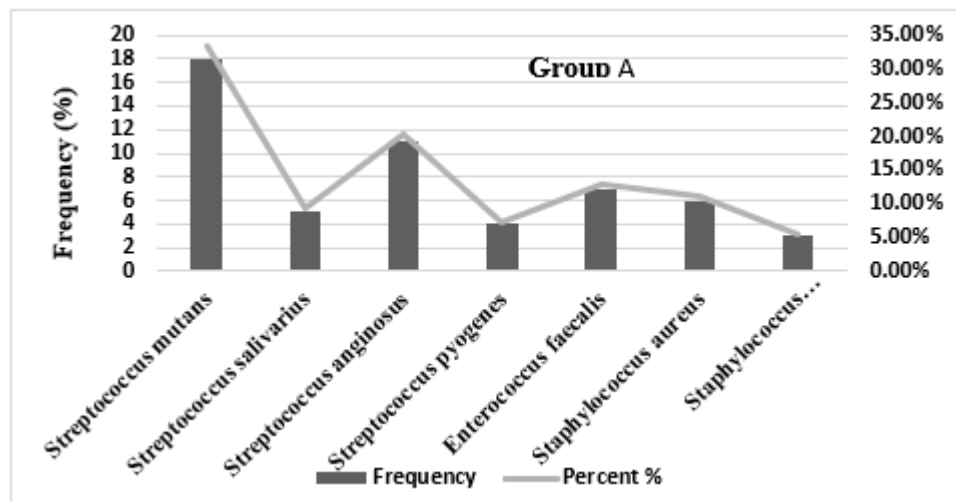


Figure (3): Bacterial Species Isolated from the Gums of the Patients (Group A).

As mentioned above, with regard to the presence of gingivitis, according to data in Table (2), the most affected age group in Group A was > 60 years old with a percentage of 43.3%, followed by the age group of 50–60 years old 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 control group), 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).

On the other hand, the most common bacterial species available in the oral cavity of the participants arranged in group B (the control group) was *Proteus mirabilis* 24 (47%), concentrated among the participants in the age group of 51–60 years old, followed by *Pseudomonas aeruginosa* 18 (35.4%), and finally *Escherichia coli* 9 (117.6%) Table (4), Figure (4).

Table (4): Bacterial Species Isolated from the Gums of Group B (control group).

<i>Isolated bacterial species</i>	Frequency	%
<i>Proteus merapilis</i>	24	%47
<i>Escherichia coli</i>	9	%17.6
<i>Pseudomonas aeruginosa</i>	18	%35.4

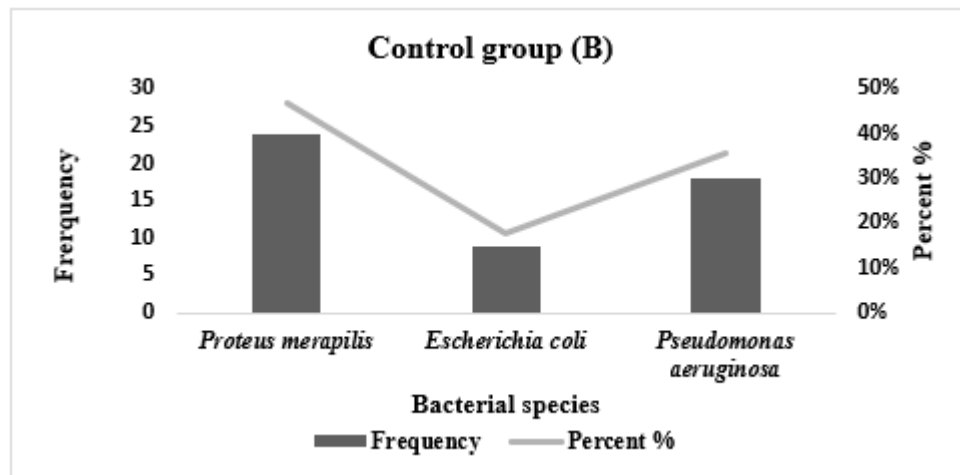


Figure (4): The Bacterial Species Isolated from the Gums of Group B (Control Sample).

Table (5) shows the distribution of isolated bacterial species from the oral cavity of the patient group (group A) according to age groups. Dependently, the highest bacterial infection was among T2DM patients in the age group (41–50) years old, with a percent of 57.1% and a frequency of four cases out of 30 patients in total. The bacterial infection determined in *Escherichia coli* was followed by a percent of 28% (Deo et al., 2019) with a bacterial species of *Pseudomonas aeruginosa* among the same age group.

The next highest percent of bacterial infection was spread among the patients belonging to the age group of 51–60 years old. 54.5% (Zhang et al., 2018) in frequency represents the infection by *Proteus mirapilis*, followed by 27.3% (Zhang et al., 2018) in frequency by *Escherichia coli*.

On the other hand, the highest percent of bacterial infection among the participants (T2DM patients) in the age group of 60 years old was 50%, with a frequency of 4 out of a total of 8 patients belonging to this age group Table (5), Figure (5).

Table (5): Distribution of Bacterial Species Isolated from the Gums of Patients (group A) According to Age Groups.

Isolated bacterial sp.	21-30 Y		31-40 Y		41-50 Y		51-60 Y		> 60 Y	
	F	%	F	%	F	%	F	%	F	%
<i>Proteus mirapilis</i>	0	0	2	50%	1	14.3%	6	54.5%	1	12.5%
<i>Escherichia coli</i>	0	0	0	0%	4	57.1%	3	27.3%	3	37.5%
<i>Pseudomonas aeruginosa</i>	0	0	2	50%	2	28.6%	2	18.2%	4	50%
Total	0	0	4	100%	7	100%	11	100%	8	100%

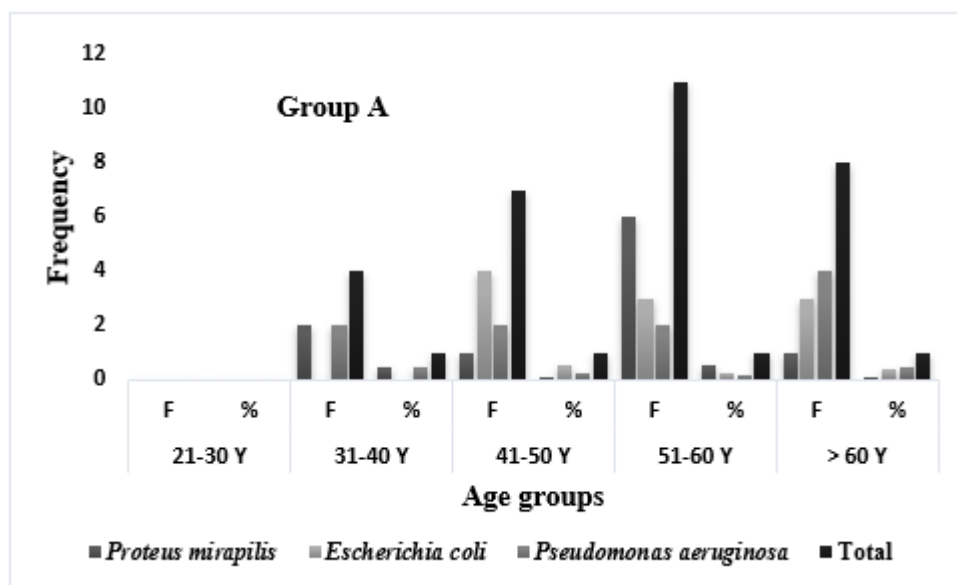


Figure (5): Distribution of Bacterial Species Isolated from the Gums of Patients (Group A) According to Age Groups.

With regard to the bacterial species isolated from the gum of the participants in group B, table 6 shows that the presence of bacteria was rare among them, especially between young ages. The highest percent among the age group of 60 years old was 38.5%, with a frequency of five participants represented by *Streptococcus pyogenes*. In contrast, *Staphylococcus aureus* has been isolated from five participants within the age group of 51–60 years old Table (6), Figure (6), with a low percent in other age groups or zero percent in most of them.

Table (6): Distribution of Bacterial Species Isolated from the Gums of Control Group (Group B) According to Age Groups.

Isolated bacterial sp.	21-30 Y		31-40 Y		41-50 Y		51-60 Y		> 60 Y	
	F	%	F	%	F	%	F	%	F	%
<i>Streptococcus mutans</i>	0	0%	2	100%	0	0%	0	0%	1	7.6%
<i>Streptococcus salivarius</i>	1	100%	0	0%	1	25%	3	30%	3	23.2%
<i>Streptococcus anginosus</i>	0	0%	0	0%	0	0%	0	0%	0	0%
<i>Streptococcus pyogenes</i>	0	0%	0	0%	2	50%	0	0%	5	38.5%
<i>Enterococcus faecalis</i>	0	0%	0	0%	0	0%	2	20%	0	0%
<i>Staphylococcus aureus</i>	0	0%	0	0%	1	25%	5	50%	4	30.7%
<i>Staphylococcus epidermids</i>	0	0%	0	0%	0	0%	0	0%	0	0%
Total	1	100%	2	100%	4	100%	10	100%	13	100%

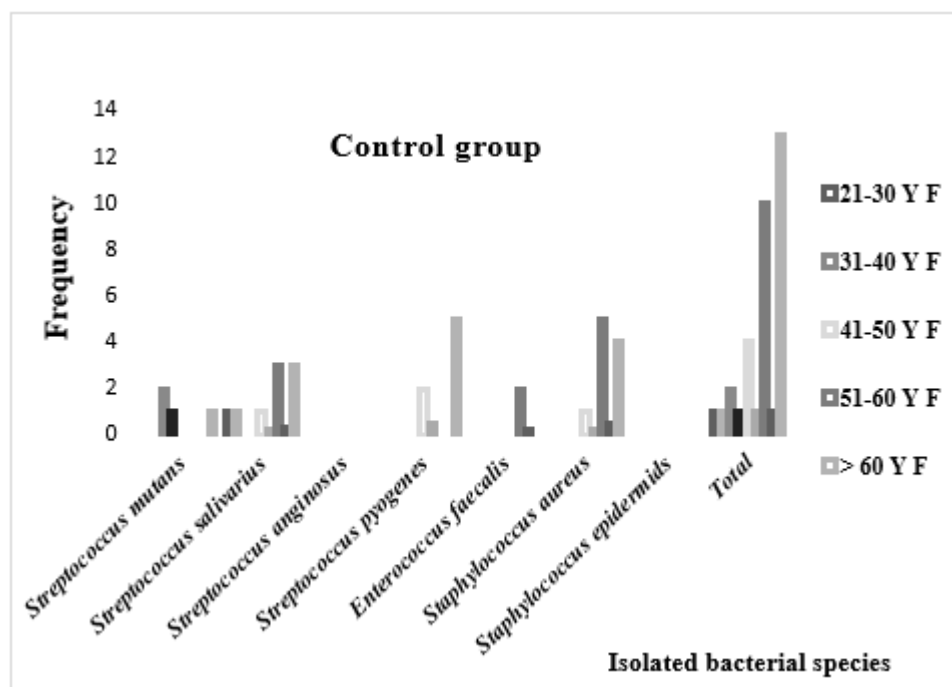


Figure (6): Distribution of Bacterial Species Isolated from the Gums of Control Group (Group B) According to Age Groups.

Discussion

The oral cavity contains a great number of microorganisms of high diversity (Kilian et al., 2016; Majumdar et al., 2014). This makes it the second-largest reservoir of different types of microbial communities (Kilian et al., 2016; Xiao et al., 2020). On the other hand, diabetes mellitus has numerous complications in the oral cavity, represented by the increased pathogenic activity of the oral microbiota, which can cause diseases such as dental caries, periodontal diseases (PD), and oral candidiasis or cause physiological disorders such as dry mouth (xerostomia and decreased salivary flow rate), lesions in the oral mucosa, altered taste, geographic tongue, oral cancer, burning mouth syndrome, and temporomandibular disorders (Verhulst et al, 2019, Rohani et al., 2019; Reddy et al., 2019).

Accordingly, this study presents the results of comparative investigations aimed at determining the microbiota that can occur in the oral environment in different human populations. The objective of the research was to identify pathogenic oral microbiota, the potential cause of health complications in patients of different population age groups, as compared with a healthy control group.

The current study identified several bacteria species recovered from periodontal sites and the oral cavity in general, namely *Streptococcus spp.*, *Enterococcus spp.*, *Staphylococcus spp.*, *Escherichia spp.*, *Proteus spp.*, and *Psoudomonas spp.*, in both diabetic (group A) and non-diabetic (group B) participants (tables 3 and 4). This result is consistent with what was published in a similar previous study in Bangladesh (Ali et al., 2021).

Generally, one of the most important observations during the current study was that the dental bacterial species isolated from diabetic individuals varied substantially from those of non-diabetic subjects. Such a different bacterial composition may result from the effect of diabetes altering the local environment within the periodontal pocket, which favors the growth of certain bacterial species.

As previously mentioned, while *Streptococcus spp.*, *Enterococcus spp.*, and *Staphylococcus spp.* were the most common bacterial species in diabetic participants, *Proteus spp.*, *Escherichia spp.*, and *Psoudomonas spp.* were the most common in non-diabetic participants. In this context, therefore, some studies have identified the association of altered gut and oral microbiota (dysbiosis) with varieties of autoimmune disorders, namely, T2DM and inflammatory bowel disease (IBD) (Mealey et al., 2000). The effects of more diversified oral bacteria in diabetic subjects are not yet apparent but probably relate to increased local or systemic inflammation. Dependently, more severe periodontitis exhibited in T2DM participants in this study was supported by earlier reports (Mealey et al., 2007; Preshaw et al., 2012).

As related to the distribution of isolated bacterial species among the participants in the current study (both groups, A and B), according to the age groups, one can observe

different scenes. Whereas in group A, isolated bacterial species were widely distributed among age groups, starting with the age group of (31–40) years old to the age group of (> 60) years old, the distribution of isolated bacterial species was different. The highest percent of bacterial species was in old age (51–60) and (> 60) years old Tables (5) and (6).

Furthermore, this study has demonstrated that *Streptococcus mutans* is the most common bacterial species found in the oral cavity of the participants with T2DM (group A). The percent of this sample was 33.3%, which is high as compared with those with non-diabetic participants. This result is consistent with the results observed in other studies (Ali et al., 2021).

Finally, this study highlighted a number of important points related to the presence of a number of bacterial species in the oral cavity of T2DM patients and in the oral cavity of healthy people. Naturally, further studies are required to understand more details about this problem and to explore different complications related to this subject of research.

Conclusion

The community of bacterial species in the oral cavity of different individuals seems to be dependent on different factors. Simultaneously, patients with DM are considered more vulnerable than others to developing some bacterial infections because their DM status has been unequivocally reported as a significant risk factor for oral diseases. The most important observation, depending on the results of the current study, was that T2DM increases the presence of some special bacterial species that may develop into pathogenic bacteria, which lead to infections under special conditions. Additionally, the presence of a larger percentage of some types of bacteria in the oral cavity of older age groups than other age groups makes these groups more vulnerable to the development of bacterial infections, especially in circumstances where they suffer from type 2 diabetes.

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