

## **Bacterial Diversity among Patients with Gingivitis and Dental Caries in Diyala Governorate**

**Iman Thamer Safad Al-Bawi, Saba J. Jawad Al-Zubaidi**

College of education for pure sciences , Diyala university . Iraq

### **Abstract**

100 bacterial isolates were isolated from the dental and gum surfaces of patients of both sexes and of different ages, as well as from mothers who gave birth to two or more children, from specialized centers and dental clinics in Diyala Governorate for the period from September 2021 to February 2022 AD. Bacterial isolates were diagnosed using the Phytex device, and they showed *Streptococcus* spp with the highest percentage of 39%, followed by *Staphylococcus epidermidis* 12%, *E.coli* 10%, *Lactobacillus* spp 9%, and *Actinomycetes* spp. In addition to bacterial species, *Candida* yeast appeared, which amounted to 12%. The results also showed that the percentage of bacterial diversity that causes gingivitis and tooth decay in females is more than males, at a rate of 59%. As for age groups, the age group came between 21-21 40)) are the most age groups that recorded bacterial diversity with a percentage of 49%, with a significant difference. While the results of bacterial diversity distributed according to the area of residence in Diyala governorate showed the highest percentage of bacterial diversity in Baquba district with a significant difference of 34%. The study also showed the percentage of bacterial diversity among mothers who had two or more children and the highest percentage was among women who they have more than two children, and the rate is 24.53%.

**Keywords:** *Streptococcus*, Bacterial Diversity, Gingivitis and Dental Caries

### **Introduction**

Dental caries is a common and important public health problem, as it is one of the most common chronic infectious oral diseases among people in the world. Its development stages begin through a series of complex chemical reactions resulting from microbial activities associated with the formation of biofilm on the dental plaque, which leads to the removal of minerals from the calcified membranes on the surface of the teeth and the decomposition of organic components (1). The energy that a person depends on in his diet, and at the same time, can cause the production of organic acids under anaerobic conditions, and these acids in turn reduce the pH of approximately (4.5) pH on the surface of the teeth, which leads to the removal of minerals (2). Dental plaque is one of the main causes of gum disease (Urzuz et al., 2008), where gingivitis is defined as a disease that affects the gum tissue, and the

inflammation is limited to the soft tissue surrounding the teeth in the gingival epithelium and connective tissue (3). Periodontal diseases rank 11th in terms of their prevalence around the world, and their prevalence reached from 20% to 50%, and in the period between 1990 to 2020, the rate of periodontal diseases reached about 57.3% (4). There are many types of bacteria in the mouth, and some of them cause tooth decay, where bacteria interfere with each other to try to form themselves in the environment, the most common of which are the oral rosococcus Streptococcus, followed by Staphylococcus spp, Lactobacillus spp, and Pseudomonas and E.coli bacteria, in addition to oral bacterial species, fungi, especially Candida yeast, recorded a presence in the mouth of 10.64% (5).

According to our follow-up to the scientific literature, it was found that there were no previous studies in Diyala Governorate specialized in studying bacterial diversity among patients with gingivitis and dental caries, as this study was the first locally, and accordingly this study aimed at the following:

- 1- Isolation and identification of bacterial types that cause gingivitis and dental caries
- 2- Studying the effect of some factors on infection rates and the epidemiological rate resulting from bacterial diversity in Diyala Governorate.

### **Materials and Methods**

110 samples of patients with dental caries and periodontitis were collected from specialized dental centers in Diyala governorate, distributed proportionally among its districts, for a period from the beginning of September 2021 to the end of February 2022, and the information about the patient was recorded in terms of gender and age. The housing and the location of the injury if it was inflammation of the gums or tooth decay, and the number of children in relation to the female mothers. Swabs were taken from decayed teeth and inflamed gums by means of sterile swabs, then the samples were planted in the laboratory and each sample according to its condition on different culture media, represented by blood agar medium, MacConkey agar medium, and Mitis Salivarius agar medium. Anaerobic under sterile conditions and incubated at 37 C for 24 hours for aerobic culture, as for anaerobic culture, the dishes were incubated at 37 C for 48 hours, and all samples were tested on gram dye for the purpose of staining bacteria and distinguishing between positive and negative for gram dye Where this dye showed the shape and arrangement of the bacteria, then the samples were diagnosed and confirmed using the Vitek 2 device.

## Results and Discussion

The samples of the current study included 75 samples from patients with dental caries, 68.2%, 35 samples from patients with gingivitis, 31.8%, 55 male samples, 50% and 55 female samples, and 50% for all age groups. The study also included mothers who had less or more than two children, and Table (1) shows the basic data for the study groups.

Table (1) shows the basic data for the study groups

	Value	Count	Percent	P value
Gender	Males	55	50.0%	1.00
	Females	55	50.0%	
Age groups	1-20	25	22.7%	0.001***
	21-40	54	49.1%	
	41-60	26	23.6%	
	>60	5	4.5%	
Living	Baquba	34	30.9%	p>0.05
	Khalja	30	27.3%	
	Baladrasa	23	20.9%	
	Khasaqin	23	20.9%	
Children	No	31	56.4%	0.001***
	1-2	10	18.2%	
	>2	14	25.40%	

The culture results also showed that 100 samples with a percentage of 90.9% showed positive growth of microbial culture and 10 samples with a percentage of 9.1% showed negative growth of microbial culture as shown in Figure (1).

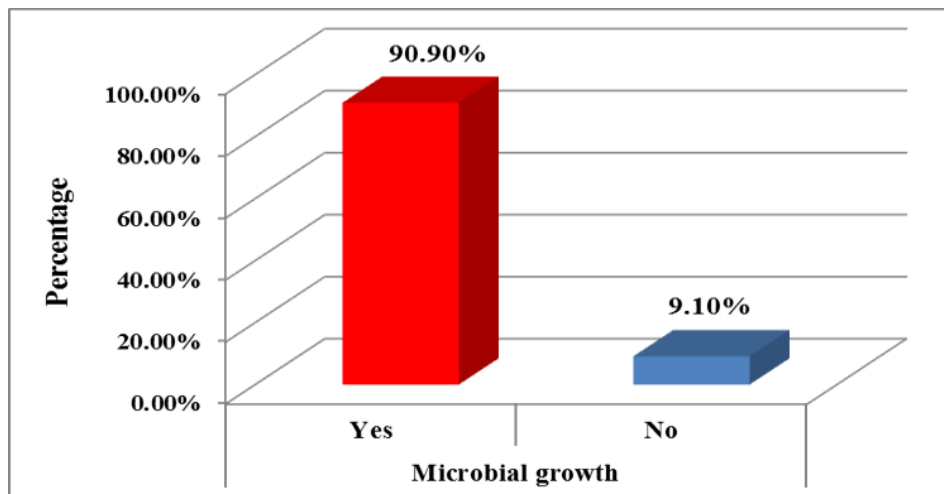


Figure (1) the percentage of microbial growth of the study groups

The results of the current study, using the Vitec device, showed that 39 isolates, and 39% of them belonged to the genus Streptococci, and that the predominance of streptococci bacteria over other isolates in the current study is consistent with the results of (6), which showed in his study that streptococci bacteria recorded the highest percentage Presence in the mouth at a rate of 33.3% as it is the main cause of dental caries lesions due to its important qualities that play an important role in increasing the incidence of infection such as its ability to convert fermented sugar into acid when competing with other bacteria and the ability to maintain diabetes metabolism under the circumstances The environment is very harsh, such as low pH, where few oral bacteria have the ability to withstand acidic conditions during the incubation period, but Streptococcus bacteria can pump proteins even in acidic conditions due to their enzymes operating at the optimum pH (7). Followed by Staphylococcus bacteria in the second place, which was 12 isolates and 12%, and this percentage is consistent with what came in (8), the results of its study showed that the prevalence of this bacteria in the mouth was 11%, and these species play An important role in dental caries and gingivitis, the cause of the spread of this bacteria is due to its being one of the important pathogens with the ability to cause opportunistic diseases due to its natural presence on the bodies of carriers on the skin and at the top of the nose and digestive and genital canals (9) It also possesses many surface antigens, enzymes, resistance mechanisms and transmission through plasmid conjugation and plasmidconjugation transformation that helps it penetrate body tissues (10). While coli bacteria were recorded. E The rate of isolation is 10%, and the reason for the low isolation rates for species belonging to the intestinal family (negative for gram-negative) is likely to be due to the scarcity of its presence in the oral cavity. Cream dye comes from infections of the respiratory and digestive system and appears in the mouth. It was followed by Lactobacillus bacteria, whose presence reached 9%. It is one of the species that resides in the mouth in small percentages in its natural state and increases with increasing acidity in the mouth, where sugar is converted into acid by the process of sugary fermentation by oral bacteria, including streptococci mutan, which provides A suitable environment for the growth and reproduction of lactobacilli bacteria that are acid-tolerant and productive, which causes calcium and phosphate withdrawal from the tooth enamel and the formation of holes and gaps in it (11). The lowest percentage recorded in the isolation results for this study was 6% and 7% for Pseudomonas and Actinomycetes respectively, and there are 5 other isolates with 5% belonging to different bacterial genera. The bacterial diversity in the oral environment of patients, which is located in the periodontal pockets, destroys the dental bone and periodontal ligaments, which leads to bleeding in the gum tissues, and the pockets in this case constitute a suitable place for bacterial growth (12). In addition to the results of bacterial diversity in the

mouth, isolates of *Candida* were obtained, which amounted to 12 samples with a percentage of 12%. Figure (4-2) shows the percentages of isolation of other bacterial species taken from infections of inflamed gums and decayed teeth. The percentage of isolation of bacteria is affected by many factors, including genetic and hormonal factors, type of food and the concentration of carbohydrates in food, which leads to an increase in infection Dental plaque and gums, especially *Streptococcus mutan* bacteria, where these bacteria have proven their ability to stick to the surface of the teeth, as well as to form a focus of adhesion with the rest of the microbes that ferment sugars in the mouth and produce acid that causes necrosis and calcium loss (13). Therefore, the oral cavity acquires changes in microbial species with a difference in the level of health awareness, daily cleaning of the teeth and the culture of the community.

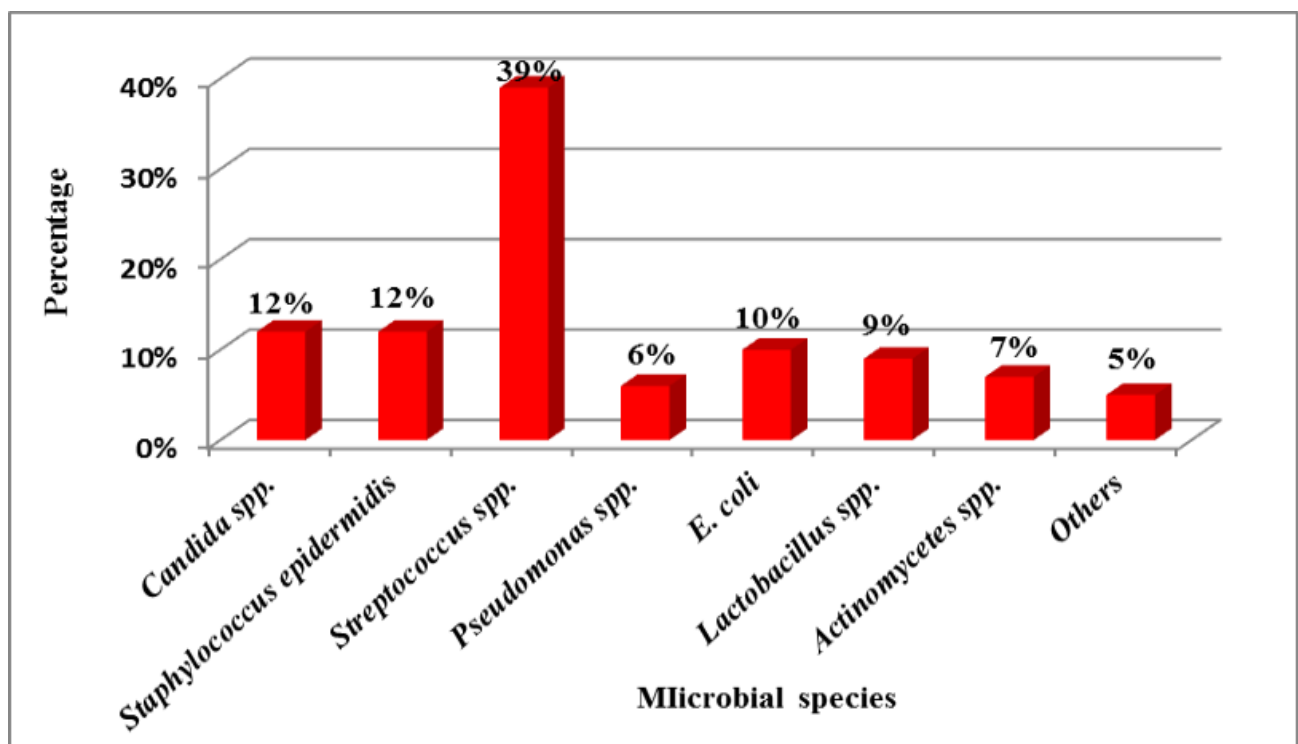


Figure (2) Percentages of bacterial species

## Epidemiology of bacterial diversity in Diyala governorate

### The relationship of bacterial diversity with sex

The results of the current study, which included 100 microbial isolates, showed that the percentage of bacterial diversity that causes gingivitis and dental caries in females is higher than males with a significant difference. *Streptococcus* isolates by sex, the results showed that the number of *Streptococcus* isolates in females is greater than in males, with a difference of 23 and 59% in females, and 16 isolates at 41% in males. The results were in agreement with (14), where it was shown in his study that the number of infected females is more than males as shows in Table (2)

Table (2) Percentage of bacterial diversity by sex

Microbial growth		Gender		Total	P value
		Males	Females		
<i>Streptococcus spp.</i>	N	16	23	39	P>0.05
	%	41.0%	59.0%	100.0%	
<i>Staphylococcus epidermidis</i>	N	4	8	12	P>0.05
	%	33.3%	66.7%	100.0%	
<i>E. coli</i>	N	4	6	10	P>0.05
	%	40.0%	60.0%	100.0%	
<i>Lactobacillus spp</i>	N	6	3	9	P>0.05
	%	66.7%	33.3%	100.0%	
<i>Actinomyces spp</i>	N	3	4	7	P<0.05
	%	42.9%	57.1%	100.0%	
<i>Pseudomonas</i>	N	5	1	6	P<0.001***
	%	83.3%	16.7%	100.0%	
<i>Candida</i>	N	5	7	12	P>0.05
	%	41.7%	58.3%	100.0%	
Other	N	4	1	5	P<0.001***
	%	80.0%	20.0%	100.0%	
Total	N	47	53	100	P>0.05
	%	47.0%	53.0%	100.0%	
	P<0.01		P<0.001	P<0.001	
	**		***	***	P<0.001***
					P>0.05

### Age related to bacterial diversity

The current study included most of the age groups of children, adults and the elderly with tooth decay and gingivitis, and the results showed that the age group (21-40) is the most diverse age group that recorded a diversity of bacteria, where the number of isolates for this group was 49 isolates, with a percentage of 49%, with a significant difference. Given that this group occupies the largest segment of society, in addition to the association of these ages with smoking problems for males and the effect of hormonal factors and puberty on both sexes. Table (3) shows the percentage of bacterial diversity in the mouth according to age groups.

Table (3) Percentage of bacterial diversity in the mouth according to age groups

Microbial growth	Age groups (years)				Total	P value 1-	
	20	21-40	41-60	>60			
<i>Streptococcus spp</i>	n	9	19	9	2	39	0.01**
	%	23.1%	48.7%	23.1%	5.1%	100.0%	
<i>Staphylococcus epidermidis</i>	n	3	6	2	1	12	P>0.05
	%	25.0%	50.0%	16.7%	8.3%	100.0%	
<i>E. coli</i>	n	0	9	1	0	10	0.001**
	%	0.0%	90%	0.0%	5.1%	100.0%	
<i>Lactobacillus spp</i>	n	4	3	2	0	9	P>0.05
	%	44.4%	33.3%	22.2%	0.0%	100.0%	
<i>Actinomyces spp</i>	n	3	2	2	0	7	P>0.05
	%	42.9%	28.6%	28.6%	0.0%	100.0%	
<i>Pseudomonas spp</i>	n	1	3	1	1	6	P>0.05
	%	16.7%	50%	16.7%	0.0%	100.0%	
<i>Candida</i>	n	3	4	4	1	12	P>0.05
	%	25%	33.3%	33.3%	8.3%	100.0%	
<i>Others</i>	n	1	3	1	0	5	P>0.05
	%	20.0%	60.0%	20.0%	0.0%	100.0%	

### Relationship of bacterial diversity to the site of oral infection

The current study showed the distribution of bacterial diversity according to samples taken from decayed teeth and inflamed gums, and the results showed that the percentage of bacterial diversity in dental samples was more than gum samples, with a difference of 30 samples, as it was 65% for 65 patients, while the percentage of bacterial diversity for people with gingivitis It reached 35% for 35 patients, the reason

for the low percentage of people with gingivitis is likely to not visit specialized dental centers and

only take antibiotics to reduce inflammation, except in cases of exacerbation of inflammation and severe infection of the gum tissue, while the frequency of people with dental caries is higher than it Symptoms that have a significant impact on daily life such as difficulty chewing and severe toothache. Table (4) shows the percentage of bacterial diversity according to the location of the infection in the mouth.

Table (4) Percentage of bacterial diversity in the mouth according to the site of infection

infection site Microbial growth	Tooth		Gums		Total	P value
	N	%	N	%		
<i>Streptococcus spp</i>	9	59%	3	16.41%	139	P<0.001***
					100.0%	
<i>Staphylococcus epidermidis</i>	4	75.0%	1	60.0%	12	P<0.001***
					100.0%	
<i>E. coli</i>	8	40.0%	2	57.1%	9	P>0.05
					100.0%	
<i>Lactobacillus spp</i>	3	88.9%	0	16.7%	7	1.00
					100.0%	
<i>Actinomyces spp</i>	3	50.0%	3	0.0%	6	P>0.05
					100.0%	
<i>Pseudomonas spp</i>	5	83.3%	0	0.0%	5	P<0.001***
					100.0%	
<i>Candida</i>	65	65.0%	0	0.0%	100	P>0.05
					100.0%	
<i>Others</i>	***		***		***	1.00
Total						P<0.05*
P value						

### Relationship of bacterial diversity with the area of residence

The current study included the distribution of bacterial diversity that causes dental caries and periodontitis according to the districts of Diyala Governorate as in Table (5), and the results showed that the highest percentage of bacterial diversity was recorded in Baquba district with a significant difference of 34 isolates and a percentage of 34%, and this is explained by the fact that Baquba district It is the center of the province and overcrowded with high population rates, as well as the large consumption of foodstuffs rich in sugars, carbohydrates and canned goods by the people of this region, followed by Al-Khalis district, in which the number of isolates was 30 by 30% for all bacterial races, as well as the record of bacterial diversity. The cause of dental caries and gingivitis in Baladrooz district was 23% for 23 isolates, while Khanaqin district had the lowest percentage among other districts by 13% for 13 isolates.



Table (5) Percentage of bacterial diversity according to the area of residence

Living		Khalis	Baladrose	Khanagin	Total	P value	
<i>Baquba</i>	N	12	14	11	2	39	
<i>Streptococcus spp</i>	%	30.8%	35.9% 1	28.2% 4	5.1%	100.0%	P<0.05
	n	4	8.3% 1	33.3% 4	3	12	
<i>Staphylococcus epidermidis</i>	%	33.3%	10% 2	40% 0	25.0%	100.0%	P>0.05
	n	4	50.0% 1	0.0% 4	1	10	
<i>E.coli</i>	%	40% n	22.2% 3	0.0% 0	10%	100.0%	P>0.05
	n	4	50% 2	0.0% 2	3	9	
<i>Lactobacillus spp</i>	%	44.4%	16.7% 3	16.7% 0	33.3%	100.0%	P>0.05
	n	4	60.0%	0.0% 23	1	10	
	%	44.4%	30	23.0%	33.3%	100.0%	P>0.05
<i>Actinomyces</i>	n	1	30.0%	P<0.001	2	6	
	%	16.7%	P<0.001	***	33.3%	100.0%	P>0.05
	n	6	***		2	12	
<i>Pseudomonas</i>	%	50% n			16.7%	100.0%	P>0.05
	n	2			0	5	
	%	40.0%			0.0%	100.0%	P>0.05
<i>Candida</i>	n	34			13	100	P>0.05
	%	34.0%			13.0%	100.0%	P>0.05
<i>Others</i>	P<0.001					P<0.001	P>0.05
	***				P>0.05	***	P>0.05
<b>Total</b>							P<0.05*
<b>P value</b>							

### The relationship of bacterial diversity in mothers who have a number of children

The results show the bacterial diversity of mothers who have a number of children, which included two categories. The first category was for mothers who had two children or more, and whose results recorded the highest bacterial diversity rate, which is 24.53%, in 13 isolates, while the second category was for mothers who have (1- 2) A child, in which the bacterial diversity ratio was 16.98%, by 9 isolates, with a significant difference. These results were in agreement with (15), where the results of her study showed that the highest rate of infection appeared in women who have (3-4) at a rate of 55.17%. Table (6) shows the percentage of bacterial diversity among mothers who have two or more children.

Table (6) the percentage of bacterial diversity among mothers who have a number of children

Microbial growth	No	N	Children		Total	P value
			1-2	>2.5		
		14	4	2		
	%	60.87%	17.39%	21.74%		
<i>Streptococcus spp.</i>	N	5	1	2		P<0.01**
	%	62.50%	12.50%	25.00%	100.00%	
<i>Staphylococcus epidermidis.</i>	N	3	2	1	8	P<0.01**
	%	50%	33.3%	16.67%	100.00%	
<i>E.coli</i>	N	2	0	1	6	
	%	66.67%	0.00%	33.33%	100.00%	
<i>Lactobacillus spp</i>	N	3	1	0	3	P>0.05
	%	75%	25%	0.0%	100.00%	
<i>Actinomycetes spp.</i>	N	0	0	1	4	
	%	0.0%	0.00%	100%	100.00%	P>0.05
<i>Pseudomonas</i>	N	4	1	2	1	
	%	75.00%	25.00%	0.00%	100.00%	
<i>Candida</i>	N	0	0	1	7	P<0.001***
	%	0.00%	0.00%	100.00%	100.00%	
<i>Others</i>	N	31	9	13	1	
	%	58.49%	16.98%	24.53%	100.00%	1.00
	P<0.001 ***		P>0.05	P>0.05	53	
					100.00%	
					P<0.001 ***	P<0.001***
<b>Total</b>						1.00
<b>P value</b>						P<0.01**

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