



# Evaluation of the effect of patient related factors on bacterial periodontitis in Salah al-Din city in Iraq

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Received 1 Apr 2024; Accepted 2 May 2024; Published 7 May 2024

## Abstract

One of the most common bacterial infections in people is periodontal disease. its primary reasons for tooth loss. The primary cause of these oral ulcers is the colonization of harmful microorganisms on the surfaces of the mouth. These microorganisms differ based on the lesion under investigation, the length of their development, and the host.

## Aim of the study

The study aimed to evaluate the bacterial causes of periodontitis and determined their antibiotic resistance and explain the relation between the bacterial spp. isolate with different patient's variables.

## Methods

90 specimens were collected from patients with gingivitis resulting from orthodontic and placement from medical clinics and health centers in Balad district in Salah al-Din Governorate after they were diagnosed in bed with gingivitis by specialist doctors. Swabs of the inflamed gums were taken and then cultured on appropriate culture media and appropriate diagnostic tests were performed, the disc diffusion method were used for antibiotics susceptibility test for all isolated bacteria.

## Results

The study showed that periodontitis was most prevalent in teenagers (65%) and in female (63.3%) with mixed diet (61.6%) and urban residence (74.7%). *Streptococcus pyogenes* was most common isolated bacteria from patients (61.9%) also other bacteria were obtained. *Strep. pyogenes* was sensitive to Ampicillin, Augmentin and Ceftazidime while resist to metronidazole and tetracycline.

## Conclusion

The periodontal status is influenced by numerous patient-related local variables. The most resistant bacteria in the current investigation, *Streptococcus pyogenes*, demonstrated considerable antibiotic sensitivity.

**Keywords:** gingivitis, swab, ampicillin, *Streptococcus pyogenes*, urban

## Introduction

Because it affects 60–90% of the world's population collectively, periodontal disease is regarded as the most common bacterial infection in humans <sup>[1]</sup>. Approximately 15–25% of adult Americans experience tooth loss, with periodontal disorders thought to be the primary cause <sup>[2]</sup>.

The primary cause of these oral diseases is the growth of harmful microorganisms on the surfaces of the mouth. These microbes differ based on the lesion under investigation, the length of their development, and the host. However, one of the most significant virulence determinants and, thus, one of the characteristics of these colonizers that has been researched the most is their capacity to proliferate in biofilms. Complex microbial colonies known as biofilms are formed on a range of surfaces. Typically, they are connected to an extracellular matrix made up of several biopolymer types <sup>[3]</sup>.

Antibiotic resistance is conferred by growth within biofilms because different antimicrobial drugs are less able to penetrate its internal structure <sup>[4]</sup>. Therefore, it is very difficult or impossible to remove microorganisms from biofilms <sup>[5]</sup>. Over 500 distinct bacterial species are found in the complex oral microbiota <sup>[6]</sup>.

The microbial community in the mouth is generally in balance, but when this balance is upset, disease can develop. We now

have a better understanding of the microorganisms that cause dental caries, periodontal disorders, and the majority of oral infections. In the absence of appropriate treatment, periodontal diseases—possibly the most prevalent chronic inflammatory problem among adults—may result in the loss of teeth. Periodontal illnesses are commonly classified into two groups: gingivitis, which affects just the gingiva, and periodontitis, which affects the tissues of the periodontium that support the teeth, including the alveolar bone and the periodontal ligament. An increase in the mass of bacteria, either Gram-positive or Gram-negative, at or under the gingival crevice might cause a nonspecific inflammatory reaction that leads to gingivitis <sup>[7]</sup>. As a result, a periodontal pocket forms, which is very conducive to additional bacterial buildup and a change in the proportionate composition of bacteria. There are periods of active and inactive tissue destruction in the episodic evolution of periodontitis. This illustrates how the host immune system and the bacterial challenge behave in opposition to one another. according to how often isolation occurs in lesion areas. The most prevalent kind of periodontitis, adult periodontitis, has been linked to a collection of approximately 10 bacterial species, including *P. gingivalis*, *S. mutans*, *S. pyogenes*, and other bacteria <sup>[8]</sup>. These bacterial species cause periodontitis, a

mixed anaerobic infection, by acting cooperatively or synergistically [9].

## Methods

### Study sample

90 specimen (60 patients and 30 control) were collected from patients with gingivitis resulting from orthodontic and placement from medical clinics and health centers in Balad district in Salah al-Din Governorate after they were diagnosed in bed with gingivitis by specialist doctors for a period from 1-11-2023 to 15-1-2024, and the patients' information was recorded. Which includes age, gender, chronic diseases, nature of food, and nature of the patient's residence. Swabs of the inflamed gums were taken using sterile swabs transport media containing until they were transported to the laboratory. The swabs were cultured on appropriate culture media and appropriate diagnostic tests were performed.

### Culturing of samples

The samples were cultured using the planned method on culture media that are suitable for bacterial growth. This media includes blood agar, MacConkey agar, mannitol salt agar, and the selective medium MSB agar. The petri dishes were incubated upside down in the incubator for a period ranging from 18-24 hours until use and for the purpose of performing diagnostic tests. The diagnosis was made according to the standard methods followed in Forbes *et al* [10].

### Culture characteristic and microscopically examination

The phenotypic characteristics of the bacterial colonies growing on nutritious agricultural media and the various selective media mentioned previously were diagnosed, such as color, shape, smell, texture, height, size of the colony and its edges. The isolates were then stained with the Gram stain and examined using an optical microscope and then identification is made by biochemical tests and confirmed by VITEK 2 compact system.

### Antibiotic susceptibility test

The method of spreading around discs on MHA (Mueller Hinton Agar) medium for this purpose Conducting susceptibility testing for bacterial isolates using the modified Kirby–Bauer method described by the World Health Organization (Humphries *et al.*, 2021) using discs with concentrations specified in micrograms which were (Ampicillin, metronidazole, Tetracycline, Amoxicillin/Clavulanic acid, Augmentin and Ceftazidime).

### Statistical analysis

The statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences), version 25 (SPSS, Chicago). Continuous data were subjected to the Shapiro Wilk test for normality, and data with a normal distribution were presented as standard deviation and analyzed using the student t test. Then, the data with non-normal distribution were presented as the mean and range and analyzed using the Mann-Whitney U test.

## Results

### Periodontitis infection distribution according to demographic characteristics and other variables

Table (1) showed that the results recorded that Periodontitis infection distribution in age groups (10-20, 21-30 and < 31) were (65%, 28.3% and 6.7%) respectively, in which young aged group scored high percentage and older patients scored lowest percentage, compared with controls groups in same age groups recorded (30%, 66.7% and 3.3%) respectively, periodontitis showed significant difference P- value between age groups.

According to sex groups recorded high rates in female (63.3%) than with the male that recorded (36.7%), Compared with the controls groups female and male recorded that (66.7%, 33.3%) respectively, there is significant differences *p*-value among gender groups in Periodontitis compared with controls groups. While according to diet which divided into two types according to the nature of the food into plant foods and mixed foods recorded that (38.3%, 61.7%) respectively, compared with control groups (16.7%, 83.3%) respectively, periodontitis was recorded that no significant difference *p*-value according to diet patients with Periodontitis divided according to residence, patients lived in urban recorded high rate (76.7%) than lived in rural recorded (23.3%), compared with control groups lived in Urban and Rural recorded (63.3%, 33.7%) respectively periodontitis was recorded that were significant difference *p*-value according to residence.

**Table 1:** The frequency distribution of patients with periodontitis infection according to demographic characteristics and other variables

Variable	Category	Periodontitis groups No. 60 N (%)	Control groups No. 30 N (%)
Age group	10-20	39 (65%)	9 (30%)
	21-30	17 (28.3)	20 (66.7%)
	<31	4 (6.7%)	1 (3.3%)
Sex	Male	22 (36.7%)	10 (33.3%)
	Female	38 (63.3%)	20 (66.7%)
Diet	Vegetarian food	23 (38.3%)	5 (16.7%)
	Mixed food	37 (61.7%)	25(83.3%)
Residence	Urban	46 (76.7%)	19 (63.3%)
	Rural	14 (23.3%)	11 (33.7%)

### Bacterial isolation and identification

A total of 90 clinical samples were obtained from patient's gingivitis. After collecting the samples all samples were grown on blood, MacConkey, mannitol salt agar and Chromogenic Medium and other simple media plates and incubated aerobically at 37 °C for 24 hours.

About (98.3%) showed positive bacterial growth which were *Streptococcus pyogenes* (61.9%), *Staphylococcus aureus* (11.1%), *Enterococcus faecalis* (7.9%), *Streptococcus mutans* (6.3%), *Klebsiella spp* and *Staphylococcus epidermidis* (4.8% %) for both, and (1.6 %) for *Streptococcus sanguinis* and *kocuria kristinae*, that was diagnosed based on biochemical tests. and the diagnosis of some bacterial species was confirmed with the VITEK device., whereas (1.7%) showed no growth, which might be attributed to antibiotic treatment or the presence of other types of causative agents which may need specialized diagnostic tests.

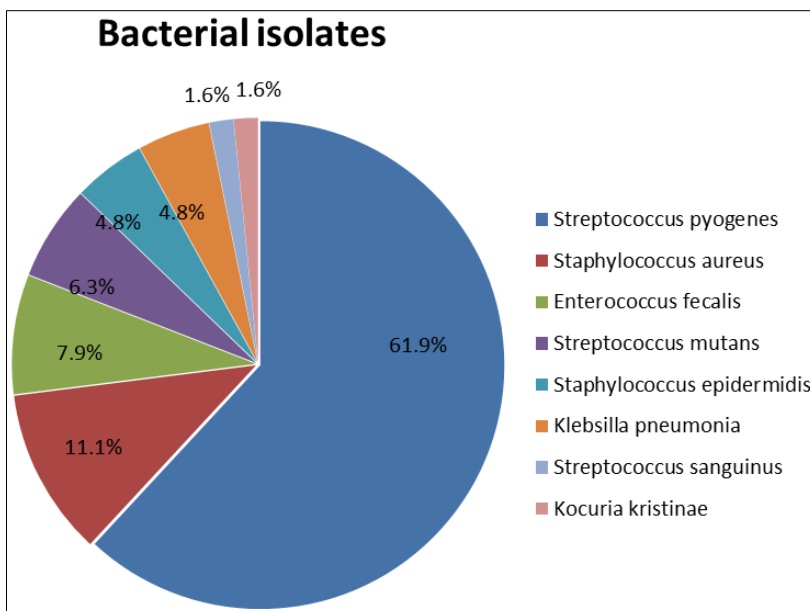


Fig 1: Bacterial isolations from periodontitis

**Relationship between bacterial spp. and demographic characteristics and other variables of periodontitis patients**

The current study showed that there was a significant difference ( $p < 0.05$ ) between age groups and diet with isolated bacteria, *Strep pyogenes* was most isolated bacteria from all

ages with different diet groups. According to the gender and residence there was no significant difference ( $p > 0.05$ ) with isolated bacteria but *strep. pyogenes* showed most isolated spp. in these groups, Table (2).

Table 2: Relationship between bacterial spp. And different variables in periodontitis patients

Variables		<i>Strep. pyogenes</i>	<i>Strep. mutans</i>	<i>Staph. aureus</i>	<i>Staph. epidermidis</i>	<i>Klebsilla pneumonia</i>	<i>Enterococcus fecalis</i>	p value
Age	10-17	66.6%	8.3%	12.5%	4.2%	4.2%	4.2%	0.047
	18-25	52%	8%	12%	8%	4%	16%	
	26-30	54.5%	0%	9.1%	9.1%	18.2%	9.1%	
Gender	Male	47.8%	8.7%	17.4%	4.4%	8.7%	13%	0.721
	Female	64.9%	5.4%	8.1%	8.1%	5.4%	8.1%	
Residence	Urban	59%	4.5%	11.4%	9.3%	6.7%	12.5%	0.740
	Rural	56.4%	6.3%	12.5%	0%	6.3%	9.1%	
Diet	Vegetarian food	47.6%	4.8%	9.5%	19%	14.3%	4.8%	0.034
	Mixed food	64.1%	7.7%	12.8%	0%	2.6%	12.8%	

**Antibiotic resistance of bacterial isolates isolated from periodontitis**

The significantly emergence of antimicrobial resistance and the absence of new antimicrobial drug development has gradually reduced the treatment choices for bacterial infection disease [11].

Table 3 shows that *Enterococcus fecalis* resistance to (Ampicillin, metronidazole, tetracycline and Augmentin) total of Bacteria (30). *klepsiella pneumoniae* resistance to (Ampicillin, metronidazole and Augmentin) total of Bacteria (20). While *Kocuria kristinae* there was no resistance to the antibiotics mentioned in the study Total of Bacteria (5). *Staphylococcus aures* and *Staphylococcus epidermidis* resistance to (Ampicillin, tetracycline and Augmentin) total of Bacteria (25, 20) respectively. As well as *strepptococcus pyogen*, *streptococcus mutans* and *streptococcus sanguinis* resistance to (metronidazole and tetracycline) total of Bacteria (185, 15 and 5) respectively.

5 milliliters of sterile normal saline was used to suspend pure colonies of bacterial isolates, and these were then compared to the 0.5 McFarland standard. To uniformly inoculate samples

from the suspension onto MHA, a sterile cotton swab was utilized, within 2–15 minutes seven antibiotics, including (Ampicillin (AM. 25μ), metronidazole (MET. 30μ), tetracycline (TE. 10μ), Augmentin (AMC 30 μ), and Ceftazidime (CAZ 30), were placed on the MHA plates inoculated with test bacteria and incubated at 37°C for 16–18 hours. The zones of inhibition were measured using a caliper and compared to the standard after an overnight incubation period. The results were interpreted as susceptible (S), intermediate (I), or resistant (R).

In this study, it was shown that the bacteria *Enterococcus fecalis* and the bacteria *klepsiella pneumoniae* is more resistant to multiple antibiotics, followed by *Staph aures* resistant to multiple antibiotics which recorded.

The *Strep. pyogenes* isolate was tested for resistant to 2 antibiotics by disk diffusion method on Mueller-Hinton Agar medium, according to the Clinical Laboratory Standard Organization (CLSI) to determine the extent of sensitivity or resistance of the bacterial isolates to antibiotics by measuring the area of inhibition diameter and comparing the results with the standard tables mentioned in [12].

**Table 3:** Antibiotic susceptibility of bacterial isolates isolated from periodontitis

Antibiotics bacteria	Ampicillin (AM. 25µ)			metronidazole (MET .30 µ)			tetracycline (TE. 10µ)			Augmentin (AMC 30 µ)			Ceftazidime (CAZ 30)			Total
	Res.	Inter.	Sens.	Res.	Inter.	Sens.	Res.	Inter.	Sens.	Res.	Inter.	Sens.	Res.	Inter.	Sens.	
<i>Enterococcus faecalis</i>	5	1	0	3	3	0	6	0	0	6	0	0	0	3	3	30
<i>Klebsiella pneumonia</i>	3	1	0	2	0	2	0	1	3	4	0	0	0	1	3	20
<i>Kocuria Kristina</i>	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	5
<i>Staphylococcus aureus</i>	4	1	0	1	4	0	3	2	0	4	1	0	0	4	1	25
<i>Staphylococcus epidermidis</i>	3	0	1	1	3	0	3	1	0	3	1	0	0	1	3	20
<i>Streptococcus pyogenes</i>	1	0	36	24	13	0	26	11	0	1	2	34	0	2	35	185
<i>Streptococcus mutans</i>	0	0	3	2	1	0	1	2	0	0	0	3	0	0	3	15
<i>Streptococcus sanguinis</i>	0	0	1	1	0	0	1	0	0	0	0	1	0	0	1	5
Total	16	3	42	34	24	3	40	17	4	18	4	39	0	11	50	305

\*\*Pearson Chi-Square = 56.687, p value = 0.0002

## Discussion

The study of revealed that the periodontal was most prevalence in the age ranged between 21 and 30 years, most of patients were resident in urban areas, these results matched with present study results [13]. Jasim *et al.* showed in their study that periodontal disease was the most common in age group 20-29 years old and only a case reported with periodontal disease in 65 years old patients. According to sex distribution of patients, periodontal disease was mostly found in males than females. The periodontitis was more common in patients they living in rural area than they living in urban area and these result agree with The current study that showed that patients with teenagers were most prevalent and disagree with gender and residence results [14].

The impact on periodontitis will vary depending on the type of carbohydrates ingested. Consuming too much sugar or refined carbs encouraged microbial dysbiosis, which in turn sparked an inflammatory response and led to the appearance of periodontitis. Furthermore, glucose promotes apoptosis and inhibits proliferation in periodontal ligament cells [15]. Salazar *et al.* [16] similarly came to the conclusion that there was a negative correlation between PD and increased fruit and whole-grain eating. The improvement of glycemic management, a known risk factor for periodontitis, may account for the preventive effect of fibers against PDs.

According to a study conducted in Baghdad, at least eight different genera were isolated from patients with periodontitis; these genera were ranked ascendingly into categories such as *Staphylococcus* species, *Enterobacteriaceae* species, *Streptococcus* species, *Acinetobacter* species, *Neisseria* species, *Bacillus* species, *Corynebacterium* species, and *Pseudomonas* species. These results were not consistent with those of our study [17].

*Streptococcus mutans* showed the predominance 62% among other isolates. Whereas, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were with a distribution of 27% and 11% respectively in different ages and both genders in study of [18]. Oher study reported that the predominant bacterial isolates were of *Streptococcus mutans* with percentage 62.2%, whilst in the Sweden study and this agree with previous study of Saleh *et al.* [19], Kryvtsova *et al.* showed that Opportunistic microorganisms play a significant role in the development of inflammatory diseases, including generalized periodontitis, *S. pyogenes*, *S. pneumoniae*, *C. albicans*, *C. glabrata* isolated from the oral cavities of patients with different ages and both

genders with mixed food eaters suffering from the inflammatory periodontium [20].

*Strep. salivarius*, *Lactobacillus salivarius*, *Strep. mutans* and *E. faecalis* are considered as key pathogens in periodontitis in study of [21] which disagree with present study s results.

The first mechanism takes place at the biofilm's surface as an antibiotic tries to pierce the slimy, sticky membrane. Because of the intricate structure of the biofilm, which is made up of DNA, protein, and exopolysaccharide, antibiotics find it difficult to pass through the matrix and get to the bacterial target inside. Furthermore, the antibiotic is more likely to deactivate at the surface level before it has a chance to disperse because of its slower diffusion. But not all biofilms have this characteristic, and it's not certain how powerful a driver this process is for AMR [22].

*S. aureus* possesses biochemical mechanisms that enable it to resist antibiotics, as the microorganism has the ability to employ several methods to develop drug resistance [23]. Resistance to all these antimicrobial agents against pathogenic microorganisms is rising in some patients due to prolonged use of antimicrobial agents by these patients [24].

Uribe-García *et al.* in their study on *Staphylococcus aureus* strains isolated from patient with periodontitis indicated that resistant to Ampicillin [25], In a study on *Staph. epidermidis* isolated from different periodontitis, reported that *Staphylococcus epidermidis* was resistant to tetracycline and Augmentin [26].

Conceição *et al.* detected resistance of *Enterococcus faecalis* to Ampicillin and tetracycline in patient with periodontitis [27]. *k. pneumonia* showed a high resistance to Augmentin, metronidazole and Ampicillin, this result agrees with [28] who found that *K. Pneumonia* had resistance to Ampicillin [29] who found that showed the highest resistance to metronidazole.

Kityamuwesi *et al.*, [30] showed the metronidazole and tetracycline were resistance at 95.7% and 87% of *Strep. pyogenes* in periodontitis, These results matched with our study that showed these antibiotics were resistance in *Strep. pyogenes*.

Reduced permeability of the drug into the cell or decreased affinity of the target PBPs could be the cause of the unusually high percentage of resistance to these antibiotics, in addition to the formation of  $\beta$ -lactamase enzyme. Beta lactam resistance is caused by a number of methods. *S. aureus* and *Streptococcus* spp., which traditionally produce penicillinase and cephalosporinase, which destroy the antibacterial agent before it

can have an effect, are examples of resistant strains that contain an enzyme called beta-lactamase, which serves to "break" the beta lactam ring, effectively nullifying the effectiveness of the antibiotic [31]. Due to the widespread use of beta-lactam antibiotics, overprescribing, and overdosing, *S. aureus* isolates have been able to develop resistance to conventional medication regimens and caused development issues.

### Conclusion

There are many local patient related factors that affect the periodontal condition. *Streptococcus pyogenes* was the most reluctant in the current study and showed high sensitive for most antibiotics.

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