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Phenotypic and Molecular Diagnosis of Some Bacterial Species Isolated from Gingivitis from Patients in Al-Rifai District

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Abstract

Gingivitis is an inflammatory, non-destructive disease mainly caused by microbial plaques. The study was proposed to explore bacterial agents causing gingivitis. Subjects were 100 patients suffering from periodontal infections as well as other dental problems who visited governmental health centers and outpatient clinics in Al-Rifai District during the period from September 2022 to March 2023. Initial characterization of the gingiva was done by examination to exclude periodontitis cases. Those diagnosed as gingivitis patients were 48 patients, comprised of 33 males and 15 females. Gingival specimens was done to proceed with identification, which was performed by cultural, microscopic, utilization of the Vitek compact system, and molecular characterization. 41 specimens gave positive culture on at least one of the used culture media, while 7 specimens showed no growth on any of those media. The positive growth cultures were either pure cultures (28) or mixed cultures (13), which were excluded from the study. The 28 pure cultures were submitted to the vitek-2 compact system. Relying on the latter, identification results showed a high frequency of species belonging to the Gram-negative bacilli. Where the highest percentage was of Enterobacter cloacae (six isolates) (21.43%%), the lowest percentage was one isolate (3.55%) for each of: Aeromonas veronii, Pantoea spp., Laclercia adecarboxylate, Klebsiella pneumonia, Acinetobacter lowifii, Pseudomonas species, Burkholderia gladioli, and Staphylococcus aureus as gram-positive bacteria, and six specimens (21.43%) were not identified. According to age groups, the highest number of infections was in the age group (41-60) with 20 infections. According to sex, the percentage of infections among males was higher 33 (68.75%) than females 15(31.25%). The results of the present study showed an obvious variation in bacterial etiogens that were responsible for the destruction of gingiva among Al-Rifai District patients.

Keywords: *Phenotypic, Molecule, Bacterial Species.*

Introduction

Gingivitis represents the most prevalent disease of the periodontium and is usually known to be a site-specific inflammatory disease resulting from the buildup of dental biofilm (Zhang et al., 2021). Occasionally, some of the bacteria found in the mouth from gingivitis can easily drip into the bloodstream, causing organ damage. The disease of periodontium may be featured as gingivitis and periodontitis (Murakami et al., 2018). Although gingivitis

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always precedes periodontitis, the former does not always develop into periodontitis. Gingivitis results from the exacerbation of an inflammatory response against the activity of the oral microbiome. Symptoms are various and range from bleeding and redness to systemic inflammation passing through loss of tooth (Batchelor, 2014 ; Onyido et al., 2011).

Plaque starts when changes occur in the oral microbiome. When turns from healthy gum to plaque formation, examination of plaque showed little variation of microbes represented by gram-positive rods and cocci. Later on, the community becomes more complex and is dominated by gram-negative rods, spirilli, and spirochetes (Kistler et al., 2013).

Several factors are responsible for the pathogenesis of gingivitis, including microbial plaques, drugs, hormonal fluctuations, malnutrition, and some systemic diseases. Other factors still control the susceptibility and regulatory capacity of microbial pathogens within plaques, like genetic variation as well as epigenetic programs (Wade, 2013).

Various methods have been utilized to determine the microbial pathogens of gingivitis, including culture-based methods and culture-independent molecular methods, which supplied highly accurate results reflecting the huge phylotype variation within the oral microbial community based on 16S rRNA sequencing (Mythri et al., 2015 ; Wade, 2013).

Good oral hygiene could reverse gingivitis. Treatment is necessary to restore the normal condition of the gingiva and prevent it from developing into periodontitis. The latter is one of the leading causes of tooth loss. (Adams et al., 2000).

Genomic Methods

1- DNA extraction

Genomic DNA was extracted from six bacterial isolates that were not diagnosed with the Vitek 2 system in order to diagnose them by PCR using the boiling method (Dashti et al.,2009).

2- Prepare the polymerase chain reaction mixture

2 microliters of the DNA template were mixed with 10 µl Master mix ,1 µl Forward primer , 1 µl reverse primer and 6 µl double-distilled water .The final volume was reduced to 20 µl (Srivastava et al.,2008).

3- PCR amplification

The PCR technique was performed by a gradient thermal cycler. The universal primers Forward 5'- AGAGTTTGATCCTGGCTCAG -3' and reverse primer 5'-CTTGTGCGGGCCCCCGTCAATTC-3') of primer targeted 16S rRNA. Amplification was performed by initial denaturation at (94°C for 3 min), followed by (30 cycles) of denaturation at (94°C for 30 sec), annealing temperature was(55°C for 30 sec) and extension at(72°C for 1 min). Final extension was at(72°C for 10 min) (Dos Santos et al.,2018; Srivastava et al.,2008).

Agarose gel electrophoresis

The agarose gel electrophoresis technique was carried out for the detection of amplicon, as mentioned by (Sambrook and Russell 2001).

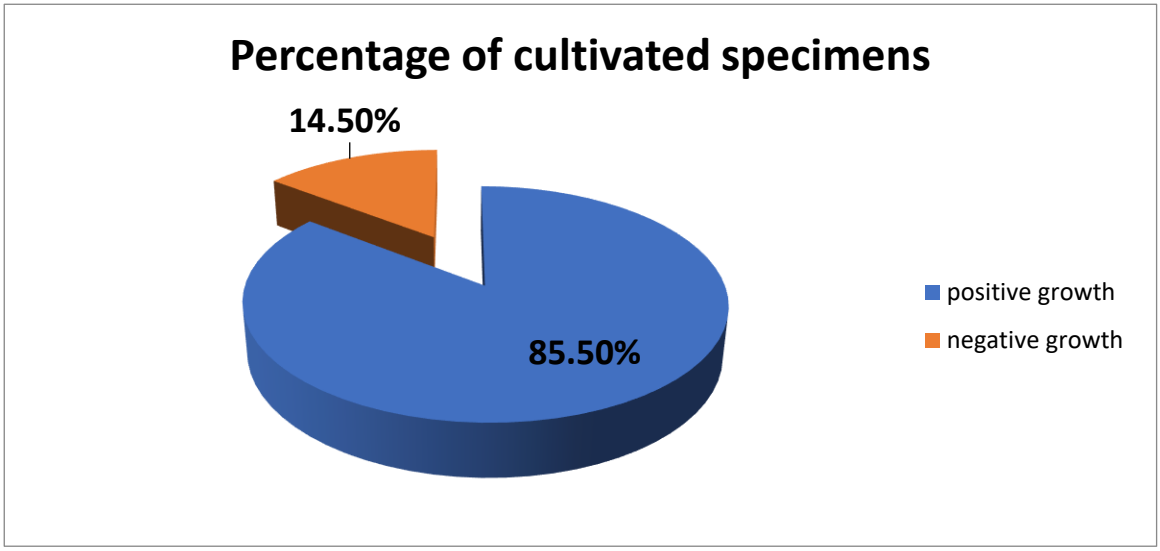
DNA sequencing of the 16S rDNA fragment

DNA samples of the 16S rDNA gene with primers (forward and reverse) were sent to Macrogen Inc (South Korea Geumchen, Seoul) and the results were then analyzed.

RESULTS AND DISSCUSSION

Isolation and Identification

In this study, forty-eight swabs have been collected from forty-eight patients diagnosed with gingivitis. Forty-one (85.5%) specimens showed positive growth on different culture, while seven (14.5%) specimens showed negative growth on different culture. (Figure -1) shows the percentage of Gingivitis specimens growth cultures



(Figure -1) shows the percentage of Gingivitis specimens growth cultures

Jaseem and Yasin (2023) found (75%) specimens showed positive bacterial growth cultures, while (25%) specimens showed no bacterial growth cultures.

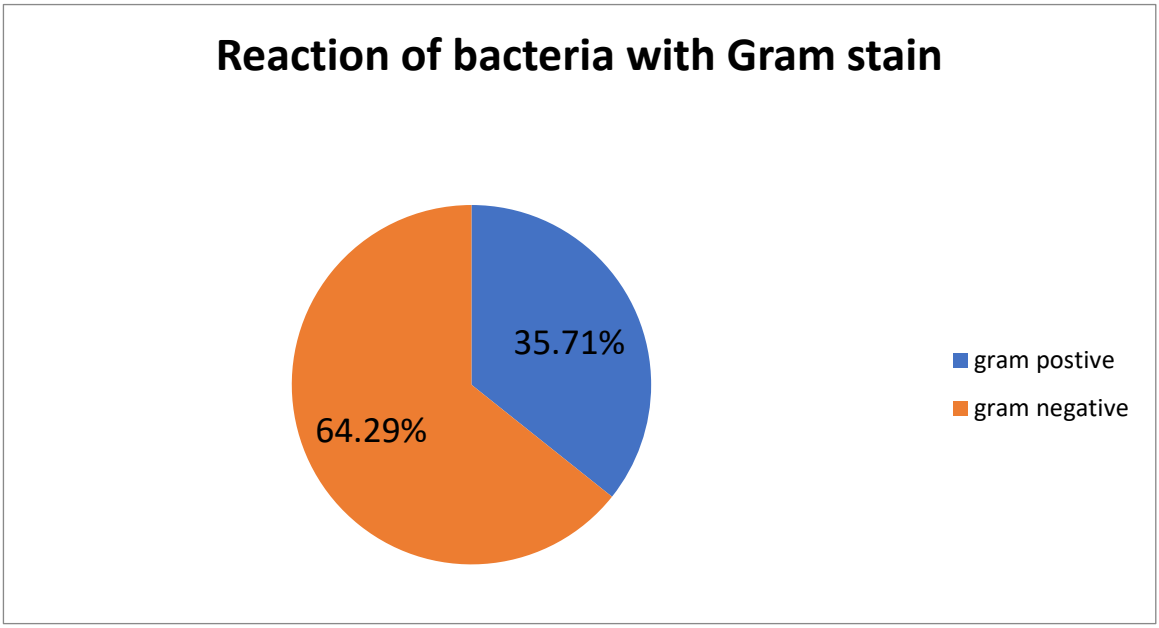
Cultures varied from pure culture, characterized by the appearance of a single, defined type of colony, reflecting their return to a single type of microorganism. The total number of such cultures was 28 (68.29%); to those that showed multiple types of colonial appearance, which means that there are multiple microorganisms forming them, represented 13 (31.70%). The latter were excluded (Table -1). Studies showed that approximately half of oral microbes cannot be cultivated or have not been cultivated in laboratory conditions (Wade, 2004). Multiple bacterial growths support the concept of the complexity of the oral cavity microbial world. In a study by Aas et al.,(2005), results estimated from 34 to more than 70 different phylotypes at the level of species in each single individual included in the study were determined by molecular-dependent methods.

(Table -1): The Number and Percentage of Positive and Negative Culture Samples

<table>
<tr><th colspan="2">No. of Positive Samples (%)</th><th>No. of Negative Samples (%)</th></tr>
<tr><td colspan="2">41 (85.5)</td><td rowspan="3">7(14.5)</td></tr>
<tr><td>No. of Pure cultures (%)</td><td>No. of mixed cultures isolates (%)</td></tr>
<tr><td>28 (68.3)</td><td>13(31.7)</td></tr>
</table>

After that, a microscopic examination of all bacterial isolates was performed using the Gram stain to differentiate between positive and negative bacteria to interact with the Gram stain and to know the shape, size and arrangement of the bacteria. The results showed that 10(35.71%) bacterial isolates were positive for the Gram stain and that 18 (64.29%)

bacterial isolates were negative for the Gram stain. (Figure -2) shows the percentage of Gingivitis gram positive and gram negative.



(Figure -2) shows the percentage of Gingivitis gram positive and gram negative

a broad spectrum of bacteria including, gram-negative bacilli were found as the predominant microbial populations in the gingival specimens. The differences observed between these results can be addressed to the area of sampling on the gingival plaques. The result obtained by this study is consistent with the results of (Azadeh et al., 2011) while don't consistent with those reported by (Leo ,1981) and (Moening, 1989) who emphasized the importance of gram positive cocci in the initiation of dental plaque formation, leading to gingivitis.

After that, the diagnosis was made using the Vitek 2 compact system to accurately diagnose all bacterial isolates .The results revealed that 10 (35.71%) of bacteria isolates which were gram positive bacteria Staphylococcus aureus 1 (3.57%), Un-identified. While gram negative were found in 18 (64.29%) isolates , represented by 6 (21.44%) of Enterobacter cloacae spp, 3 (10.72%) isolates of Klebsiella pneumonia, 2 (7.14%) Acinetobacter lowifii , Pseudomonas species and Burkholderia gladioli, 1 (3.57%) isolates of Aeromonas veronii ,Pantoea spp and Laclercia adecarboxylate respectively. (Table -2) exhibit types and numbers of bacterial isolates identified in the study.

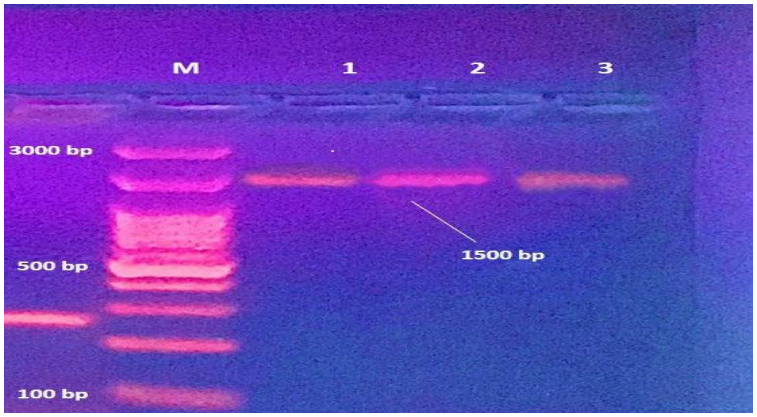
(Table-2): Types and Numbers of Bacterial Isolates Collected in This Study.

No	Bacterial isolates	No (%)
1	Enterobacter cloacae spp	6 (21.44)
2	Klebsiella pneumonia	3 (10.72)
3	Acinetobacter lowifii	2 (7.14)
4	Pseudomonas species	2 (7.14)
5	Burkholderia gladioli	2 (7.14)
6	Aeromonas veronii	1 (3.57)
7	Pantoea spp	1 (3.57)
8	Laclercia adecarboxylate	1 (3.57)

9	Staphylococcus aureus	1 (3.57)
10	Un-identified	9 (32.14)
	Total	28(100)

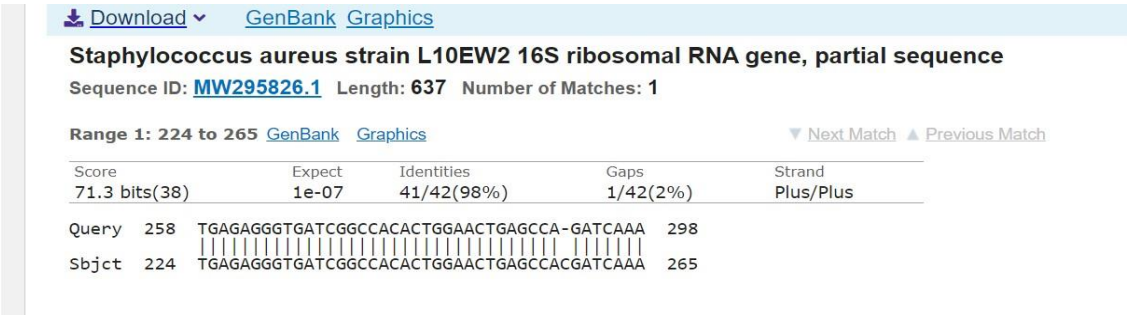
This study is in agreement with Alghamdi, (2022) who showed in isolation of species of different families including six families of the gram positive bacteria; Leuconostocaceae, Listeriaceae, Streptococcaceae, Staphylococcaceae, Bacillaceae, Corynebacteriaceae, and 5 families of gram negative; Neisseriaceae, Enterobacteriaceae, Pseudomonadaceae, Yersiniaceae, and Moraxellaceae,. The overall dominated family observed was Enterobacteriaceae (19.36%) having Escherichia coli as the most prevalent specie (53.06%) followed by Klebsiella pneumoniae (28.57%). Al-Shammarie and Maaroof (2020) Perkowski et al., (2019) also found many different bacterial species that cause gingivitis.

After that Molecular identification of those which could not be identified by vitek-2 system. Where there were 9 isolates, the vitek -2 system gave un -identified results for them. Those were submitted to molecular identification by 16S rRNA . only three gave positive result by PCR that gave the specific product size after gel electrophoresis. (Figure-1).

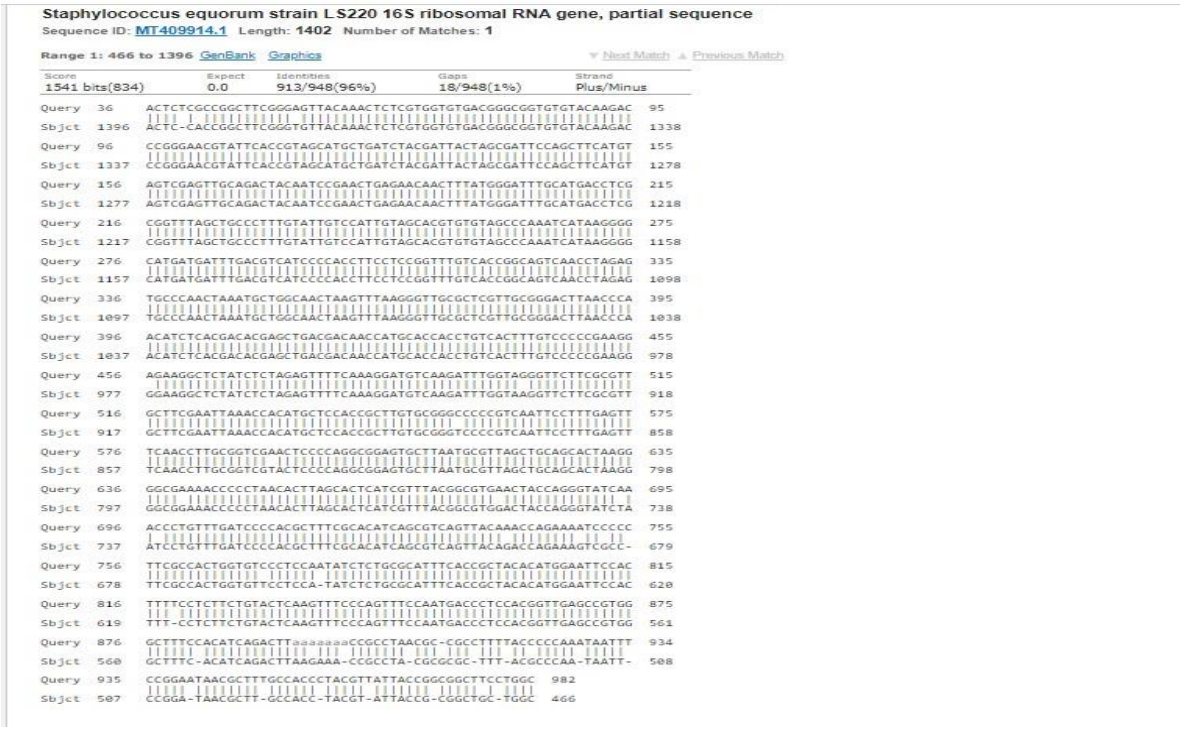


(Figure -3): Gel Electrophoresis of 16SrRNA Sequence result after PCR

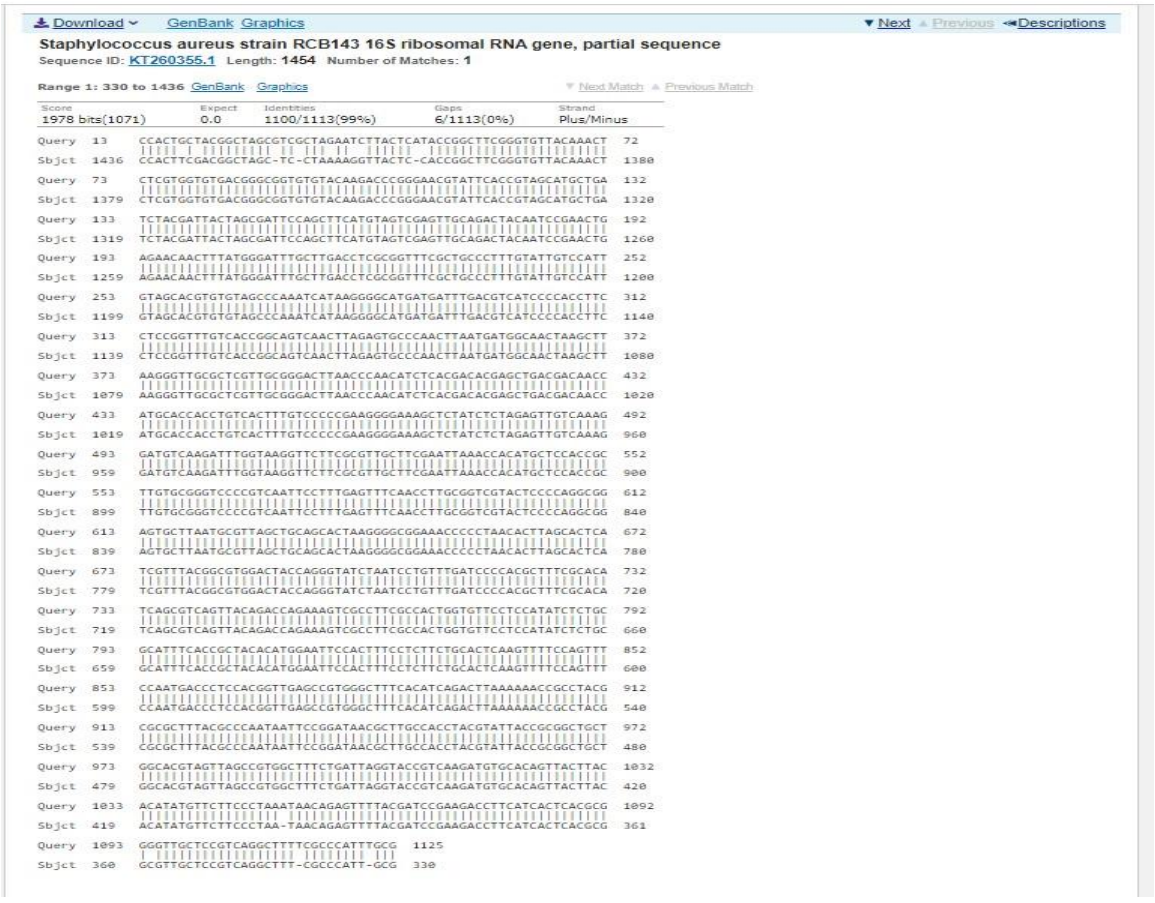
DNA Sequencing results showed that two of them were S. aureus and on was S.equoeumas shown in figure (4), (5) and (6).This method is one of the most important ways to identify the identity of pathogenic bacteria. This method also detects the occurrence of genetic mutations in resistance genes and other genes, by identifying the sequence of the nitrogenous bases of the gene. Thus, the number of diagnosed bacterial isolates in this study became 22 (78.57%) bacterial isolates out of 28 bacterial isolates, while the number of undiagnosed isolates was 6 (21.43%) bacterial isolates.



(Figure -4) Sequence analysis of the 16SrRNA gene of bacteria S. aureus in isolation number 1



(Figure -5) Sequence analysis of the 16SrRNA gene of bacteria S.equoeum in isolation number 2



(Figure -4) Sequence analysis of the 16SrRNA gene of bacteria S. aureus in isolation number 3

In a study by Al-Abdul et al., (2017) performed in AL-Basrah showed that Staphylococcus spp. Were the most commonest isolates. Similarly in another study in Kufa study S. aureus was the commonest in acute gingivitis.

The objective of this study was to determine the number and frequencies of bacterial populations associated in dental plaques among Al-Rifai District patients with gingivitis. Some other studies have also been conducted to profile the pathogenic bacteria isolated from gingivitis plaques among different groups of populations in different cities of Iraq (Ibrahim and Hussien, 2018 ; Jabuk et al.,2015; Najm and Younis,2009). However, in current study,

Distribution of Patients According Age and Sex

According to age groups, the highest number of infections was in the age group (41-60) where it was 20 infections, while the other age groups had the same infections number 14 infections each (Table-3).

In general the results of this study have been conclude that the infection increases with the advanced stages of age , and this is consistent with many studies (Ababneh et al.,2012; Stamm, 1986).

(Table -3): Distribution of Gingival Patients According to Age

Age group	No. of Patients
1years -20	14
21-40	14
41-60	20
Total	48

As for infections distribution among males and females, the percentage of infections in males was higher 33(68.75) % males than 15(31.25) %females as shown in (Table- 4). At different age stages there an obvious lower frequency of gingivitis in females as compared with males despite the fact that females are influenced by hormonal changes. This may be attributed better teeth health care as well as the presence of physiologic difference between the sexes (Newman et al ., 2006). More recent native study revealed that males showed higher gingival index than females with no significant differences which may be due to the population samples were taken from Dentistry College (Wais et al ., 2023). It was also the same in a study in Sullaimaniya (Hamid et al ., 2020; Kinane et al ., 2017 Humphrey et al ., 2008)

(Table -4) Distribution of Gingival Patients According to Sex

Sex	Number of Patients (%)
Male	33 (68.75)
Female	15 (31.25)
Total	48 (100%)

Conclusions

The results of present study indicate that a wide range of communicable pathogenic bacteria are responsible for gingivitis and its progression among residents of Al-Rifai District. The oral microbiome is a complex community which could be used further to monitor health status.

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