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Research Article

# Molecular Identification of Adhesion Gene *Fima* in *Porphyromonas Gingivalis* Isolated From Periodontitis Patients in Misan City

Zahraa F Faroq[1], Mohammed A Abd Ali[2], Sami Kh Jabar[3]

[1,2]Department of Biology, College of Science, University of Misan, Misan, Iraq.
[3]College of Medicine, University of Misan, Misan, Iraq.
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## I N F O

**Corresponding Author:**
Mohammed A Abd Ali, Department of Biology, College of Science, University of Misan, Misan, Iraq.
**E-mail Id:**
waleedabas22@uomisan.edu.iq
**Orcid Id:**
https// orcid. org/0000-0001-9691-4058
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## A B S T R A C T

*Introduction: Porphyromonas gingivalis* is an anaerobic, gram-negative bacterium that promotes oral dysbiosis and alters host defence mechanisms, contributing to the pathogenesis of periodontitis. The goal of the present study was to isolate and identify pathogenic *P. gingivalis* from gingivitis and periodontitis patients.

*Method:* Fifty swabs were collected from periodontitis patients attending Special Dental Center, Misan City, Iraq. *Porphyromonas gingivalis* was identified by PCR and its presence was clinically correlated with periodontal disease. The resistance of *P. gingivalis* was also tested against antibiotics (ciprofloxacin 5 mcg, carbenicillin 25 mcg, cefoxitin 30 mcg, doxycycline 30 mcg, and nalidixic acid 30 mcg).

*Results: P. gingivalis* was found to be associated with periodontal diseases. The strains were resistant to the antibiotics used in this study.

*Conclusion: P. gingivalis* is responsible for causing gingivitis and periodontitis in humans. The higher age groups are more commonly affected by it. Its strains were found to be resistant to the antibiotics used in this study.

**Keywords:** *Porphyromonas Gingivalis*, Virulence Factors, *Fima* Gene, Periodontitis

## Introduction

The genus *Porphyromonas* includes several species of obligatory gram-negative, anaerobic, opportunistic pathogens, producing black-pigmented colonies and possessing multiple virulence factors. It belongs to the Porphyromonadaceae family which colonises dental plaque biofilms in the oral cavity.[1] The bacteria associated with periodontal disease have been studied since the advent of the science of microbiology. Gave the first detailed description of sub-gingival plaque and revealed considerable diversity of bacterial plaque is also linked to the aetiology of periodontal disorders, in which the host responds inappropriately to an increased microbial load surrounding the gingivae. It has been estimated that more than 700 bacterial species can be identified within the sub-gingival plaque.[2]

*Porphyromonas gingivalis* promotes oral dysbiosis and alters host defence mechanisms, contributing to the pathogenesis of periodontitis.[3] They are found in the mucous membranes of humans and infect epithelial cells of the oral cavity.[4]

*P. gingivalis* is a main pathogenic bacterium that causes the initiation and progression of periodontitis. It has been strongly implicated in chronic periodontitis.[5] The obligate pathogenic bacteria *P. gingivalis* colonises for multispecies plaque biofilms in the surface of gingival margin have also identified the deep crypts of the tongue as a habitat of *P. gingivalis*.[6] Interestingly, *P. gingivalis* has not only been found in higher loads in the sub-gingival biofilm in periodontitis subjects compared to healthy individuals, but their virulent properties also vary among strains isolated from each condition. In previous studies, it has been reported that clinical isolates of *P. gingivalis* obtained from healthy and periodontitis subjects show differences in their virulence, for example, strains from periodontitis patients exhibit higher resistance to antimicrobial peptides.[7] and a higher ability to decrease the apoptosis of host cells and therefore, to persist intracellular.[8] In recent years, there has been a great interest in Polymerase chain reaction (PCR)-based tests which use the bacterial small sub-unit 16S ribosomal ribonucleic acid (rRNA) for the detection of bacterial pathogens. Molecular analysis based on PCR of 16S rRNA gene is revolutionising.[9] The present study has been conducted to detect the adhesion gene in *Porphyromonas gingivalis* isolated from periodontitis patients and to analyse the effect of antibiotics on the isolates.

## Material and Methods

In this research, swabs of bacteria were collected from 50 periodontal patients (28 male and 22 female) aged 11 to 70 years and attending Special Dental Centre, Misan City, Iraq. Specimens of gingival sulcus fluid were collected with sterile absorbent paper tips inserted into periodontal pockets or gingival sulci from November 21 to February 6, 2023. After collection, the samples were immediately taken to the laboratory of microbiology in the Biology Department, College of Science, and University of Misan. Used in experimental design and statistical analysis Statistical differencs Chi-Square.

## Isolation and Detection of Bacterial Strains

The PCR detection of *P. gingivalis* by 16S rDNA was conducted using Mix, Promega Corporation, USA. Each PCR mixture contained GoTaq Green Master Mix (10 µl), forward primer (1 µl), and reverse primer (1 µl).[10]

### The gDNA Extraction

The gDNA for each isolate was extracted using the kit supplied by Geneaid according to the manufacturer's instructions. The gDNA was detected by electrophoresis on 1% agarose gel which was examined by the Gel Documentary System.

### Polymerase Chain Reaction (PCR)

PCR technique was used to amplify the diagnostic 16S rRNA gene for bacterium DNA. It was also used to diagnose the genes of resistance to vancomycin, *fime* A (II) and *fime* A (III) prefixes. The primers set targeted region was discovered to be directly related to ability in *P. gingivalis* in the current investigation. However, *fime* A was amplified for the detection in the final identification shown in Table 2 and Table 3. PCR was performed in a volume of 50 µl according to the leaflet attached with the kit supplied by the manufacturer (Promega)

### Design of Primer for *fime* A Gene

Specific *fime* A (II) and *fime* A (III) primers were designed according to the *P. gingivalis* 16S rDNA gene sequence. The parameters of primer design were as follows: size: 30–35 bp, product size: 100–500 bp, GC content: 20%–30%, and melting temperature (Tm): 50–100 $^{O}$C . Three primer pairs were selected for examination.

### Antibiotics Susceptibility Assay

The antibiotic activity was evaluated using the disc diffusion method according to.[11] Antibiotic activity testing was used to determine the biological activity against several strains of *Porphyromonas gingivalis* bacterial isolates obtained from periodontitis patients in Misan City. Mueller-Hinton agar was used to grow *Porphyromonas gingivalis* isolates. The resistance of the isolates to five antibiotics (ciprofloxacin 5 mcg, carbenicillin 25 mcg, cefoxitin 30 mcg, doxycycline 100 µg, and nalidixic acid 500 µg) was analysed.

## Ethics Statement

The present study was approved by the Committee of Medical Ethics. All subjec ts gave their written informed consent for the study.

## Results

Swabs of bacterial isolates were collected from 50 periodontal patients (28 male and 22 female) attending Special Dental Center, Misan City, Iraq. Twenty-six bacterial strains were isolated from these swabs containing every type of *fimA* gene. The biochemical tests of *P. gingivalis* isolates showed catalase-negativity, oxidase-negativity, and absence of motility (Table 1). Tables 2 and 3 show the universal and specific primers used in PCR amplification in this study. Table 4 shows the agewise distribution of the subjects with *fime A* gene in the 26 strains of P. gingivalis. The isolates were incubated for 7 to 14 days (Figures 1a and 1b). The colonies that appeared were observed under an immersion oil microscope (Figure 1). PCR was used to identify *P. gingivalis* pathogenic bacterial isolates. DNA amplicons were observed with a detection limit of 1 pg of DNA (Figure 2). Figure 3 presents the Agarose gel electrophoresis of these DNA bands for the bacterial isolates. Figure 4 shows the PCR products of the specific primers used in this study as seen on Agarose gel electrophoresis. Figures 5 and 6 show the results of gel electrophoresis of

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the specific primers binding fim A gene region on the *P. gingivalis* isolates (8, 9, 10, and 11) and (14, 16, 17, 19, 21, and 22), respectively. Only *P. gingivalis* isolates number 21 have 2 types of genes (fim A gene types II 295bp and types III genes 234bp). The association of parameters of clinical periodontitis with the presence of *P. gingivalis* has been depicted in Table 5. The percentages of fim A gene *genotypes* in the various strains in this study were calculated and have been presented in Table 6. The various strains of *P. gingivalis* were found to be resistant to the antibiotics used in this study (ciprofloxacin, carbenicillin, cefoxitin, doxycycline, and nalidixic acid) (Table 7 and Figure 8).

**Table 1.Microscopic and Biochemical Tests for *Porphyromonas Gingivalis* Isolates**

| Biochemical Tests | Result of Biochemical Tests |
|---|---|
| Gram stain | Negative |
| Catalase test | Negative |
| Oxidase test | Negative |
| Motility | Negative |

**Table 2.Universal Primers Used in Pcr Amplification in The Present Study**

| Gene | | Sequence | Length (pb) | Annealing temperature | Size bp | Number of cycle |
|---|---|---|---|---|---|---|
| 27 | Forward | 5- AGAGTTTGATCCTGGCTCAGA-3 | 20 | 60 | 1500 | 55 |
| 1492 | Reverse | 5- GGTTACCTTGTTACGACTT-3 | 19 | 54 | 1500 | |

**Table 3.Specific Primers Used in PCR Amplification in the Present Study**

| S. No. | Gene | | Sequence | Length (pb) | Annealing Temperature | Size (pb) |
|---|---|---|---|---|---|---|
| 1 | *P. gingivalis* | F | 5-AGGCAGCTTGCCATACTGCG-3 | 20 | 64 | 404 |
| | | R | 5-ACGTTCGATTTCATCACGTTG-3 | 20 | 64 | 404 |
| 2 | *P. gingivalis* | F | 5-CGTGGACCAAAGATTCATCGGT-3 | 21 | 66 | 256 |
| | | R | 5-CTTTACTCCCCAACAAAAGCA-3 | 22 | 66 | 256 |

F: Forward, R: Reverse

**Table 4.Presence of *fimA* Gene in 26 Strains of *Porphyromonas gingivalis* in the Present Study**

| S. No. | Age (Years) | Female (43%) | | Male (57%) | | Presence of fimA Gene |
|---|---|---|---|---|---|---|
| | | Number | Percentage | Number | Percentage | |
| 1 | ≤ 10 | 3 | 6.9 | 2 | 3.5 | - |
| 2 | 11–20 | 11 | 25.5 | 7 | 12.2 | + |
| 3 | 21–30 | 11 | 25.5 | 22 | 38.7 | - |
| 4 | 31–40 | 10 | 23.2 | 17 | 29.8 | ++ |
| 5 | 41–50 | 5 | 11.8 | 5 | 8.7 | ++ |
| 6 | 51–60 | 2 | 4.6 | 3 | 5.2 | +++ |
| 7 | 61–70 | 1 | 2.3 | 1 | 1.7 | + |
| 43 Total | | 43 Total | - | 57 Total | Total | 100 |

Chi- square = 4.907 *(p< 0.01 )
+: Present, -: Absent

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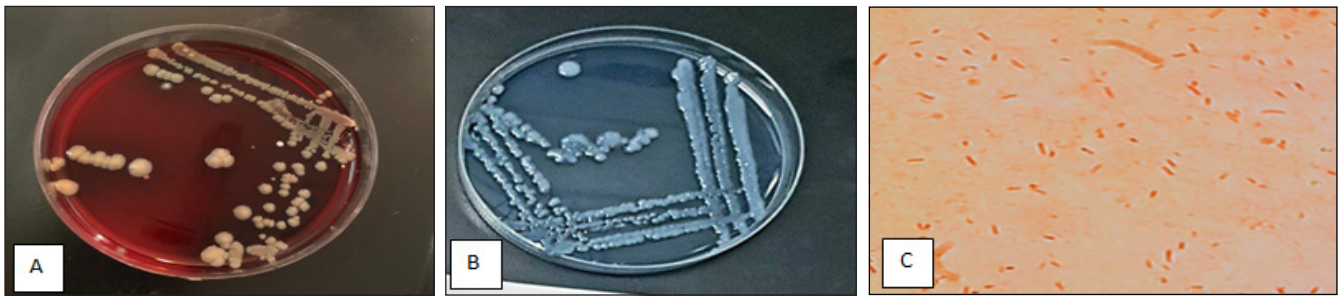


**Figure 1.*Porphyromonas gingivalis* Colonies on (a).Blood Agar (b).Mitis Salivarius Agar after 7 Days of Incubation at 37 °C in an Anaerobic Chamber. (c).*Porphyromonas gingivalis* Isolates 100X Under Immersion Oil Microscope**

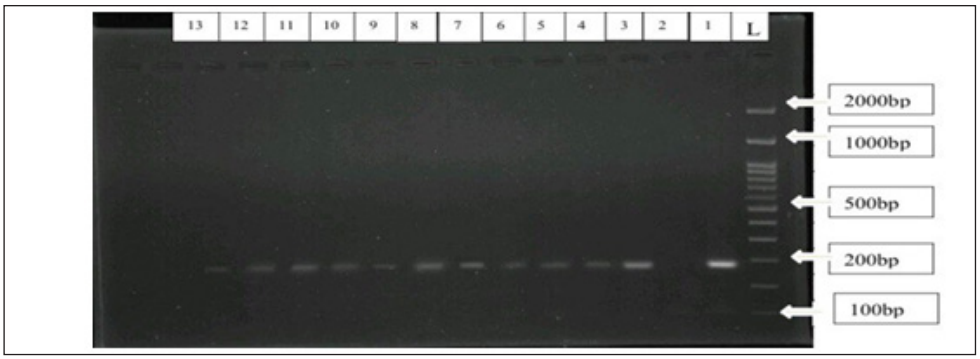


**Figure 2.PCR Products of Specific Primers Seen on Agarose Gel Electrophoresis, L: Ladder**

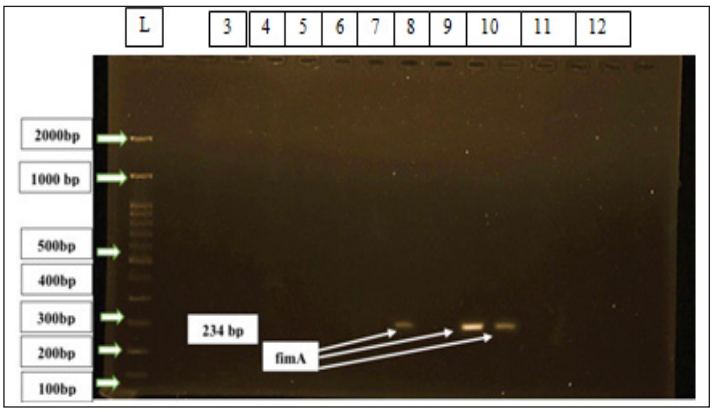


**Figure 3.Gel Electrophoresis of Specific Primers Binding fim A gene Region on the *P. gingivalis* Isolates (8, 9, 10, and 11) Through Polymerase Chain Reaction**

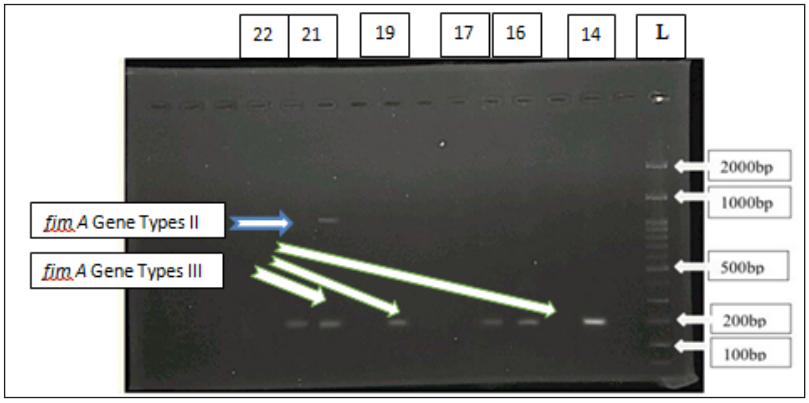


**Figure 4.Gel Electrophoresis of Specific Primers Binding fim A gene Region on the *P. gingivalis* Isolates (12, 13, 14, 16, 17, 18) Through Polymerase Chain Reaction**

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**Table 5.Percentages of fim A gene Genotypes in 26 *P. gingivalis*-Positive Periodontitis Patients in Misan City**

| Number/ Percentage of Isolates (26, 100%) | With fimA Type II Gene | With fimA Type III Gene | With fimA Type II and Type III Genes | Without fimA Type II and Type III Genes |
|---|---|---|---|---|
| Number | 1 | 9 | 10 | 16 |
| Percentage | 3.84 | 34.61 | 38.46 | 61.53 |

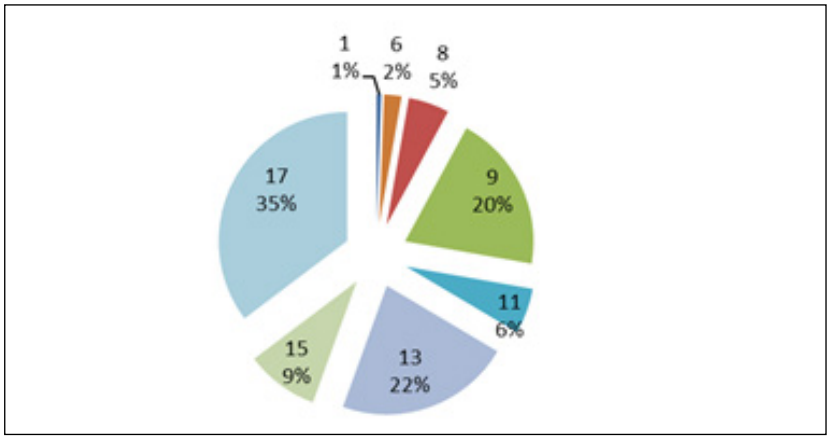


**Figure 5.Percentages of fim A gene Genotypes in 26 *P. gingivalis* Strains Isolated from Periodontitis Patients in Misan City**

**Table 7.Effect of the Five Types of Antibiotics Used in the Present Study on *Porphyromonas gingivalis***

| Antibiotics, Concentration (µg /disk) | Effect on *Porphyromonas gingivalis* |
|---|---|
| Ciprofloxacin, 5 | R |
| Carbenicillin, 25 | R |
| Cefoxitin, 30 | R |
| Nalidixic acid, 500 | R |
| Doxycycline, 100 | R |

## Discussion

Periodontitis occurs as a result of sub-gingival plaque due to specific bacteria, especially gram-negative anaerobic bacteria Studies have shown that *v* have a major role in periodontitis diseases [15]. In the present study, *P. gingivalis* infection was found to be more common among the elderly (≥ 30 years old) subjects. Previously, the *P. gingivalis* detection rate was observed to significantly increase with age, while the rates of *T. denticola* and *T. forsythia* were comparably high across all age groups[16]. *P. gingivalis* is called an opportunistic pathogen and is known to be a key pathogen responsible for the cause and progress of periodontal disease [17]. Microbial culture is considered the gold standard for new microbial detections in periodontics. Conventional methods rely on the presence of viable microorganisms and require that samples are immediately tested in a laboratory in order to maximise bacterial survival. The present investigation found an increase in the prevalence of *P. gingivalis* with an increasing probing pocket depth (PPD) *P. gingivalis*, an anaerobic gram-negative oral cavity pathogenesis bacterium, is a member of more than 500 bacterial species that live in the oral cavity In this study, the various strains of *P. gingivalis* were found to be resistant to all five antibiotics. This is in contrast with the study in which 32 samples of *P. gingivalis* strains were found to be sensitive to the used antibiotics[18].

## Conclusion

The findings of the present study indicate the association between the diseases of gingivitis and periodontitis and the presence of *P. gingivalis*. The strains of *P. gingivalis* were resistant to all the used antibiotics (ciprofloxacin 5 mcg, carbenicillin 25 mcg, cefoxitin 30 mcg, doxycycline 100 µg, and nalidixic acid 500 µg). The study suggests that according to the molecular analysis, the genes of fimbriae, particularly the two type's *fimA* II Gene and *fimA* III Gene, are among the most significant factors that promote the disease of gingivitis and periodontitis. It was seen that PCR detection when used with specific primer sets, can identify

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the bacterial isolates of *P. gingivalis*. At the same time, this study also showed that people more than 30 years of age were affected the most with gingivitis and periodontitis.

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**Conflict of Interest:** None

## References

1. Ashely G. The investigation of novel virulence genes in *Porphyromonas gingivalis* [dissertation]. University of Sheffield, School of Clinical Dentistry; 2021. [Google Scholar]
2. Chigasaki O, Takeuchi Y, Aoki A, Sasaki Y, Mizutani K, Aoyama N, Ikeda Y, Gokyu M, Umeda M, Ishikawa I, Izumi Y. A cross-sectional study on the periodontal status and prevalence of red complex periodontal pathogens in a Japanese population. J Oral Sci. 2018; 60(2):293-303. [PubMed] [Google Scholar]
3. De Lillo A, Ashley FP, Palmer RM, Munson MA, Kyriacou L, Weightman AJ, Wade WG. Novel subgingival bacterial phylotypes detected using multiple universal polymerase chain reaction primer sets. Oral Microbiol Immunol. 2006; 21(1):61-8. [PubMed] [Google Scholar]
4. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A, Wade WG. The human oral microbiome. J Bacteriol. 2010; 192(19): 5002-17. [PubMed] [Google Scholar]
5. Díaz L, Hoare A, Soto C, Bugueño I, Silva N, Dutzan N, Venegas D, Salinas D, Perez-Donoso JM, Gamonal J, Bravo D. Changes in lipopolysaccharide profile of *Porphyromonas gingivalis* clinical isolates correlate with changes in colony morphology and polymyxin B resistance. Anaerobe. 2015;33:25-32. [PubMed] [Google Scholar]
6. Pfau EA, Avila-Campos MJ. *Prevotella intermedia* and *Porphyromonas gingivalis* isolated from osseointegrated dental implants: colonization and antimicrobial susceptibility. Braz J Microbiol. 2005; 36(3):281-5. [Google Scholar]
7. Koliarakis I, Messaritakis I, Nikolouzakis TK, Hamilos G, Souglakos J, Tsiaoussis J. Oral bacteria and intestinal dysbiosis in colorectal cancer. Int J Mol Sci. 2019; 20(17):4146. [PubMed] [Google Scholar]
8. Jandik KA, Bélanger M, Low SL, Dorn BR, Yang MC, Progulske-Fox A. Invasive differences among *Porphyromonas gingivalis* strains from healthy and diseased periodontal sites. J Periodontal Res. 2008; 43(5):524-30. [PubMed] [Google Scholar]
9. Ribeiro Junior JC, Tamanini R, Soares BF, de Oliveira AM, Silva FG, da Silva FF, Augusto NA, Beloti V. Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. Semin Cienc Agrar J. 2016;37(5):3069-78. [Google Scholar]
10. Hajishengallis G, Jenkinson HF, Lamont RJ. Oral microbiology and immunology. 2nd ed. USA: ASM Press; 2014. 120 p.
11. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. Microbiol Mol Biol Rev. 1998;62(4):1244-63. [Google Scholar]
12. Leeuwenhoek A. Arcana nature, Delphis Bartavorum, 1965. Reprinted in facsimile, Brussels. Culture et civilization 1966 cited from Carranza's Clinical Periodontology. 9th ed. Philadelphia: Saunders; 2003. 5 p.
13. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, Prochazkova J, Duskova J. Porphyromonas gingivalis: major *periodontopathic pathogen* overview. J Immunol Res. 2014; 2014:476068. [PubMed] [Google Scholar]
14. Olsen I, Lambris JD, Hajishengallis G. *Porphyromonas gingivalis* disturbs host-commensal homeostasis by changing complement function. J Oral Microbiol. 2017 Jun 30;9(1):1340085. [PubMed] [Google Scholar]
15. Salcetti JM, Moriarty JD, Cooper LF, Smith FW, Collins JG, Socransky SS, Offenbacher S. The clinical, microbial, and host response characteristics of the failing implant. Int J Oral Maxillofac Implants. 1997;12(1):32-42. [PubMed] [Google Scholar]
16. Soto C, Bugueño I, Hoare A, Gonzalez S, Venegas D, Salinas D, Melgar-Rodriguez S, Vernal R, Gamonal J, Quest AF, Perez-Donoso JM, Bravo D. The *Porphyromonas gingivalis* O antigen is required for inhibition of apoptosis in gingival epithelial cells following bacterial infection. J Periodontal Res. 2016;51(4):518-28. [PubMed] [Google Scholar]
17. Wade WG. Characterisation of the human oral microbiome. J Oral Biosci. 2013; 55(3):143-8. [Google Scholar]
18. Yang HW, Huang YF, Chou MY. Occurrence of *Porphyromonas gingivalis* and *Tannerella forsythensis* in periodontally diseased and healthy subjects. J Periodontol. 2004; 75(8):1077-83. [PubMed] [Google Scholar]

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