International Journal of Health Systems and Medical Sciences

ISSN 2833-7433
Volume 2 | No 8 | Aug -2023

Isolation and Molecular Detection of Some Anaerobic Bacteria From Patients With Periodontitis

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Abstract:
This study aimed conducted for isolation and Molecular detection of some anaerobic bacteria from patients with Periodontitis. Transport and collection: 78 Patients with periodontitis between the ages of 16 and 69 from Dental Clinics in the city of Baghdad were recruited between 2021 and 2022 for this study. After placing a paper point (size 30-45 mm.) inside a pocket with a depth of 3 mm. and leaving it there for 60 seconds, we were able to obtain an adequate sample of GCF for analysis. Next, Thioglycollate broth was added to the paper point. Within 2 hours of collection, all samples were sent to the bacteriology lab where they were cultured at 37 °C for 24-48 hours. Genomic DNA micro Kit was used to isolate the bacterial genome. Amplification using global bacterial 16S rDNA primers. From a total of 78 samples, 34 unique anaerobic bacteria were isolated. Bacteriological techniques were used to identify the five species of bacteria (representing four genera) that were isolated. The Gramme stain test was used for initial confirmation of the microbiological isolates found in both diabetes and non-diabetic individuals, and the findings showed that 24 of the bacterial isolates were Gramme positive cocci, 5 were Gramme positive anaerobic bacilli, and 3 were Gramme negative anaerobic Cocci. The results displays the gender breakdown of our patient population, which breaks down to 15 male (42.9% of the total) and 25 females (58.1% of the total), with no statistically significant differences ($P > 0.05$) between the sexes. Streptococcus salivasrius was found to be the most resistant species in this study, accounting for 18 (52.9%) of the isolates, followed by Lactobacillus salivarius with 7 (20.6%) isolates, Streptococcus mutans with 5 (14.7%), and Veillonella Spp. with 2 (5.5%). However, only 2.5% of diabetes individuals have the bacterium species Enterococcus faecalis. All these bacteria were confirmed by using PCR.
In conclusion, the present investigation found that Streptococcus salivarius was the most resistant in all individuals, followed by Lactobacillus salivarius.
Keywords: **Anaerobic, Periodontitis, bacteria, Molecular.**

Introduction

Loss of alveolar bone and attachment of supporting connective tissue occurs in periodontitis, an infection of the tissues that hold teeth in place. Since sub-gingival plaque bacteria are the principal etiologic agents of periodontitis, the illness is classified as a poly-microbial infection, with the

interplay of the particular pathogens being more important to disease progression than are individual species (1, 2, 3).

Periodontitis, which affects the gums, periodontal ligaments, and alveolar bone, is a more severe form of gingivitis, the milder form of periodontal disease (4). Gingivitis causes inflammation of the gums but does not cause any permanent damage to the teeth or their supporting structures. However, not all cases of gingivitis progress into periodontitis because patients with gingivitis may recover, whereas those with periodontitis always have the disease (5, 6).

First, there is necrotizing periodontitis; second, there is periodontitis as an appearance of a systemic disease; and third, there is a single category known as " periodontitis," formerly known as chronic or aggressive periodontitis. These classifications were established by a global workshop in 2017 (7, 8, 9).

Both necrotizing ulcerative gingivitis and periodontitis belong to the umbrella term necrotizing periodontitis (NPD), and have comparable aetiologies, clinical manifestations, and therapies (8–10), NPD is characterised by discomfort, bleeding, and ulceration of the interdental papillae (8). NPD lesions are thought to harbour *Prevotella intermedia*, *Selenomonas spp*., *Treponema spp.*, as well as *Fusobacterium spp*., according to culture investigations (11,12).

Systemic periodontitis is the second form of this illness, and it is treated with the same drugs that impact the peri-dontium as are used to treat malignant instances (7). Several systemic illnesses, both hereditary and non-genetic, may induce periodontal inflammation, which significantly contributes to peridontal tissue loss because they can alter the plaque's influence on the host by altering the microbe-host balance (13).

This study aimed conducted for isolation and Molecular detection of some anaerobic bacteria from patients with Periodontitis.

MATERIALS AND METHODS

Transport and collection: 78 Patients with periodontitis between the ages of 16 and 69 from Dental Clinics in the city of Baghdad were recruited between 2021 and 2022 for this study. After placing a paper point (size 30-45 mm.) inside a pocket with a depth of 3 mm. and leaving it there for 60 seconds, we were able to obtain an adequate sample of GCF for analysis. Next, Thioglycollate broth was added to the paper point. Within 2 hours of collection, all samples were sent to the bacteriology lab where they were cultured at 37 °C for 24-48 hours.

In a variation of the Hungate method (14), bacteria grown in thioglycollate broth were inoculated onto a schaedler agar slant using a loopful of the broth, which was then flushed with filtered CO2 and N2 using a sterile cupper needle for 3-7 days. After that, we counted the colonies and labelled the isolates (15, 16).

The morphology of the bacterial colony was further characterised by Gramme staining and microscopic examination of the developing colonies on Shaedler agar.

Genomic DNA micro Kit (Geneaid, Korea) was used to isolate the bacterial genome. Amplification using global bacterial 16S rDNA primers 27F 5' AGAGTTTGATCCTGGC-3' and 1492 R 5'- GGTTACCTTGTTACGACTT-3' 45 was used to identify the genome of the bacterial isolates, as detailed in the user handbook. PCR amplification required a total volume of 20 l, consisting of 0.5 l of F Primer, 0.5 l of R Primer, 1.5 l of DNA template, and 12.5 l of Nuclease-free water in a 25 l Master Mix tube. The initial denaturation temperature was 92 degrees Celsius for 2 minutes, followed by 30 cycles of 94 degrees Celsius for 30 seconds each, followed by 45 seconds of annealing at 51.8 degrees Celsius and 1.5 minutes of extension at 72 degrees Celsius. The last extension was done for 5 minutes at 72 degrees Celsius. For 1 hour at 60V in the casting tray with 2% agarose gel produced in 1TBE containing 1 l of ethidium bromide per 100 ml of agarose

solution, we ran 3.5 l of 1 kb DNA ladder and 6 l of 16S rDNA. Digital photographs of the items taken under UV light revealed bands of 1500 bp characteristic of the 16S rDNA gene.

Results:

From a total of 78 samples, 34 unique anaerobic bacteria were isolated. Bacteriological techniques were used to identify the five species of bacteria (representing four genera) that were isolated. The Gramme stain test was used for initial confirmation of the microbiological isolates found in both diabetes and non-diabetic individuals, and the findings showed that 24 of the bacterial isolates were Gramme positive cocci, 5 were Gramme positive anaerobic bacilli, and 3 were Gramme negative anaerobic Cocci.

Table 1 displays the gender breakdown of our patient population, which breaks down to 15 male (42.9% of the total) and 25 females (58.1% of the total), with no statistically significant differences ($P > 0.05$) between the sexes.

Table. 1. Numbers and percentages of patients on the basis of sex

Patients	No. Of patients	No. of positive	Percentage
Male	35	15	42.9
Female	43	25	58.1
Total	78	40	51.3

Streptococcus salivasrius was found to be the most resistant species in this study, accounting for 18 (52.9%) of the isolates, followed by *Lactobacillus salivarius* with 7 (20.6%) isolates, *Streptococcus mutans* with 5 (14.7%), and *Veillonella Spp*. with 2 (5.5%). However, only 2.5% of diabetes individuals have the bacterium species *Enterococcus faecalis* (Table 2). All these bacteria were confirmed by using PCR.

Table 2. Isolates frequencies of various bacteria

Anaerobic bacteria	No. of Positive	Percentages
Streptococcus salivarius	18	52.9
Lactobacillus salivarius	7	20.6
Streptococcus mutans	5	14.7
Veillonella spp.	2	5.9
Enterococcus faecalis	2	5.9
Total	34	100%

Discussions:

Different types of anaerobic bacteria were found in diabetic and non-diabetic patients' periodontal pockets, as shown by the microbial isolates. *Streptococcus salivarius* was the most common of these, followed by *Lactobacillus salivarius*, *Streptococcus mutans*, *Veillonella* Spp., and *Enterococcus faecalis*. Daniluk found that *Veillonella* spp., *Gemella morbillorum*, *Prevotella intermedia*, *Streptococcus consellatus*, *Fusobacterium*, *Peptostreptococcus* spp., and *Staphylococcus saccharolyticus* were the most frequent bacterial species; these findings practically contradict his findings (17).

The following bacterial species were identified in the continuing research: Aggregatibacter actinomycetemcomitanse (26.8%), Porphyromonas gingivalis (21.9%), Caponocytophaga spp.

(16.7%), Eikenella corrodens (13.2%), Prevotella intrmedia (10.5%), Prevotella disiens (3.1%), Peptostreptococcus micros (2.9%), Caponocytophaga ging (18).

Recent research has identified the following bacteria as the most common and key pathogens in periodontitis: Peptstreptococcus prevotii, with 15 (8.3%) cases, followed by Peptostreptococcus tetradius, Prevotella melani, Fusibacterium mortiferum, Prevotella intermedia, Wolinella spp., and prevotella disiens, with 1 (0.5%) case (5).

Susceptibility testing research is useful for monitoring the antibiotic responsiveness of individual isolates and providing data for screening for shifting resistance trends. Testing for anaerobes' sensitivity was difficult for several reasons, such as the need for special conditions, the organisms' pickiness, and their sluggish growth rate (19, 20).

Periodontitis is a chronic inflammatory disease of the gums that may be treated with antibiotics; nevertheless, their overuse and the development of antibiotic-resistant bacterial isolates have led doctors to emphasise the need of using them sparingly. The majority of periodontal diseases may be resolved with mechanical treatment alone; however, antibacterial medicines can augment therapy, particularly in individuals who do not respond well to mechanical therapy (21).

Conclusion:

The present investigation found that *Streptococcus salivarius* was the most resistant in all individuals, followed by *Lactobacillus salivarius*.

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