P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2023,(17), (2):48 – 56

Using Multiplex Polymerase Chain Reaction (m-PCR) Methods to detect six Pathogenic Bacteria in Human Oral Cavity

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ARTICLE INFO

Received: 01 / 05 /2023
Accepted: 16 / 05 / 2023
Available online: 12 / 12 / 2023

DOI: 10.37652/juaps.2023.140052.1060

Keywords:
Oral cavity bacterial, Biofilm, Periodontal diseases, Multiplex PCR

ABSTRACT

According to estimates, the human oral cavity can harbour 700–1000 different species of bacteria, which are associated with periodontal and dental problems such as dental caries. Therefore, bacterial infections in the oral cavity may lead to significant health consequences, therefore early and correct identification is essential for effective treatment. This study has tried to create a multiplex PCR (m-PCR) procedure to detect six pathogenic bacteria frequently found in oral cavity infections: *Streptococcus oralis*, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Veillonella parvula*. To confirm the sensitivity of the m-PCR assay, the assay was able to detect as little as 250 pg/µL of genomic DNA from each target bacterium, demonstrating high sensitivity. In conclusion, we have developed a rapid and reliable m-PCR assay for the simultaneous detection of six pathogenic bacteria frequently found in oral cavity infections. This assay has the potential to be a valuable tool for the early and accurate diagnosis of oral cavity bacterial infections, leading to more effective treatment and improved patient outcomes.

INTRODUCTION

According to estimates, the human oral cavity can harbor 700–1000 different species of bacteria, which are associated to periodontal and dental problems such dental caries [1, 2]. Most, if not all the dental caries and periodontal diseases are associated with certain signs, symptoms and medical conditions that are make greater in size, and amount of complexity of the oral microorganisms[3, 4]. Approximately 100 and 300 different strains of bacteria are present in the oral cavity of every person, which are living as biofilms in the oral cavity particulally on the either soft and hard tissues or both [2, 5]. Matrix and also called biofilm structure in oral cavity is a several sequential stages of microbiota activiteis which are start from 1- initial bacterial attachment to a surface of teeth, 2- microcolony formation by cell-to-cell adhesion and then 3- biofilm maturation and final stage will be 4 - dispersal or detachment (strategies to colonize new areas)[6].

The processes of adherence are a complex interactions between various bacteria by useing a differnt steps such as the exchange of signals and metabolites and the generation of growth-stimulating or growth-inhibiting substances which can lead to control the biofilm formation [7].

Mutualistic interactions are thought to promote the formation of mixed-species communities in circumstances whereby each species might be present without the other [8]. In these interactions, *Streptococcus oralis* appears to be particularly significant and can promote the development of model mixed-species communities in human saliva[9, 10]. The most bacteria that can related to periodontal diseases are mostly gram-negative anaerobic bacteria and possibility consist of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and others[11].

The most research has demonstrated *that Streptococcus oralis*, *Streptococcus mutans*, *P. gingivalis*, *P. intermedia* and others have ability to

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inhabit oral cavity [12]. There is evidence that a number of oral bacteria for example *Streptococcus oralis, Streptococcus mutans* belongs to the oral viridans group streptococci. One of the most important oral microbiota in the oral cavity is *S. oralis* and it pioneer bacteria engaged in primary dentition colonization as well as it can be isolated from diffrent location of oral cavity. [13] . *S. oralis* is believed to be associated to the development of human cardiovascular disorders such as infectious endocarditis as well as atherosclerosisdue to the bacterial cell wall of *S. oralis* which is contains coaggregation receptor polysaccharides (RPS) [14]. Receptor-like protein structures function as receptors for surface adhesins with lectin-like characteristics observed on other oral bacteria of in the oral biofilm community. [15]. The structural, functional and molecular properties of streptococcal RPS support a recognition role of these cell surface molecules in oral biofilm formation[16, 17]. According to research, the cell wall polysaccharides found in *S. oralis* and other streptococci of the viridans work together as receptors for lectin-like adhesins located on other components of the oral biofilm community [18].

New evidence indicates that the growth of Candida albicans in the oral cavity of mice encourages the formation of mucosal biofilms by *S. oralis* [19, 20]. *S. oralis* in an S. gordonii dominated biofilm could enable *S. oralis* for introduction of a more favourable partner, such as Actinomyces naeslundii, to join the biofilm community [21]. In the early stages of dental plaque development, It would prove advantageous for a bacteria species to be ability to grow without the help of other microorganisms In addition, encouraging growth through reciprocal cooperation across many species and genera might be critical of diseases development [22]. To battle oral infectious illnesses, it may be critical to understand the interaction between S. gordonii and *S. oralis* in the early stages of oral biofilm development. Nonetheless, the specific roles of *S. oralis* species in the production of biofilms in the oral cavity remain unknown.

At the present time, the semi-quantitative methods that are included cultivation technics using selective and differentiation media, immunogenic methods are the most important way to identify of periodontal and cariogenic bacteria. The using quantitative methods for example DNA sequences, polymerase chain reaction (PCR), or real-time PCR, and shotgun whole metagenome sequencing will be more efficient to identify of periodontal and cariogenic bacteria due to depend on genetic material of bacteria (16S rRNA sequence analysis). Therefore, the most important target of this study was to develop high sensitivity method to detect dental caries and periodontal diseases using Multiplex PCR.

Material and Methods

1- Bacterial strains and growth conditions

S.oralis, S. gordonii, P. gingivalis, S. oralis, F. nucleatum and *V. parvula* that have been used in this study, were routinely cultured under static conditions at a temperature of 37°C using THYE medium. The composition of the THYE medium consists of Todd Hewitt Broth (30 g L-1) and Yeast Extract (5 g L-1). Solidified THYE medium was also used, which contains Bacto Agar (15 g L-1) to provide a solid surface for the bacteria to grow on. Alternatively, the bacteria were cultured in BHYG medium, which contains Brain Heart Infusion (37 g/L), Yeast Extract (5 g/L), and sodium glutamate (2.5 g/L). This medium may have been used for specific experiments or to culture different bacterial strains. All the culture media used were sterilized by autoclaving at 121°C for 15 minutes to eliminate any potential sources of contamination before use all material that have been used were supplied from Sigma-Aldrich, UK. In this study, the following oral bacteria strains were used, *Streptococcus gordonii, Streptococcus oralis, Streptococcus mutans, P. gingivalis, Fusobacterium nucleatum* and *Veillonella parvula.* All bacteria were grown on liquid and solid media (with 1.5% w/v agar that is generally used to solidify growth media for culturing microorganisms) and then sterilized by autoclaving for 15 min at 121°C and 15 psi. S. gordonii, and *S. gordonii* were frequently cultivated in Todd Hewitt Yeast Extract (THYE) medium (20 mL), which included 30 g/L Todd Hewitt Broth and 5 g/L Yeast Extract. *F. nucleatum* was cultured in FAA containing 46 g/L Fastidious Anaerobic Broth (FAB [Oxoid, Leicester, UK]), 2.5 g/L L-glutamic acid, (w/v) Neopeptone, and 5 g/L yeast extract (all Sigma-Aldrich,

UK). *V. parvula* was routinely cultured in BHYGL medium comprising 37 g/L brain Heart Infusion (Melford Laboratories Ltd., UK) 5 g/L yeast extract, 2.5 g/L L- glutamic acid monosodium salt hydrate, and 14 mL ≥88% (v/v) DL-lactate (all Sigma-Aldrich, UK). The bacteria were cultivated anaerobically in a anaerobic jar at 37°C and -5% CO2 environment for 18-48 hours without shaking. The pH of *V. parvula* culture was adjusted to pH 7.5 with NaOH before autoclaving. De-ionized water was used to make all media.

Table1: Shows strains bacteria that are used in this study.

Strains	Description	Source
S. gordonii	**All bacteria were wild types**	**(Delisl *et al*., 1977)**
F. nucleatum		**ATCC**
S. gordonii		**Howard Jenkinson, Bristol**
P. gingivalis		**(Delisl *et al*., 1977)**
V.parvula		**Rob Palmer NIH**
S. gordonii		**Howard Jenkinson, Bristol**

2- DNA extraction

In this study, genomics DNA were purified from a 20 mL culture of all bacteria. The MasterpureTM DNA purification kit was used in accordance with the manufacturer's instructions, with an additional step to ensure effective disruption, to purify chromosomal DNA from *S. gordonii*, S. gordonii, *V. parvula*, *P. gingivalis* and *F. nucleatum*. It was put into practice with the following changes: *S. gordonii*, A. oris, and *V. parvula* were cultivated for 18 hours at 37°C in BHYG broth and BHYGL broth, respectively. For 24 to 48 hours, *F. nucleatum* and *P. gingivalis* were grown in FAB. The supernatant was discarded after the cells were collected. The pellet was re-suspended in 150 µl of spheroplasting buffer (20 mM Tris-HCl, pH 6.8, 10 mM MgCl2, and 26% w/v raffinose.5H2O) that had been pre-warmed to 37°C.

The bacteria were cultured at 37°C for 30 minutes after adding 250 g/mL lysozyme (Sigma Aldrich) and then 250 g/mL lysozyme was added (Sigma Aldrich) with 5 g/ml of Mutanolysin (Sigma Aldrich), which were reconstituted at 10,000 U mL-1. After that cells were incubated, and then loaded in screw-cap Eppendorf tubes with 150 µl of 2x T&C Lysis Solution (Epicentre), subsequently placed into a bead-lysis device (Qiagen Tissue Lyser Ltd., UK) at 40-50 Hz for 3-6 min using 20–60 mg of acid-washed glass beads (0.1-0.2 mm). The manufacturer's instructions were followed when utilizing the MasterPureTM G+ and G- bacteria DNA Purification Kit (Epicentre® Biotechnologies), which has all the components required to isolate genomic DNA from Gram positive bacteria. DNA was suspended in 25 l of elution buffer after extraction (10 mM Tris pH 8.5). Thermo Scientific's NanoDrop ND-1000 Spectrophotometer was used to measure DNA concentrations using the DNA-50 present and then the extracted DNA was checked by agarose gel electrophoresis prior to sending. To be used in later processes, DNA was kept at -20°C.

3- Analysis of DNA concentration by Nano-drop

The optical density (OD) ratios at 260/280 and 260/230 nm, as well as 1% agarose gel electrophoresis, were used to evaluate the quantity and purity of chromosomal DNA. DNA concentrations in the samples were measured using a UK-made spectrophotometer called the NanoDrop ND-1000. The NanoDrop stage was then loaded with 1-2 µl quantities of DNA material. A program that is part of the NanoDrop system can compute concentrations and purity automatically. By measuring the absorbance at 260 nm as ng/µl, the content of DNA is determined; 1 OD260 unit equals 50 g/mL for DNA. The range of 1.8 to 2.0 that was considered to be pure for DNA from guanidine thiocyanate contamination was employed as a secondary measure of DNA purity.

4- DNA gel electrophoresis

To measure the size of DNA, electrophoresis on agarose gel was performed (FMC Bioproducts, Rockland, USA). Depending on the DNA size of the products under investigation, agarose gels dissolved in an electrophoresis buffer were prepared at a concentration of 1-2%. For standard PCR products, agarose gels between 1-2% (w/v) were prepared. To see DNA, agarose was dissolved in TAE buffer (40 mM Tris-acetate, 1.0 mM EDTA, pH 8.0), and Gel Red Nucleic Acid Gel Stain (10,000x in water; Biotium) was added at a rate of 0.1 g/mL (5 l per 50 mL). In order to determine the size of the products on the gel during gel electrophoresis, the relevant DNA ladders (or DNA molecular weight markers) were utilised: HyperLadder

100 bp (100-1013 bp; Bio-line, UK); HyperLadder 1 kb Plus; HyperLadder 25 bp (25-500 bp); (250-12,007 bp; Bioline). After mixing, loading dye (40 percent (w/v) sucrose, 100 millimolar EDTA, 8.0 pH, and 0.01percent (w/v) bromophenol blue) and DNA sample were loaded to the well. Gels were run for 120–180 min at 250–300 mA and 85–100 V. The gel's molecules were then observable after the electrophoresis procedures were finished by exposing the gel to long-wave ultraviolet (UV) light from a transilluminator. Using a gel documentation technique, 5.51 Mpixel images of DNA were collected (G: BOX Trans illuminator, Syngene).

5- Primer design

The National Centre for Biotechnology Information's (NCBI) genomic DNA sequences was used to design primers. PCR primers were created using Primer3Plus for m-PCR. Most primers were made to produce products between 359 and 1030 bp in length and most had melting temperatures between 50 and 60 °C, guanine:cytosine contents between 40 and 60 %, and lengths between 15 and 25 bp. For the purpose of designing primers for other PCR applications, NCBI software (National Center of Biotechnology Information) was used. Eurogentec acquired and created oligonucleotide primers. Stock solutions were kept at -20 °C after being diluted to a final concentration of 10 M. Table1 contains a list of the primer sequences.

Table 2: Describe the m- PCR primers that are utilized in this study.

Bacteria Name		Primer Sequence	bp	Source
P.gingivalis	F	TTGGTGAGGTAACGG CTCAC	359	This Study
	R	CTCAGGTTTCACCGC TGACT		
F.nucleatum	F	ACAGAGTTGAGCCCT GCATT	479	This Study
	R	TTGCCTCACAGCTAG GGACA		
S. oralis	F	ACGCATTTCACCGCT ACACA	599	This Study
	R	GGGTGAGTAACGCGT AGGTA		
V.parvula	F	GAAGGCGACTTTCTG GACGA	703	This Study
	R	ACTTCCGACTTTCGT GGTGT		
S. gordonii	F	TGAGTGCAGAAGGGG AGAGT	872	This Study
	R	CACCTTCCGATACGG CTACC		
S. gordonii	F	ACCAGAAAGGGACGG CTAAC	1030	This Study
	R	CCTTCCGATACGGCT ACCTT		

6- Release of DNA from whole cells using Gene Releaser

The method of gene release works via rupturing cell membranes and releasing DNA into the solution. The process of extracting DNA begins by picking a single colony of bacteria from an agar plate using a sterilized toothpick and subjecting it to the Gene Releaser kit (BioVentures, Inc. Murfreesboro, USA). The isolated colony was transferred to the bottom of a PCR tube, and 20µl of re-suspended Gene Releaser was added, which was briefly vortexed to mix. The thermal cycling Gene Releaser methodology was performed as per the manufacturer's instructions (refer to Table 2 6). After centrifugation at 5,000 g for 1 minute, the supernatant was collected in a fresh PCR tube, and 1-3 µl was used as the PCR template for further amplification. The PCR protocol was followed as mentioned in table2. Finally, the insoluble material was discarded, and only the supernatant was used as the template for PCR amplification.

Table3: Steps of gene Releaser program and conditions that are utilized to release genomic DNA from a single colony

Step	Temperature	Time
1.	65 °C	30 sec.
2.	8 °C	30 sec.
3.	65 °C	90 sec.
4.	97 °C	180 sec.
5.	8 °C	60 sec.
6.	65 °C	180 sec.
7.	97 °C	60 sec.
8.	65 °C	60 sec.
9.	80 °C	Hold

7- Conventional polymerase chain reaction

The Expand High Fidelity PCR System (Roche) kit and ReddyMixTM (ABgene) kit have been commonly used in 50 µl reactions to amplify small DNA fragments. The ReddyMixTM kit was used to perform standard PCR reactions according to the manufacturer's guidelines. On the other hand, the Expand High Fidelity PCR System employed Tgo DNA polymerase isolated from Thermococcus gorgonarius, which has proofreading activity and reduces the risk of incorrect base incorporation for more effective PCR reactions. The protocols and recommended annealing temperatures for the kit is presented in Tables 4, based on the primers used. The DNA Engine PTC-200 (MJ Research) or T100

Thermal Cycler (Bio-Rad) thermocyclers were used for all PCR experiments.

Table 4: Expand high fidelity PCR system reaction mix. This PCR system is a mixture of Taq DNA polymerase and a DNA polymerase with proofreading activity for high yield and fidelity

Component	Volume	Final Conc.
Water, PCR-grade	Add to 50 µl	
5x Expand Long Range Buffer with 12.5 mM MgCl2	10 µl	1X
PCR Nucleotide mix (dNTP 10Mm each)	2.5 µl	500 µM
Forward primer	Depend on concentration	0.3 µM
Reverse Primer	Depend on concentration	0.3 µM
Expand Long Range Enzyme mix (5ULµl)	0.7 µl	3.5 U
Template	Depend on concentration	Up to 500 ng
Total Volume	50 µl	

8- Sensitivity of the m-PCR assay

To determine the sensitivity of the m-PCR assay. The genomes of the six strains were extracted and further diluted to concentrations of approximately 120 ng/µL, 100 ng/µL, 75 ng/µL, 50 ng/µL, 25 ng/µL, 2.5 ng/µL, 1.25 ng/µL, 750 pg/µL, 500 pg/µL, and 250 pg/µL. One microliter of each dilution was used as the template DNA in the m-PCR to determine the sensitivity of the test.

9- Detection of Six Pathogenic Bacteria .

The m-PCR method that has excellent capacity to detect six pathogens in oral cavity specimens from infected patients has been evaluated. The researchers collected saliva samples and combined them with phosphate-buffered saline before culturing them in LB broth for 4 hours. The samples were then boiled for 5 minutes to extract the genomic DNA required for the m-PCR detection process. To extract the genomic DNA, a DNA extraction mini kit (Tiangen Biotech Co., Ltd.) was used as per the manufacturer's instructions. In addition, the researchers prepared six bacterial genome mixtures beforehand, which included Streptococcus gordonii, Streptococcus oralis, Streptococcus mutans, *P. gingivalis*, Fusobacterium nucleatum and Veillonella parvula. These bacterial genome mixtures have been utilized as a positive control for the multiplex PCR methods.

Results and discussion

1- Analysis of DNA concentration by Nano-drop

It is essential to evaluate the DNA sample's quality which can be determined using the A260/A280 ratio acquired from the Nano-Drop test. A ratio of around 1.8 indicates that DNA supposes to be pure while lower ratios may indicate the presence of contaminants such as proteins, whereas a A260/A230 ratio with more than 2.2 may indicate the presence of RNA or other impurities. Therefore, further purification steps may be required if the A260/A280 ratio is outside the expected range.

All the results were between 1.8-2.2 rations which can suggest that all samples are of high quality and suitable for use in various molecular biology techniques, including gene expression profiling, microarray analysis, or RNA sequencing. It also suggests that the sample has been prepared carefully, with minimal contamination from organic compounds that could interfere with downstream applications. However, although an appropriate A260/A280 ratio was a significant criterion for assessing the quality of nucleic acid samples, it shouldn't be the only element to consider. Additional variables such as DNA or RNA integrity, purity, and concentration can all have an impact on sample quality and applicability for downstream applications. As a consequence, while evaluating the quality of nucleic acid samples for their unique experimental objectives, researchers require examine numerous criteria.

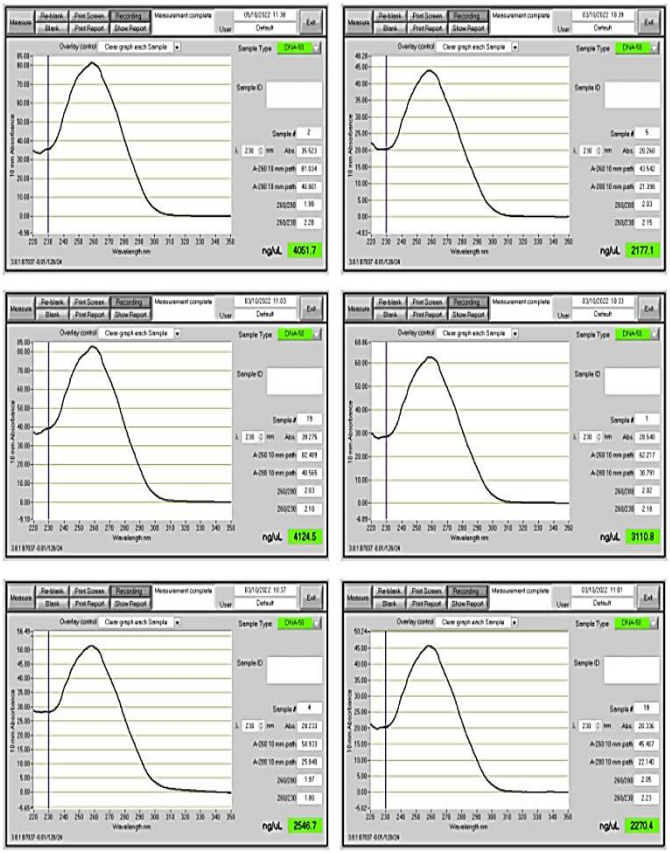

Fig 1: The purity of the DNA sample. The A260/A280 and A260/A230 ratios can be used to assess the purity of the sample. All bacteria sample were extracted very well.

2- Amplification of target genes

The primers that were designed for the m-PCR assay were successful in amplifying specific regions of six target genes from the bacterial genome mixtures. The amplified region of gene of all bacteria were 16Srna. The amplified regions were 1030 bp for the Streptococcus gordonii, 872 bp for the Streptococcus mutans, 703 bp for the Veillonella parvula, 599 bp for the Streptococcus oralis, 479 bp for the Fusobacterium nucleatum, and 359 bp for the Porphyromonas gingivalis. Agarose gel electrophoresis was used to visualize the different sizes of PCR products that were produced for each target gene, allowing for size discrimination. The PCR products appeared as distinct bands in each lane, indicating that the primers successfully amplified the desired regions of the target genes. A visual representation of the results can be seen in Figure 2. The m-PCR results demonstrate that the designed primers successfully amplified particular regions of six target genes from bacterial genome mixes.

The 16S rRNA gene a frequently employed marker gene for bacterial identification and taxonomy has been amplified in every species of bacteria. It should be noted that the PCR results for each target gene were varied in size indicating that the primers were unique to each bacterial species and did not amplify non-target DNA. The diameters of the PCR products also make it easy to discriminate between the many bacterial species in the combination. The presence of distinct bands in each lane of agarose gel electrophoresis indicates that the PCR products were of good quality and had not been damaged. The quality of PCR results is critical for applications that follow like sequencing and cloning. In general, the successful amplification of distinct target gene areas confirms that m-PCR assay's utility for detecting and characterizing bacterial species in a mixed sample. This test might be utilized in a variety of domains, including clinical microbiology, the microbiology of the environment, and food microbiology.

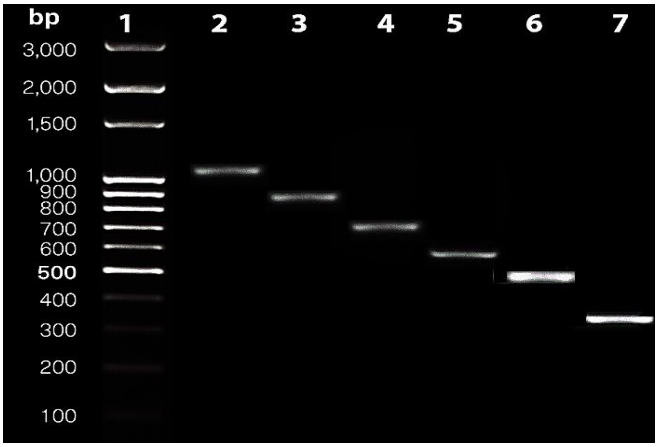

Fig2: Amplification of the multiplex PCR target gene. Lane M: 3000 base pair (bp) DNA marker; Lanes 2-7: 1030 bp *Streptococcus gordoni*, 872 bp *Streptococcus mutans*, 703 bp *Veillonella parvula*, 599 bp *Streptococcus oralis*, 479 bp *Fusobacterium nucleatum*, and 359 bp *Porphyromonas gingivalis*.

3- Sensitivity of the multiplex-PCR assay

Figure 3 demonstrates the detection small amount of genomic DNA concentrations for six bacterial species (Streptococcus oralis, Streptococcus mutans, *P.* gingivalis, Fusobacterium nucleatum and Veillonella parvula). In accordance with Figure 5, the detection limit of genomic DNA quantities for all six bacterial species was approximately 500 pg. It means that the method is capable of recognizing bacterial genomic DNA in

amounts as small as 500 pg in a sample. It is crucial to highlight that all studies were done in triplicate, which improves the dependability of the data. In general, our findings show that the test has a high sensitivity for identifying bacterial genomic DNA, it making it a valuable tool for detecting and quantifying bacterial species in complicated samples. Different parameters, such as the efficiency of DNA extraction and PCR amplification, might impact detection limits, which may need to be tuned for individual applications.

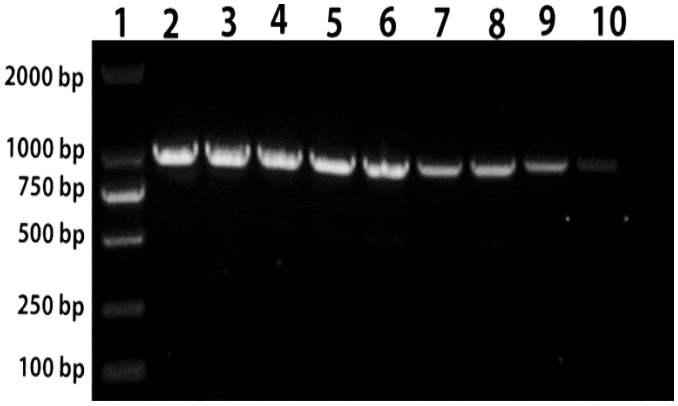

Fig3: Multiplex PCR sensitivity testing for bacterial genomic DNA detection. Lane M: 3000 bp DNA marker; a Lanes 2–10: the concentration of Porphyromonas gingivalis. The DNA concentration were 120 ng, 100 ng, 75 ng, 50 ng, 25 ng, 2.5 ng, 1.25 ng, 750 pg, 500 pg and 250 pg, respectively.

4- Detection of Six Pathogenic Bacteria using M-PCR

The results showed that the band sizes for each bacterial species in the m-PCR assay present in the tested samples as well as the specific band sizes provided are S. gordonii 1030 bp, *S. oralis* 599 bp, *S. gordonii* 872 bp, *P. gingivalis* 359 bp, *F. nucleatum* 479 bp, and *V. parvula* 703 bp (See Fig4). The results of this study indicate that the DNA from all six of these harmful microbes had been found in the examined samples. The M-PCR assay is a quick and accurate method of recognizing several species of bacteria in a single response which makes it especially helpful for diagnosing periodontal diseases and dental caries. It is essential to emphasize, however, that the presence of particular kinds of bacteria is not always indicative of the presence of a disease or illness. To evaluate the importance of the bacterial presence and design suitable treatment regimens, more clinical and microbiological examination is required. This result was similar to the study that have published by [23]. In this study was developed a multiplex PCR examine that are detected 5 bacterial infections, including Streptococcus pneumoniae, Legionella pneumophila Haemophilus influenza, S. aureus and Mycoplasma pneumoniae.

The study also took into account the necessity to produce different sizes of PCR products for quick recognition of the pathogen during electrophoretic separation. This is significant because it enables straightforward identification of bacterial species based on the size of the PCR result during gel electrophoresis. The study concludes the use of the m-PCR assay is an excellent choice for public health laboratories for the reason to its high throughput, accuracy, and low cost. The result of this test has the potential to assist the quick and precise detection of these pathogens, which can help to avoid the spread of infections and improve patient outcomes.

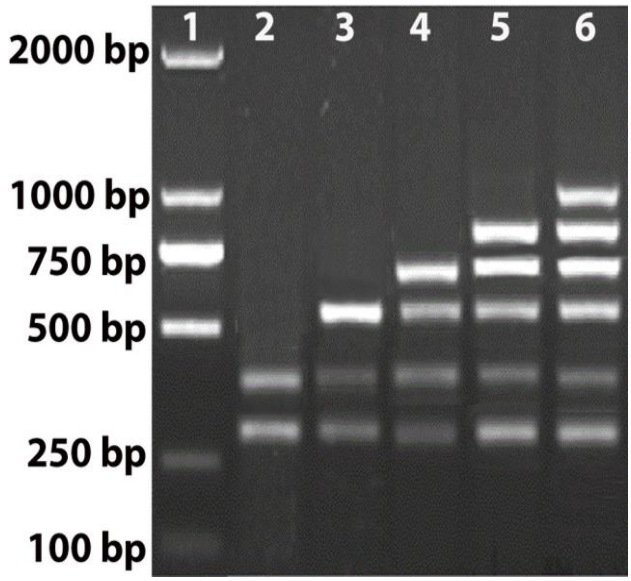

Fig4: Determination of bacteria using the multiplex PCR. Lane 1: 2000 bp DNA marker; a **Lane 2**: the template of m-PCR contains 2 bacterial genomes *P. gingivalis* and *F. nucleatum*. **Lane 3**: the template of m-PCR were *P. gingivalis*, *F. nucleatum* and *S. oralis*, **Lane4**: the template of m-PCR were *P. gingivalis*, *F. nucleatum*, *S. oralis* and *V. parvula*; **Lane 5**: the template of m-PCR were *P. gingivalis*, *F. nucleatum*, *S. oralis*, *V. parvula* and *S. gordonii*; **Lane 6**: the template of m-PCR were *P. gingivalis*, *F. nucleatum*, *S. oralis*, *V. parvula*, *S. gordonii* and *S, gordonii*.

Conclusion

In conclusion, we have developed a rapid, sensitive and specific m-PCR assay for the simultaneous detection of six pathogenic bacteria commonly found in the oral

cavity. The m-PCR assay could detect as low as 250 pg of genomic DNA of each bacterium, demonstrating high sensitivity. The specificity of the assay was confirmed by testing various bacterial species. Moreover, bacterial species were also tested to validate the specificity of the m-PCR detection system. The m-PCR assay established in this study is rapid, cost-effective, and reliable, making it a valuable tool for the clinical diagnosis of bacterial infections in the oral cavity. The assay can be used for rapid detection and identification of the six target bacteria, which is critical for effective treatment and prevention of infections. Furthermore, the m-PCR assay can also be used for surveillance and monitoring of oral bacterial pathogens, enabling early detection and control of outbreaks.

Overall, this study provides a new and efficient method for the diagnosis and surveillance of bacterial infections in the oral cavity, which could significantly improve clinical outcomes and public health.

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إستراتيجيات واعدة لتقليل خطر انتقال فيروس SARS-CoV2 أثناء إجراءات طب الأسنان والإجراءات الطبية

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الخلاصة:

وفقًا للتقديرات، يمكن أن يؤوي تجويف الفم البشري 700–1000 نوع مختلف من البكتيريا، والتي ترتبط بمشاكل اللثة والأسنان مثل تسوس الأسنان. لذلك، قد تؤدي الالتهابات البكتيرية في تجويف الفم إلى عواقب صحية كبيرة، وبالتالي فإن التحديد المبكر والصحيح ضروري للعلاج الفعال. حاولت هذه الدراسة إنشاء إجراء متعدد تفاعل البوليميراز المتسلسل (m–PCR) للكشف عن ستة بكتيريا ممرضة توجد بشكل متكرر في التهابات تجويف الفم : Veillonella ،Fusobacterium nucleatum ،Porphyromonas gingivalis ،Streptococcus mutans ،Streptococcus oralis parvula. ولتأكيد حساسية مقايسة m–PCR، كان الفحص قادرًا على اكتشاف أقل من 250 بيكوغرام/ ميكرولتر من الحمض النووي الجيني من كل بكتيريا مستهدفة، مما يدل على حساسية عالية. في الختام، قمنا بتطوير اختبار m–PCR سريع وموثوق به للكشف المتزامن عن ستة بكتيريا ممرضة توجد بشكل متكرر في التهابات تجويف الفم. هذا الاختبار لديه القدرة على أن يكون أداة قيمة للتشخيص المبكر والدقيق للالتهابات البكتيرية في تجويف الفم، مما يؤدي إلى علاج أكثر فعالية وتحسين نتائج المرضى.

الكلمات المفتاحية : جرثومي تجويف الفم، بيوفيلم، أمراض اللثة، Multiplex PCR