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Molecular Study of Some Virulence Factor For Porphyromonas gingivalis Isolated From Some Periodontal Disease Patients in Maysan City/Iraq

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By

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بسم الله الرحمن الرحيم

هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَّرَهُ مَنَازِلَ

لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابَ مَا خَلَقَ اللَّهُ ذَلِكَ إِلَّا

بِالْحَقِّ يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُونَ

صدق الله العظيم

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“Supervisor Certification”

This is certified that this thesis entitled:

(Molecular study of some virulence factors Porphyromonas gingivitis isolated from some periodontal disease patients in Maysan)

Presented by (Zahraa Farqad Faroq) was prepared under my supervision at the Department of Biology, College of Science, University of Misan, as partial fulfillment requirements for the degree of Master in Biology.

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Dedication

I dedicate the fruit of my effort kneeling to

To God Almighty

To giving incarnate, to my role model in my life, to the luminous lamp that illuminates my darkness

My mother and father

To my super heroes, to my support that does not tend to the buds of hope, to the spirit of life

My sisters and brother

To the one who gave me his effort and time

My supervisors

Zahraa

Abstract

Porphyromonas gingivalis, a gram-negative, obligately anaerobic, non-motile, and non-spore-forming bacteria can create a local infection in the periodontal tissue around implants. The consortium's bacterial species are in regular dialogue with one another; they refer to themselves as the red complex. The aims of this study to isolate and identify of the pathogenic obligate anaerobic Porphyromonas gingivalis from oral cavity of some patients with gingivitis and periodontal disease in Misan governorate center for age groups between 9-70 years of patients attending the Specialized Dental Center, Child Teaching Hospital and Dijla Dental Center in Al-Amarah City South Center Iraq. This was done using conventional, Vietk 2 compact and biochemical tests, and then it was identification by molecular methods by means of the diagnostic gene 16S rRNA by chain reaction (PCR) and specific primers that used in this study for diagnose Porphyromonas gingivalis bacterial isolates and the work of the evolutionary tree of the studied isolates bacteria based on the NCBI Gene Bank.

Samples were collected from 21 November to 6 February 2023.A total of 100 samples were collected from anaerobic bacterial isolated belonging to 50 patients with gingivitis and periodontal disease and 50 control in Misan governorate center for seven age groups between ≤ 10-70 years. (≤ 10, 11-20, 21-30, 31-40, 41-50, 51-60, 61- 70 years old). including 43 Female (43%) and Male (57%) and the highest sequestration rate was 100% of male in the age groups (21-30) and (31- 40) to identify bacterial isolates that cause gingivitis and periodontitis dental diseases. The present study indicated that Porphyromonas gingivslis pathogenic bacterial isolates Gram- negative strains were the most frequent.

Virulence is the ability of an organism to infect a host and cause a disease. Porphyromonas gingivalis plasma membrane serves as a dynamic interface between

the oral pathogen and its surroundings. Virulence components include outer membrane vesicles (OMVs), lipopolysaccharides (LPS), gingipains, hemolysins, and hemagglutinins. Fimbriae, tiny, filamentous structures on the surface of most strains of P. gingivalis, multiply outside the outer membrane and aid in the formation of biofilms, attachment of bacteria to host cells, and invasion.

The antibiotic sensitivity for Porphyromonas gingivalis, that were isolated from some patients in Misan City. These isolates strains of Porphyromonas. gingivalis, were tested against some antibiotics such as Cefoxitin (fox 30mcg), Doxycycline (DO 30mcg), Ciprofloxacin (CIP 30mcg), Nalidixic acid (NA 30mcg). The strains of Porphyromonas gingivalis showed a total (100%) resistant for all antibodies used in the experiment.

In this study was diagnoses the virulence gene. Fim A gene two type (234 bp) and (294 bp) of Porphyromonas gingivalis- specific primer Showing by gel-electrophoresis results of evaluating specific primer binding fim A gene region on the Porphyromonas gingivalis through Polymerase chain reaction (PCR) and Fim A gene of Porphyromonas gingivalis- universal primers. Showing by gel-electrophoresis results of universal primers primer binding fim A gene region on the Porphyromonas gingivalis through Polymerase chain reaction (PCR).

Finally the phylogenetic tree (Mega) with the real branch length leading of P. gingivalis clade (marked by the gray rectangle). The tree with the branch length leading to P. gingivalis shortened 100 times (the dashed line) to show detailed relationships between P. gingivalis strains and isolates. Joined names of strains and isolates indicate their 100% sequence identity. Neighbor-joining phylogenetic tree of strain P. gingivalis Bootstrap values (expressed as a percentage of 1000 replications) > 65% are shown at the branch points 16Sr RNA primer.

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List of Abbreviations

Abbreviation	Key
%	Percent
μl	Microliter
16SrRNA	Ribosomal Ribonucleic Acid
AgPD	Aggressive Periodontitis Disease
AST	Antibiotics Susceptibility Testing
AT	Annealing Temperature
Bp	Base Pairs
CPD	Chronic peridontitis Disease
CPS	Capsular polysaccharides
DNA	Deoxyribonucleic Acid
DW	Distilled Water

EPS	Extracellular polymeric substances
F	Forward
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase human
GCF	Gingival Cervricular Fluid
GD	Gingivitis Disease
GT	Granulation tissue
IgG	Immunoglobulin G
L	Liter
LPS	lipopolysaccharides
L-rrA protein	Leucine-Rich Repeat protein
Ml	Milliliter
NCBI	National Center for Biotechnology Information
NPD	Necrotizing Periodontal Disease
ºC	Degree Celsius
OHRQOL	Oral Health-related Quality of Life
OMVs	Outer membrane vesicles
P. gingivalis	Porphyromonas gingivalis
PCR	Polymerase Chain Reaction
pH	Power of Hydrogen
PP	periodontal pockets
R	Reverse
RNA	Ribonucleic acid

TBE	Buffer - 10X (Tris-borate-EDTA)
TLR2	Toll-like receptor 2
Tm	Melting Temperature

Chapter one

Introduction

1.1 Introduction

The oral cavity is place to a diverse community of living organisms, and its ecosystem encompasses both the oral mucosa and the tooth enamel. The oral microorganisms exhibit significant and swift variations in composition and activity depending on the host and diet, sensitivity to pH changes, interactions between the bacteria, and gene mutations (Tuominen & Rautava, 2021). The host features, food, bacterial adhesion, bacterial transmissibility, and other ecological factors that change as a person age and develops affect the microbiota of the oral cavity.

As a result, there are obvious differences between the quantities and types of microorganisms found in dental plaque from children, adolescents, and adults. Gingivitis, periodontitis, dental caries (tooth decay), and endodontic abscesses are some of the signs of bacterial dysbiosis in the mouth that can affect adults. Acute infections in the oral cavity are rather uncommon, despite widespread microbial colonization, which can be explained by the continual interaction between the microbiota and the host's immune system. (Tuominen and Rautava,2021). The deterioration of periodontal tissues, such as gingiva and alveolar bone, characterizes periodontal disease, which has been linked to various systemic diseases (Agnello et al.,2017).

Children's gingivitis is less severe than adults' in terms of severity the same quantity of dental plaque. According to epidemiological studies, gingivitis is uncommon in children under the age of six. then a steady rise in frequency until it reaches a peak about the time of puberty. Minor indications of gingivitis are noted if dental hygiene is not maintained in preschoolers. Regions of inflamed gingival tissue in the primary teeth of humans. There is no association between plaque burden and the size of the human's irritated gingival tissue fundamental dental system.

Chapter One Introduction

Gingivitis or periodontitis must be completely absent from any site for there to be periodontal health. The presence of symptoms of plaque-induced gingival irritation in at least one location is what is known as gingivitis. when there were no other causes of redness, swelling, or bleeding on probing—symptoms of gingival inflammation. Gingivitis was diagnosed in those who showed no evidence of attachment loss (such as pockets, gingival recession, or bone loss) Porphyromonas gingivalis, a gram-negative anaerobic black-pigmented bacterium, is a major periodontitis pathogen. It forms a "red complex" with Tannerella forsythia and Treponema denticola (How et al. 2016).

A biofilm is a three-dimensional microbial colony that develops on a surface and responds to its surroundings. Sixty percent to eighty percent of all microbial diseases are caused by biofilms, making them a novel challenge for disease identification and treatment. Biofilms are communities of bacteria attached to a surface and consist of extracellular polysaccharides (EPS), proteins, lipids (Li et al.,2020).

1.2 The Aims of the Study

Some virulence factors of Porphyromonas gingivalis isolated from patients with periodontal disease in the city of Misan, Iraq,

1- Identification of Porphyromonas gingivitis by some biochemical test, vitek 2 compact system and molecular techniques.

2- Antibiotic susceptibility testing for all Porphyromonas gingivalis strain.

3- Detection Porphyromonas gingivalis by 16s rRNA and specific primer gen sequence and virulence gene by RCR

4- Determine the frequency of different Porphyromonas gingivitis fim A genotypes and their impact on the virulence of periodontitis.

Chapter Two

Literatures Review

2. Literatures Review

2.1 Oral Health and Oral Microbiota

A healthy mouth is described as having the "multifaceted capacity to talk, smile, smell, taste, touch, chew, swallow, and transmit a variety of emotions via facial expressions with confidence and without pain, discomfort, or illness of the craniofacial complex" by the FDI World Dental Federation. Therefore, it is linked to the most basic features of one's psychological and physiological well-being. It encapsulates the physiological, social, and psychological factors that are crucial to one's quality of life and is dynamic, changing in response to the individual's experiences, perceptions, expectations, and adaptability to circumstances (Glick et al.,2016). Oral Health-related Quality of Life (OHRQOL) is a result that is focused on the individual and is based on multidimensional and subjective ideas (Graziani et al., 2020).

Health and sickness are profoundly influenced by the microbiome, the biological community of commensal and pathogenic bacteria that share our bodies and environments (Collins and Dixon,2005). A diverse environment of bacteria known as the commensal microbiota coexists with humans. They are crucial for human development, nutrition, and immunological function and are mostly found in five bodily regions: the stomach, oral cavity, skin, nose, and vagina (cheng et al., 2021).

Even though "microbiota" and "microbiome" are commonly used interchangeably, there are some subtle differences between the two. Oral microbiota refers to the non-pathogenic bacteria present in the mouth (oral microflora).

The term "Oral Microbiome" is used to describe the collective genome of the bacteria that reside in the mouth cavity. These bacteria are commensal, meaning that

they live on or within another creature without harming or helping it. The oral cavity is place to the second-largest collection of microbes in the human body. Oral bacteria can thrive on the oral mucosa and in the hard and soft tooth tissues (Al-Taweel ,2017).

Bacteria thrive in the warm, moist environment of the mouth and nasal passages. The oral cavity maintains a constant 37ºC, making it an ideal environment for bacterial growth. The pH range of human saliva, 6.7, is ideal for the vast majority of bacterial species. Biofilms, groups of bacteria protected by an extracellular polysaccharide coating, colonize the tooth enamel, gum tissue, and other hard surfaces of the oral cavity (Kebede et al.,2018).

Because of the symbiotic nature of their interaction, these creatures are classified as commensals, when compared to other human microbial communities, the oral microbiota is the second most diverse and complicated microbiome after the gut. The primary tenants are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria. Dental health requires a homeostatic balance between the oral microbiota (made up of more than 700 bacterial species) and the human immune response (Verma et al., 2018). To wit: (Cheng et al.,2021; Deshmukh,2019).

In the mouth, an antibacterial layer shields both living and nonliving surfaces. For example, periodontal biofilm can serve as a source for the transmission and development of systemic infections; as a consequence, it is necessary to control this balance between the host and bacteria in oral to preserve oral cavity homeostasis (Bianchi et al.,2020) The direct or indirect impacts of inflammation by oral bacteria are considered to contribute to neurological diseases. (Slocum et al.,2016).

2.1.1 Communal Oral Population

Communal Oral Population is estimated that there are at least 700 distinct species of bacteria living in the human oral cavity, with the gingival sulcus being the most well - studied colonization place in the oral mucosa. The gingival sulcus is the space between the gums and the teeth's hard surface where colonies of bacteria live and interact with the mucosal epithelial cells (Chawla and Sarkar,2019).

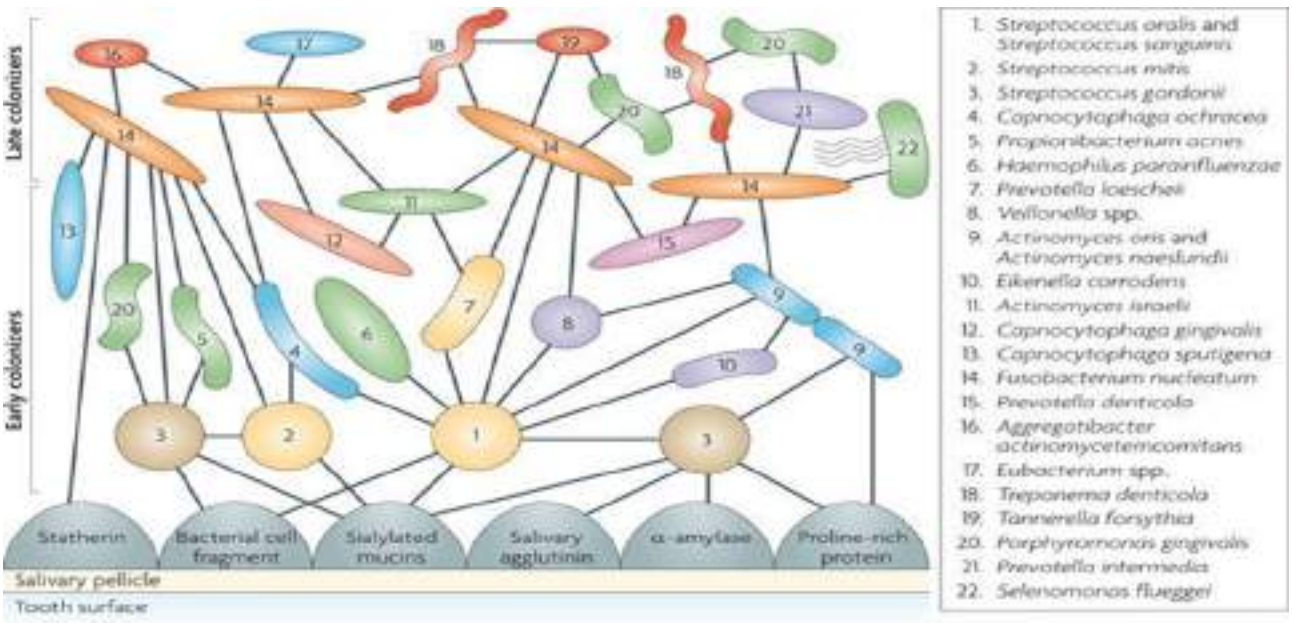

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Figure (2-1) displays a spatial and temporal model of oral bacterial colonization, with the first colonizers shown binding to corresponding salivary receptors in the acquired pellicle, followed by subsequent colonizers and bridge bacteria (Mohammed, 2018).

The primary occupants of the mouth cavity among the commensal populations are bacteria. They include Streptococcus species, Actinomyces species, Veillonella species, Fusobacterium, Porphyromonas, Prevotella species, Treponemes,

Nisseriae, Haemophilus species, Eubacteria species, Lactobacteria species, and Capnocytophaiken species in healthy mouths. Staphylococci, Propionibacterium spp., and Peptostreptococci (Gupta et al.,2008). Nutrition for oral bacteria comes from a variety of sources, including starch and sucrose from the host's meal, glycoprotein from rough secretions, extracellular microbial products of adjacent bacteria, and internal food storage granules from saliva (Holloway et al.,2014)

Bacteriophages, the most common type of virus found in the mouth cavity, replicate within bacteria before eventually eliminating them. Although their precise function in the mouth is unknown, viral communities can benefit their host bacteria's evolution (Clay-Williams et al.,2020).

About 150 different species of the genus Candida, and between 40 and 60 percent of healthy individuals have it living in their oral microbiota. This Candida can cause infection by exploiting weaknesses in the host's defenses, either locally or systemically (Xu and Darwazeh,2014). Opportunistic fungal infections have become more common recently, most likely as a result of several factors, such as broad-spectrum antibiotics, immunosuppressive treatments, blood transplants, cancer, diabetes, and AIDS (Wen et al.,2022; Patil et al 2015).

Other symbionts include the protozoa Entamoeba gingivalis and Trichomonas tenax. The oral microbiota facilitates microbial interactions between various species and has a symbiotic connection with its human host via interacting with the mouth cavity. The oral microbiota plays a crucial role in the development and maintenance of an effective immune response in the mouth. The host immune system regulates and protects the oral commensal microbiota, much as it does the host against pathogenic microorganisms. (Zinn, 2019).

2.1.2 Oral Hygiene and Risk Factors:

In oral hygiene about 770 distinct bacterial species, forming the oral microflora or mouth microbiome (Ye and Kapila, 2021). Bacteria in this area include both harmless commensals and dangerous pathogens. Commensals, bacteria that live in the host but often do not cause illness, may transform into pathogens under optimal circumstances (Cugini et al.,2021).

Prevention of cavities is important for general health since the mouth is a portal to the rest of the body. Poor nutrition, smoking, and alcohol use are all linked to an increased risk of oral health problems, as are obesity, diabetes, chronic lung disease, cancer, and cardiovascular disease (Peres et al., 2019). Dental checkups and treatments are put off due to a lack of knowledge, interest, awareness, and memory, leading to high prices and poor priority (Rocha et al., 2018). Oral problems are complicated by a several factors, and poor hygiene is one of them inadequate oral hygiene results in the development of dental plaque, a thin biofilm that can adhere to the tooth surface and harbor a wide range of bacteria and, on occasion, desquamated epithelial cells (Abebe,2021).

Dental caries and gum disease are major contributors to edentulism. Dental caries and periodontal disorders are the most prevalent oral diseases and are considered a substantial disease burden worldwide because they can go unnoticed for lengthy periods before causing painful exacerbations (Frencken et al., 2017).

2.2 Biofilm:

In practically every ecosystem on Earth, microbes colonize surfaces. Biofilms are described as aggregates of cells encased in self-produced extracellular polymeric substances (EPS) that form at a phase boundary, (Tuck et al.,2022), Biofilms have been extensively discussed in the literature due to their diversity, ubiquity, and significant impact in numerous diseases depending on their location and the species involved, biofilms can either be useful to or destructive to human civilization (Peças et al.,2018; Tuck et al.,2022).

Dental biofilms emerge as communities of interacting bacteria that are physically and functionally organized. These interactions, which can also be antagonistic or synergistic, result in the formation of a biofilm that shields the tooth surfaces from non-oral microbial invasion. An environment with a broadly neutral pH is the consequence of a biofilm's active balancing act between sluggish rates of acid creation and compensating alkali generation. Such circumstances limit the proliferation of germs linked to periodontal and caries disorders while stabilizing the composition of species linked to health. H_2O_2 and bacteriocins are two compounds that microbes in a biofilm- associated with health can create, which may inhibit the growth of germs linked to disease. (Sanz et al.,2017).

Most oral diseases may be traced back to bacterial biofilms, which are also responsible for the cariogenic activity that causes tooth decay and significantly shortens the lifetime of dental prostheses and restoratives. Bacteria, algae, and fungi form biofilms, which are multicellular communities that attach to and form layers on both living and nonliving surfaces. Biofilms are communities of microorganisms that work together to guard against environmental stress, promote synergistic interactions, sustain

survival during famine, and prevent the displacement of extracellular enzymes. Surface charge, surface energy, roughness, and topography have all been shown in vitro to affect the binding force between the underlying material and the biofilm (Engel et al., 2020).

A well-developed biofilm has communication capabilities through quorum sensing, nutrition production, sharing, matrix production, etc. The structure and the biofilm are difficult targets for the immune system and medications due to their durability. Many times, mechanical manipulation is thought to be the most efficient method for removing a biofilm. (Holmer,2022)

2.2.1 Stages of Biofilm Formation:

The word "biofilm" was first used in a professional context in the middle of the 1980s, but a complete knowledge of how they developed was not achieved until much more recently. There are five distinct phases of oral biofilm formation (Kriebel et al.,2018).

1. Pathogenic bacteria getting inside the mouth.

2. The gathering of harmful and healthy microbes, and the production of salivary pellicles.

3. Biofilm-forming bacteria proliferate noticeably, a mucilaginous layer forms, dysbiosis sets in, and adherence becomes permanent.

4. the completion of dysbiosis and the transfer of drug-resistant genes between pathogenic bacteria and the healthy oral microbiota.

5. The bacterial spread and biofilm maturation.

Biofilm, an irreversible mucilaginous coating produced on the teeth, begins with the invasion of pathogenic bacteria into the oral cavity. Bacteria use extracellular polymeric compounds to withstand mechanical stress and antibacterial drugs (EPS)

The spread of germs that are resistant to many antibiotics or have become resistant due to gene transfer between commensal and pathogenic bacteria. There are several steps involved in the creation of a biofilm. Bacterial biofilm production includes early adhesion and a conditioning layer of organic and inorganic chemicals. Biofilms are formed when bacteria colonize a surface, multiply, form clusters, and release extracellular polymeric substances (EPS). Biofilms occur when bacteria reproduce and stick together; when they reach a certain thickness, they burst, releasing planktonic bacteria, which then colonize other surfaces (Funari and Shen,2022).

2.3 Porphyromonas gingivalis:

Due to its virulence properties, P. gingivalis, a gram-negative, obligately anaerobic, non-motile, and non-spore-forming bacteria (How et al., 2016). can create a local infection in the periodontal tissue around implants. The consortium's bacterial species are in regular dialogue with one another; they refer to themselves as the red complex. Biofilm formation and F. nucleatum are both physically connected to P. gingivalis (Mohanty et al.,2019).

Root surfaces, gingival crevicular fluid, and the surfaces of gingival epithelial cells lining the subgingival crevice are the three microenvironments in which P. gingivalis lives almost exclusively. Biofilm samples from periodontal pockets of individuals with periodontitis contain it at rates exceeding 85% of the time. However, it can also be found in patients with or without periodontal disease in the tonsil region, tongue, and buccal mucosa. It is asaccharolytic, meaning it doesn't get its energy from digesting carbohydrates, but it may ferment amino acids by dissolving the proteins in connective tissue. Iron is also necessary for the survival of P. gingivalis. (How et al.,2016).

Porphyromonas gingivalis is a bacterium that causes chronic periodontal disease and is found in subgingival plaque or saliva. It is a part of the periodontitis-associated core pathogenic bacteria (red complex). Therefore, several branches of oral healthcare have dedicated resources to understanding and combating P. gingivalis to curb the spread of the bacteria inside the mouth. (Damgaard et al., 2019). The periodontal bacteria Porphyromonas gingivalis (P. gingivalis) has been associated with an increased risk of developing Alzheimer's disease (AD) (Ryder,2020). Upon incubation for 3–7 days on blood agar plates supplemented with phenol red, this rod-shaped to pleomorphic, non-motile, obligate anaerobe, opportunistic pathogenic bacterium forms black-pigmented colonies (hemin, vitamin K1) (Endres et al.,2023).

Characterizing microbial populations is made easier by the availability of culture-dependent and culture-independent molecular techniques, respectively. These methods are sometimes referred to as "culture-neutral" approaches. Metagenomic research then reveals the underlying genetic characteristics and possible functions of the oral microbiota (Quince et al.,2017).

Similar to this, ribonucleic acid (RNA) meta-transcriptomic techniques support the evaluation of gene expression in mixed bacterial populations of the oral cavity (Duran-Pinedo,2021). These methods have been applied to examine how P. gingivalis interacts with several other bacterial species and assess its impacts on the microbial community in the biofilm environment (Zhang et al.,2019). One of the primary agents responsible for periodontitis in people with subgingival plaque is Porphyromonas gingivitis. The pathogenesis of periodontitis is assumed to be significantly influenced by it (Xu et al,.2020; Zheng et al.,2021).

In particular, a group of microorganisms known as the red complex consortium works together. Porphyromonas gingivitis and F. nucleatum have been linked to

biofilm development due to their physiological similarities. To attract and bind human epithelial cells, T. forsythia expresses a Leucine-Rich Repeat protein (LrrA protein) (Mohanty et al.,2019). Coaggregation is the interaction of numerous species of plaque-forming bacteria, such as A. naeslundii, S. gordonii, S. oralis, and Porphyromonas gingivitis fimbriae are involved (How et al.,2016). Porphyromonas gingivitis may attach to and integrate into phagosomes after attaching to the surface of the host cell. After autophagy occurs in a cell, replication is stimulated while apoptosis is prevented. Furthermore, Porphyromonas gingivitis responds effectively to oxidative stress because of its virulence traits. Many virulence factors present in P. gingivalis are known to directly and indirectly damage periodontal tissue by acting as an inflammatory mediator (Xu et al.,2020).

Porphyromonas gingivitis can avoid being eliminated by the immune system, profit from the inflammatory response, penetrate host cells, and exhibit virulence traits that allow it to persist under adverse circumstances for longer. Additionally, virulence factors influence coaggregation, biofilm development, and dysbiosis of the oral microbiota, all of which contribute to the destructive periodontal tissue loss, alveolar bone resorption, and systemic disease risk associated with periodontitis (Zheng et al.,2021; Stobernack et al.,2018; Xu et al.,2020).

2.3.1 Virulence Factors:

Virulence is described as an ability of an organism to infect the host and cause a disease. Virulence factors are the molecules that assist the bacterium colonize the host at the cellular level (Sharma et al.,2017)

The P. gingivalis plasma membrane serves as a dynamic interface between the oral pathogen and its immediate surroundings. Growth is made easier by the successful intake of nutrients, and bacterial survival is guaranteed by successful

tissue colonization. They are extremely reliant on the virulence factors produced or secreted by Porphyromonas gingivitis (Chen et al.,2023).

Virulence components of P. gingivalis include outer membrane vesicles (OMVs), lipopolysaccharides (LPS), gingipains, hemolysins, and hemagglutinins. Fimbriae are tiny, filamentous structures seen on the surface of most strains of P. gingivalis. They multiply outside the outer membrane of Porphyromonas gingivitis and aid in the formation of biofilms, the attachment of bacteria to host cells, and the invasion of bacteria into host cells (Jia et al., 2019; Xu et al., 2020; Asegawa and Nagano,2021).

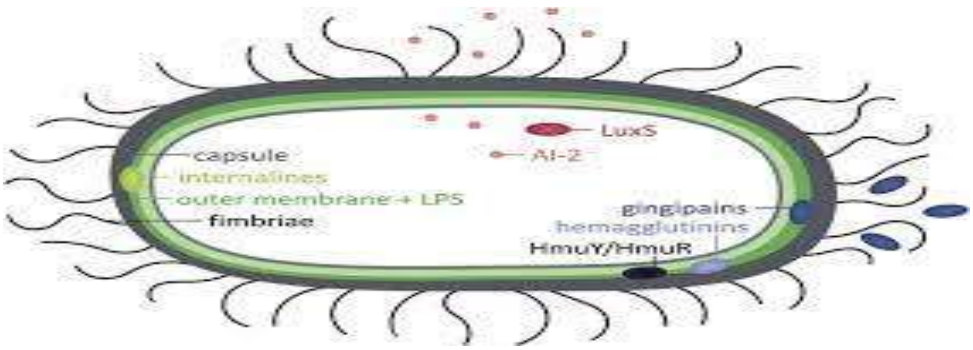


Figure (2-2) The major virulence factors of Porphyromonas gingivalis and a general overview of their involvement in pathogenicity (Gerits et al.,2017)

Long and short fimbriae are seen in Porphyromonas gingivitis. Short fimbriae are generated from Mfa1 subunits, while lengthy ones are built from FimA protein subunits. Multiple researchers (Xu et al., 2020; Asegawa and Nagano, 2021). Have the fimbriae of P. gingivalis depend on a broad range of components, including statin, fibrinogen, fibronectin, lactoferrin, and several proline-rich proteins and glycoproteins, to adhere to host tissues and cells. For instance, (Xu et al., 2020) Porphyromonas gingivitis uses its fimbriae to communicate with other oral bacteria, attach to host tissues and cells, and produce biofilm (Xu et al., 2020; Lamont and Jenkinson, 2000). Porphyromonas gingivitis attaches its long fimbriae to human

GAPDH to gain entry to host cells and provoke an immune response (Sojar and Genco, 2005; Xu and colleagues,2020).

They bind to Toll-like receptor 2 (TLR2) and stimulate bone resorption by activating and amplifying the production of proinflammatory cytokines such as IL-8, TNF-, and NF-B. (Xu et al.,2020; Jia et al.,2019) Fimbriae on Porphyromonas gingivitis helps the bacteria adhere to host tissues and cells, communicate with other oral bacteria, and create biofilms. To further avoid the host's complement system's protection against gram-negative bacteria, P. gingivalis employs elongated fimbriae (Xu et al.,2020).

2.3.1.1-Capsule:

A bacterial cell's outermost component is an envelope-like capsule. Polysaccharides and water make up this substance, which helps bacteria endure adverse conditions. K-antigen, or the Porphyromonas gingivitis capsule, prevents phagocytosis and intracellular death of bacterial cells (Xu et al.,2020).

Bacterial capsules are made up of homo- or heteropolymers of carbohydrates made up of monosaccharide units. Negative charges are created when monosaccharides are attached to the carbon backbone via carboxyl or phosphate groups. Phospholipids are depleted by capsular polysaccharides (CPS) in gram-negative bacteria, allowing glycolipids to serve as anchors (Chen et al.,2023).

Because bacterial capsules safeguard the structure of the outermost bacteria that interacts with dendritic cells, they have an impact on how dendritic cells mature. According to studies, the Porphyromonas gingivitis capsules block host response, which enables germs to survive and proliferate by evading the host's immune system (Singh et al.,2011)

2.3.1.2 Hemolysin:

At least five hemagglutinating molecules recognized virulence factors for many different types of bacteria, are produced by Porphyromonas gingivitis. The production of hemagglutinins on the bacterial cell surface may aid in colonization by enhancing bacterial binding to oligosaccharide receptors in human cells. Since P. gingivalis requires heme for proliferation, the attachment of bacterial cells to erythrocytes may also have a nutritional function (Lepine& Progulske,1996).

Heme sources include erythrocytes, gingival fluid, and hemoproteins found in saliva. Hemagglutinin, hemolysin, and gingipains are the primary heme-supply mechanisms used by Porphyromonas gingivitis, but other bacteria's heme acquisition systems can also be used. The form, growth, and pathogenicity of P. gingivalis are all significantly affected by the presence of heme. The morphology and number of fimbriae of heme-deficient cells resembled those of cocobacilli. Heme is necessary for Porphyromonas gingivalis to promote growth and virulence, but too much of it can be harmful to the cell, particularly when there is too much proteolysis going on. The proteolytic activity of Kgp can cause hemoglobin to release heme. (Smalley and Olczak, 2017).

2.3.1.3 Gingipains:

Gingipains are cysteine proteases that cleave a broad range of host proteins in the plasma, extracellular matrix, and connection to immune cells. They are surface-expressed or secreted, Lipopolysaccharide having Porphyromonas gingivalis results

in a subpar immunological reaction. Additionally, Porphyromonas gingivalis can produce diverse populations of lipids (Palm et al.,2015)

Gingipains, cysteine proteinases attached to Porphyromonas gingivalis cell surface, are the subject of ongoing research into the pathogenicity of this organism (Silva & Cascales,2021). Lysine-gingipain, arginine-gingipain A, and arginine-gingipain B are three types of gingipains that function by cleaving the genetic sequences of proteins from lysine or arginine residues, (How et al.,2016; Chopra et al., 2020; Nara et al.,2021). The Porphyromonas gingivitis primary gingipains are either discharged into the surrounding environment or adhere to other cell surfaces. Their purpose as virulence factors is to assault vital extracellular matrix components, disrupt the function of the epithelium's barrier, and open the way for Porphyromonas gingivitis to infiltrate subepithelial tissues, which contributes to the breakdown of iron-binding proteins and the degeneration of periodontal tissues (Silva & Cascales,2021).

Therefore, it can be concluded that the RgpA DNA vaccine induces both cellular and humoral immune responses, which protect against Porphyromonas gingivitis. Research on the efficacy of the HRgpA DNA vaccine in preventing gingipain-induced virulence and bone loss is ongoing. Anti- Porphyromonas gingivitis -specific IgG levels are significantly increased as a consequence (Jain et al., 2018).

2.3.1.4 Fimbriae:

Porphyromonas gingivitis fimbriae is a thin, stringy surface protrusion that aids in the bacterium's adhesion to host cells and other bacteria. By using its fimbriae, Porphyromonas gingivitis can cling to and participate in the production of biofilms on early bacterial colonies. There are two kinds of fimbriae; type I (major) fimbriae are encoded by the fimA gene and are also called fimbrillin or FimA; type II (minor)

fimbriae are encoded by the mfa1 gene and are also called Mfa subunit protein (Enersen et al., 2013). Porphyromonas gingivitis fimbriae is a thin, stringy surface protrusion that aids in the bacterium's adhesion to host cells and other bacteria. By using its fimbriae, Porphyromonas gingivitis can cling to and participate in the production of biofilms on early bacterial colonies. In the experimental periodontitis model, the unique function of fimbriae is to cause bone deterioration. FimA and Mfa1 from Porphyromonas gingivitis are potent inducers of pro-inflammatory chemicals (How et al.,2016).

Fimbriae, which are pilus appendages formed by gingipains and connected to the outer cell membrane, are required for the formation of biofilms, as well as the assault and infection of target cells while avoiding the host's defenses. Gipains can also enmesh the circulating proteins (Gerits et al.,2017; Silva & Cascales,2021). Based on the differences in genotypes, FimA is categorized into six groups (FimA I, IB, II, III, IV, and V): FimA I, IB, II, III, IV, and V (Zheng et al.,2011). Both short and long fimbriae are produced by gingipains, and both types, by their ability to adhere, aid in the formation of poly-species biofilms. Porphyromonas gingivitis long fimbriae not only help it adhere to host tissues, but they also link with toll-like receptors and block the inflammatory response they trigger. Porphyromonas gingivitis long fimbriae make it easy for the bacterium to aggregate with other oral infections. Candida albicans, a common species found in the mouth, depletes oxygen inside the polymicrobial biofilm, therefore shielding Porphyromonas gingivitis from high-oxygen situations.

As reported by (Silva & Cascales, 2021). Nothing is known about whether or not Porphyromonas gingivitis can translocate to other parts of the body. However, a recent in vitro study showed promising outcomes concerning the motility of P. gingivalis. The bacterium, which is less virulent but more fimbriated, showed a

multimodal motility pattern over its entire life cycle under lab conditions. Bacteria of were seen to be mobile throughout testing, as evidenced by their ability to roll across adjacent cells.

Porphyromonas gingivitis was also shown to be mobile and rollable on the surface of erythrocytes. Porphyromonas gingivitis consumes several different metabolites, allowing it to thrive and spread. Gliding motility rather than active motility is likely responsible for Porphyromonas gingivitis ability to translocate to neighboring and distant places through means of proteolysis, cell dispersion, cell-on -cell rolling, and subdiffusive cell-driven motility. (Moradali et al., 2019).

2.3.1.5 Lipopolysaccharides (LPS):

Bacterial lipopolysaccharides include hydrophobic domains known as lipid A or endotoxins, distal polysaccharides (O-antigens), and core oligosaccharides. (Ogawa & Yagi ,2010). Lipopolysaccharides from Porphyromonas gingivitis have the function of interfering with the innate host by causing leukocytes to be distributed around bacterial colonization. A failure in the host defense system and the presence of bacterial colonies in the periodontal tissue are the root causes of periodontal disease. In periodontal ligament stem cells, which are important in tissue regeneration, Porphyromonas gingivitis lipopolysaccharides induce the production of pro-inflammatory cytokines and inhibit osteoblastic growth and mineralization. (How et al., 2016).

Lipopolysaccharide heterogeneity leads to immunological dysregulation by causing the opposing immune response. Lipopolysaccharide A subpar immunological response is triggered by P. gingivalis. Additionally, P. gingivalis can produce different types of lipid A (Kato et al.,2014).

2.3.1.6 Hemagglutinins (Hag)

Non-fimbrial adhesion hemagglutinin B is being considered for use in an immunization program (HagB). Hemagglutinin is the bacterial protein responsible for adhesion and invasion. Heme, an essential growth factor, is taken up by the host cells, where it then agglutinates and lyses the erythrocytes. When mice were inoculated in the nose with a virulent strain of Salmonella typhimurium expressing the HagB gene, the animals developed systemic and mucosal antibody responses. Increases in these responses are indicative of the formation of a memory T-cell or B-cell response. Furthermore, subcutaneous vaccination of rats with recombinant HagB protected them against P. gingivalis strain-induced periodontal bone loss. It should be able to deploy human antibodies against hemagglutinin in immunotherapy. To wit: (Kaizuka et al., 2003).

Hemagglutinins, as adhesins, have been shown to significantly affect the virulence of a several pathogenic microbial species in a several studies (Alonso et al., 2002; Chen and Duncan, 2004; Ishibashi et al., 2001; Kuramitsu et al., 2003). These hemagglutinins on the bacterial cell surface have been shown to function as fimbrial adhesins or non-filamentous surface molecules to facilitate bacterial attachment to host cells (Han et al., 1996).

2.4 Periodontal Diseases

Deterioration of the bone and gums that hold teeth in place is the hallmark of periodontal disease, also known as periodontitis, a chronic multifactorial inflammatory sickness linked to dysbiosis plaque biofilms. The term "periodontium" is used to describe the whole network of tissues that help keep teeth in place. This network consists of the alveolar bone, cementum, gingiva, and periodontal ligament (Helmi et al.,2019)

Periodontal disease, an inflammatory illness affecting the soft and hard structures that support teeth, is the main cause of tooth loss and is the sixth most common condition globally (Peres et al.,2019). Bacterial plaque accumulation is the most significant risk factor for gingival inflammation, leading to periodontal damage if prolonged (Scribante et al.,2022). This host-microbe interaction maintains the integrity of the periodontium by ensuring a balance in the periodontal tissue, Nevertheless, dysbiosis, or shifts in the subgingival microbiota toward those linked to disease. (Curtis et al.,2020).

Hemostasis, inflammation, proliferation, and maturation are the four separate but overlapping phases of periodontal wound healing. Granulation tissue (GT) is created in the setting of persistent inflammation to reconstruct the area, which has a highly dense network of blood vessels and capillaries, enhanced fibroblast and macrophage cellular density, and unevenly spaced collagen fibers (Gousopoulou et al.,2023)

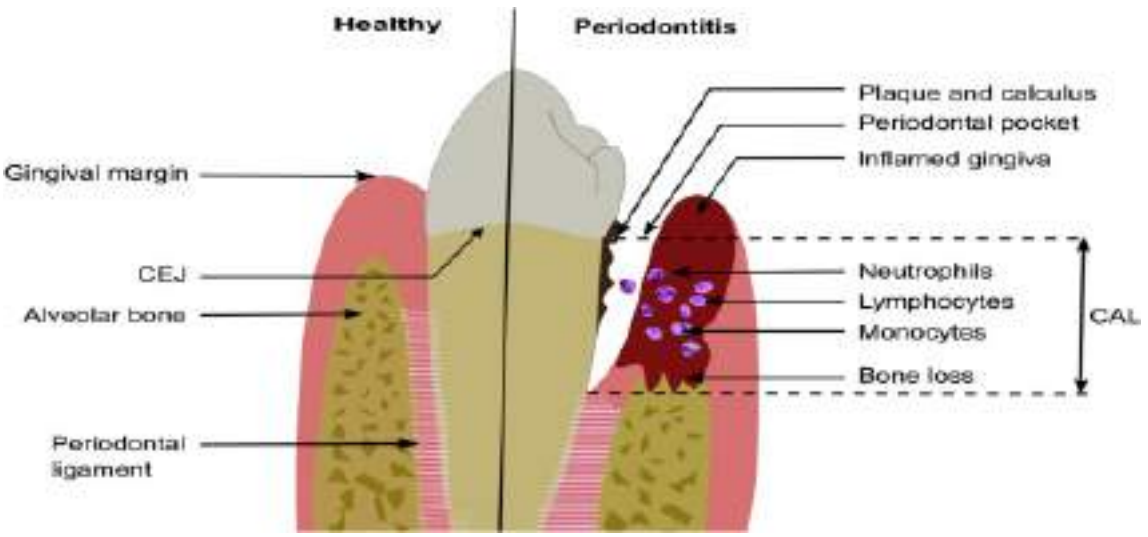


Figure (2-3): The Anatomic of Gingiva In Healthy And Periodontal Diseases (Cheng et al.,2017).

When microbial populations adapt to the changed nutrients available, their functional properties change. The dysbiosis microbiota can then resist or downregulate the host's immunological and inflammatory response. (Sanz et al.,2017). periodontal pockets (PP) develop instead of a healthy gingival sulcus periodontal pocket. Due to their mucosal wall, which is formed by an ulcerated epithelium with exposed connective tissue and its vascular ramifications, and their dental root wall, which facilitates the establishment of complex subgingival biofilms, PP has distinct subgingival habitats after pathological alveolar bone loss and migration of the supracrustal attachment tissues apically (Badran et al.,2020).

2.4.1 Pathogenesis of Periodontal Diseases:

Bacterial infection is what first causes periodontal disease. (Dede et al.,2023). Typically, oral microbial stimulation of periodontal tissue results in the coordinated release of host defensive mediators. (Zhu et al.,2022). When pathogenic bacteria invade periodontal tissues in large numbers, they weaken the immune system and cause the tissues to produce an abundance of inflammatory mediators, which may lead to tissue death (Zhu et al., 2022; Zhuang et al., 2019).

Invasion of the mucosal barrier and epithelial cells, as well as the generation of lipopolysaccharide, are shared features of the oral pathogens Actinobacillus actinomycetemcomitans (A.a.), Tannerella forsythia (T. forsythia), and Porphyromonas gingivalis (P. gingivalis), (Kinane et al.,2017). The primary oral pathogen linked to periodontitis is Porphyromonas gingivalis (P. gingivalis) (Nagashima et al.,2017). Fimbriae, gingipains, and lipopolysaccharides are the three main virulence factors expressed by Porphyromonas gingivitis (Behm et al.,2019). Half of the adult population suffers from periodontal disease nowadays (Cao et al.,2019). Periodontitis is encouraged by the presence of dental plaque and the bacterial biofilm it produces, which in turn leads to the formation of pockets, the

loss of clinical attachment, and the degeneration of alveolar bone (Nitta et al.,2017). Root surface contamination from calculus and biofilm may be reduced with non-surgical treatments including scaling and root planing. Clinical inflammation and periodontal pocket depth are reportedly reduced by both treatments (Cobb and Sottosanti,2021).

Socransky and Haffajee identified six types of periodontitis-causing bacteria, which they named the red, orange, yellow, green, blue, and purple complexes. The most prevalent periodontal pathogen is the red complex member Porphyromonas gingivalis. It's responsible for periodontal disease thanks to the virulence factors (such as gingivalin) that it secretes and releases into the mouth (Jepsen et al., 2021). When periodontal tissues are damaged, a reduction in fibrinolysis and a buildup of fibrin can maintain neutrophil activation, exacerbate local immunopathology, and worsen periodontitis Tooth loss is not a result of gingivitis (Dagnino et al., 2020; Silva et al.,2021).

This is so that the alveolar bone is not affected, just the gingival soft tissue is. But if neglected, it could worsen and turn into the more serious illness known as periodontitis. When this occurs, pockets are left behind at the base of the tooth where the soft gingival tissue had previously been. Plaque bacteria in the mouth may easily colonize these spaces and spread illness. The degradation of bone and connective tissue is hastened by the poisons released by invading bacteria (Nicholson,2022).

2.4.2 General Characteristics of Periodontal Diseases:

Gums that are red, swollen, and bleeding are the main signs. As the condition worsens, the teeth become loose, primarily as a result of the formation of periodontal pockets and the loss of alveolar bone (Yang et al.,2023).

2.4.3 Diagnostic Criteria of Periodontal Diseases:

- Clinical evaluation and molecular genetic testing (for variations in the innate immune system-related genes C1R and C1S)
- The complete absence of gingival attachment is regarded as pathognomonic
- The majority of children are diagnosed through family history.

(Johnson et al.,2021; Rinner et al.,2018).

2.4.4 Clinical of Periodontal Diseases:

There are many clinical of periodontal diseases such as:

severe gingival swelling, detached gingiva loss, gingival weakening and recession, rapid loss of the alveolar bone, and early tooth loss (Johnson et al.,2021; Rinner et al.,2018).

2.4.5 Stages in the Progression of Periodontitis Diseases:

There are many stages in the progression of diseases such as:

Stage I Gingivitis and periodontitis coexist in a borderline state and Comments There is some attachment loss. In regular dentistry practice.

Stage II existing periodontitis compromised tooth support, reacts to management that is quite straightforward.

stage III significant attachment damage and some tooth loss, has no complex requirements to restore function.

stage IV severe tooth loss and extensive attachment injury, loss of chewing ability. Teeth with excessive mobility and deep periodontal diseases, stabilization or

restoration of masticatory function are necessary for case management (Tonetti et al.,2018).

2.4.6 Type of Periodontal Disease:

In 1999, researchers classified periodontitis into four subtypes: necrotizing, chronic, aggressive, and as a sign of a systemic illness, localized (affecting less than 30% of teeth) or generalized (affecting more than 30% of teeth) periodontitis is present in each of the three most common forms of periodontitis (periodontitis, necrotizing periodontitis, and periodontitis as a direct manifestation) (Tonetti et al., 2018). The major cause of periodontitis is the accumulation of bacteria and their subsequent colonization of oral tissues. Genetic predisposition, systemic diseases, dental plaque, and tartar accumulation, insufficient cleaning of teeth and restorations, poor diet, and unhealthy habits like smoking and excessive drinking all play a role in the development of this condition. (Mehrotra, 2019 & Mehta, 2021).

2.4.6.1 Gingivitis Disease (GD)

Gingivitis is the first stage of periodontal disease, gingivitis is a reversible inflammation that affects only the gingiva (Niemiec et al.,2020). Plaque-induced gingivitis is the most typical type, a lack of vitamin C or carbohydrates with a high glycemic index can also cause nutritional gingivitis, which is characterized by gingival inflammation brought on by the generation of pro-inflammatory cytokines and an increase in oxidative stress. A high omega 6 to omega 3 fat ratio also encourages the inflammatory response and may speed up the development of gingivitis by decreasing the protective mechanisms at the gingival interface (Rathee et al., 2022).

Although it is generally known that it more frequently affects pregnant women, this is likely due to the increased bodily fluid and blood flow, as well as the tendency for dilated blood vessels (Rathee & Jain, 2022; Soma et al.,2016).

Differentiating plaque-induced gingivitis from periodontitis is the fact that all tissue alterations are reversible if the biofilm on the tooth is removed, because it may lead to periodontitis, an even more serious disorder marked by inflammation of the gums, loss of supporting connective tissue, and even bone, gingivitis is of great clinical importance. This is true even if the tissue alterations brought on by gingivitis may be restored (Huang et al.,2021).

2.4.6.2 Chronic Periodontitis Disease (CPD)

Chronic periodontitis disease a multifaceted inflammatory illness, the development of which is affected by a broad range of variables. Infectious agents such Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans are among these causes (Colombo & Tanner, 2019; Lamont et al., 2018). The interaction between the microbial infection and the human sensitivity to pathogenic stimuli leads to this complex sickness (Chen et al., 2018).

Chronic periodontitis is a common oral illness that affects adults and is mostly caused by inflammation of the periodontal tissues caused by an excess of dental plaque. Chronic periodontitis develops from initial gingivitis, sometimes progressing quickly (referred to as "bursts of destruction"). The causes include food impaction, poor restorations, and other factors.

Poor breath (halitosis), loose teeth, bleeding or red gums during brushing, and persistent swelling of the gums are all indicators of gum disease (Pham & Phan, 2020). The only remaining treatment choices are surgical, such as teeth extraction among others, when the chance to treat the disease in its early stages is lost. Unfortunately, these types of care are pricey, and some patients are unable to pay for them (Cheah et al,2020; Aral et al.,2020).

2.4.6.3 Aggressive Periodontitis Disease (AgPD)

Multiple teeth are affected by the devastating illness known as aggressive periodontitis, it starts at a young age, progresses quickly, and causes a distinct loss of periodontal tissue without any underlying systemic disorders. The fact that severe periodontitis tends to run in families suggests that a host predisposition, in addition to infection with specific bacteria, plays an essential role in the etiology of this illness (Fine et al.,2018).

AgP is a polygenic condition, which means that many different gene loci interact to cause it. Autosomal recessive inheritance, which has been related to the development of AgP, often causes nonprotective inflammatory responses that lead to dysbiotic microbial changes. It is a genetic condition with several subtypes that may be triggered by different environments (Toker et al.,2017) A.actinomycetemcomitans, Porphyromonas gingivalis , Tannerella forsythia, Selenomonas sputigena, and Treponema denticola are all associated with AgP, according to numerous research(Nagpal et al., 2016).

2.4.6.4 Necrotizing Periodontal Disease (NPD)

Clinically relevant because untreated NPD, which affects less than 1% of the population, can quickly advance into necrotizing stomatitis or cancrum oris (noma), a potentially fatal infection that causes oral soft and hard tissue gangrene, NPD is

rare, and risk factors for poor oral health include tobacco use, poor nutrition, HIV infection, uncontrolled diabetes, cancer, and stress (Dufty et al.,2017).

Chapter Three

Materials & Methods

3.1 Materials

3.1.1 Equipment's and Materials

Table (3-1): Equipment used in this study

No	Equipment	Supplier	Origin
1	Anaerobic Jar	Oxoid	England
2	Autoclave	Hiarayam	Japan
3	Electrophoresis system	Cleaver	England
4	Eppendorf centrifuge		Germany
5	Electrophoresis	Bioneer	Korea
6	Hood	Lap Tech	France
7	Incubator	Fisher scientific	U S A
8	Light Microscope	Olympus	Philippine
9	Magnetic stirrer Hot plate	Heidolph	Germany
11	Mini vortex	Apogent	USA
12	Oven	Memmert	Germany
13	PCR thermal cycler	Bioneer	korea
14	Sensitive balance	Denver	Germany
15	UV light transilluminator	Cleaver	England
16	Vitek 2 compact system	BioMerieux	France
17	Water Bath	Memmet	Germany

Table (3-2): Tools used in this study

No.	Tools	Supplier	Origin
1	Beaker	General	USA
2	Burner	Himedia	India
3	Conical Flask(100	HBG	Engeland
4	Cotton Swap	AL-Rawbi	China
5	Cylinder (50,100,250)	HBG	Engeland
6	Forceps	Himedia	India
7	Gloves	Broche	Malaysia
8	Micropipettes	DraonMED	China
9	Microscope cover slides	Citogias	China
10	Paper point		
11	Para film	Jenway	Germany
12	Petri Dishes	Bio zek medical	Holland
13	Plain tubes	Afco	Jordan
14	Plastic ruck	Meheco	China
15	Screw cap bottles	Pyrex	England
16	Slides	Superestar	India
17	Test Tube	ALS	Canada
18	Universal Pipette Tips	Globe scientific	Germany
19	Wire loop	Himedia	India

3.1.2 Media and Chemicals

3.1.2.1 Media that Prepared in Lab

Table (3-3): Preparation of Culture Media for Isolating Bacteria with the Name of the Company Manufacturer and Country of Origin

NO	Media	Supplier	origin
1	Blood Agar Base	Oxoid	England
2	Brain Heart Infusion	Titan Biotech. LTD	India
3	MacConkey Agar	Oxoid	England
4	Mitis Salivarius Agar	Titan Biotech. LTD	India
5	Muller-Hinton agar	Titan Biotech. LTD	India
6	Nutrient Agar	Titan Biotech. LTD	India
7	Nutrient Broth	Titan Biotech. LTD	India
8	Thioglycollat Broth	Titan Biotech. LTD	India
9	Trypton Soya Agar	Titan Biotech. LTD	India

3.1.2.2Chemicals

Table (3-4): All Chemicals Used in the Study with the Name of the Company Manufacturer and Country of Origin

No	Chemicals	Supplier	Origin
1	DNA extraction kit	Geneaid	Korea
2	DNA marker (100- 2000 bp)	Takara	China
3	TBE buffer 5x	Promega	USA
4	Absolute ethanol	Scharlan	Spain
5	Agarose	Promega	USA
6	Nuclease free water	Bioneer	Korea
7	Master mix	Bioneer	Korea

3.1.3 Stain

Table (3-5): All Stain Used in the Study with the Name of the Company Manufacturer and Country of Origin

NO	Stain	Supplier	Origin
1	AnaeroGen ™3.5 L Gas Back	Oxoid Ltd	England
2	Ethedium bromide	Promega	USA
3	Gram Stain	Titan Biotech (LTD)	India
4	Vitek2 Kit Gram Negative ID Kit ANC REF Kit	Biomerieux	France

3.1.4 Antibiotic Disks

Table (3-6): Antibiotic Disks Used in the Study with the Symbol, Concentration mcg \ disk and Country of Origin

No	Antibiotic Disks	Symbol	Concentration mcg\ disk	Origin
1	Cefoxitin	fox	30mcg	India
2	Ciprofloxacin	CIP	5mcg	
3	Doxycycline	Do	30mcg	
4	Nalidixic acid	NA	30mcg	

3.1.5 Primers Used in PCR Amplification in this Study

Amplification of isolates using universal and specific primers for all samples.

Table (3-7): Sequence of universal Primer that Use in this Study

No	Primer		Sequence	Length pb	AmpliconSize (bp)
1	16s rDNA	F	5-AGAGTTTGATCCTGGCTCAG-3	20	1500
2	16s rDNA	R	5-GGTTACCTTGTTACGACTT-3	20	1500

Table (3-8): Sequence of specific Primer that Use in this Study

No	Primer		Sequence	Length pb	Amplicon Size (bp)
1	P.gingivalis	F	5′ -AGGCAGCTTGCCATACTGCG -3′	20	404
2	P.gingivalis	R	5′ -ACGTTCGATTTCATCACGTTG-3′	20	404

Table (3-9): Sequence of universal Primer that Use in this Study to diagnose virulence gene

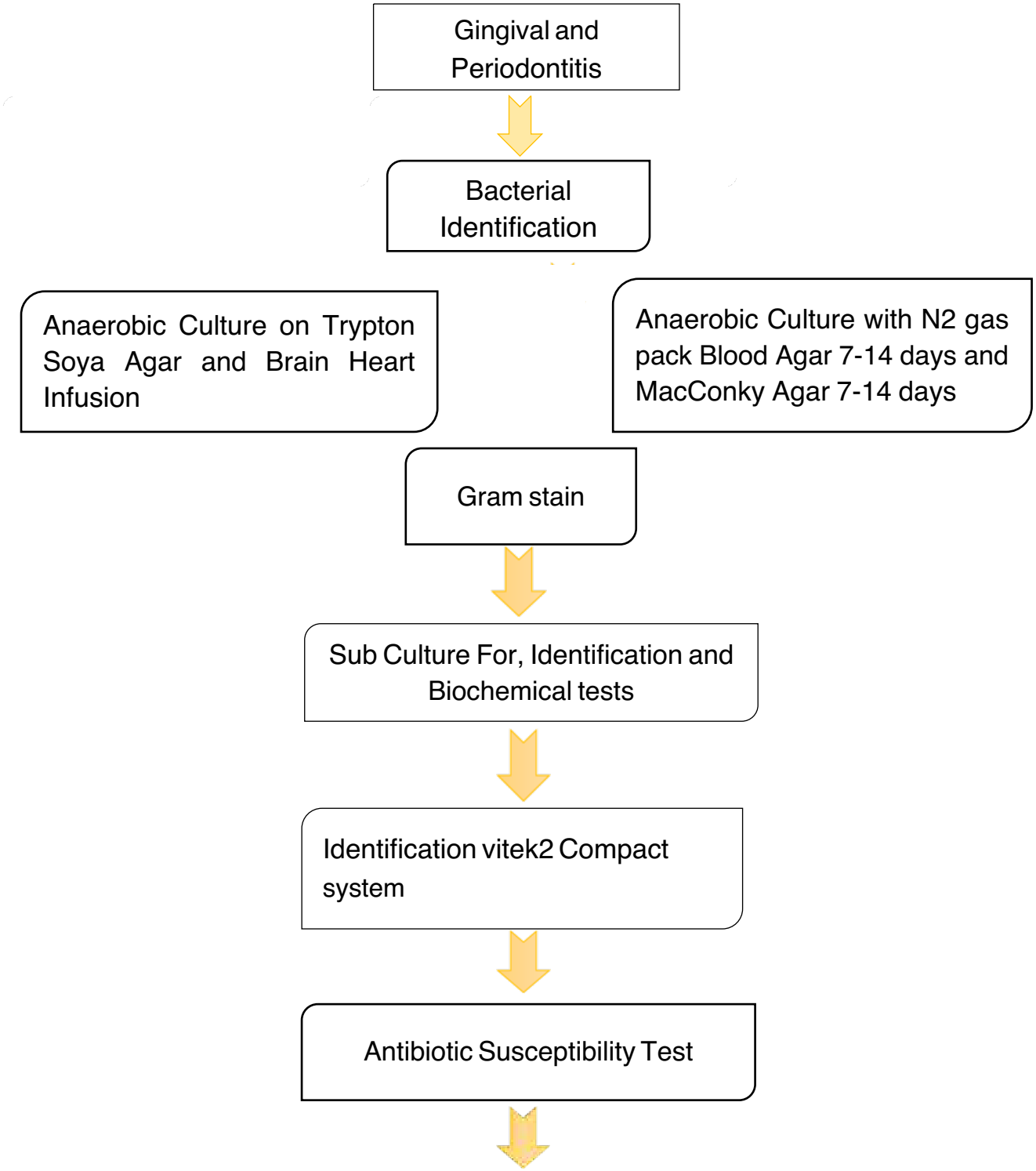
No	Primer		Sequence	Length pb	Amplicon Size (bp)
1	Fimbria	F	5′- AGTTCTTGCCTGCCTTCAAA-3′	20	250-500
2		R	5′-AACCCCGCTCCCTGTATTCCGA-3′	22	250-500

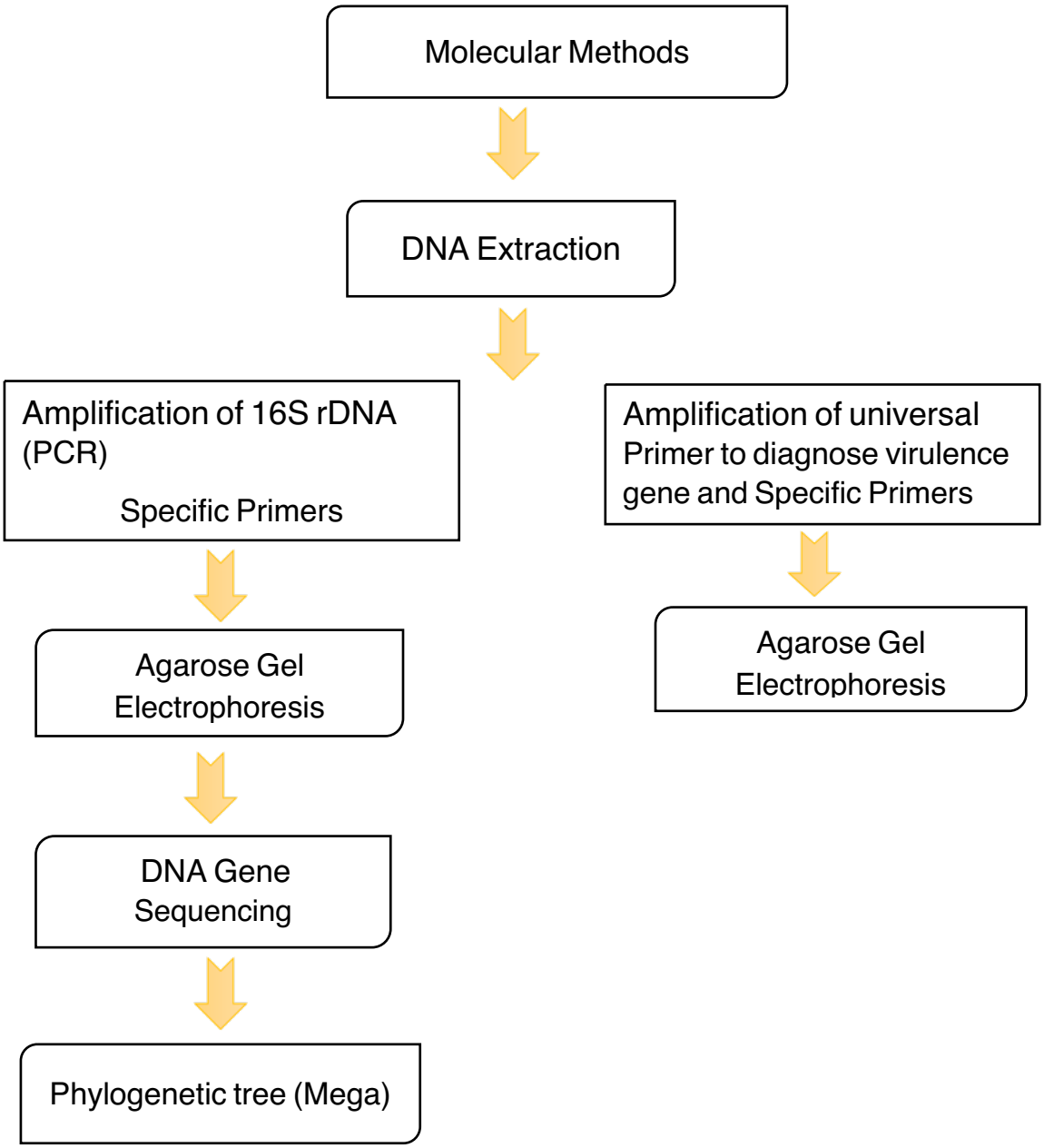
Table (3-10): Sequence of specific Primer that Use in this Study to diagnose virulence gene

No	Primer		Sequence	Length pb	Amplicon Size (bp)
1	Fimbria	F	5′ -ATA ATG GAG AAC AGC AGG AA -3′	20	100-250
2		R	5′ -TCT TGC CAA CCA GTT CCA TTGC -3′	22	100-250

Steps To Isolate and Diagnose Bacterial Porphyromonas gingivalis

100 sample Periodontal Disease sample (50 Pathogen and 50 control)





3.2Methods

3.2.1 Creation of the Media

The preparation of culture media followed the manufacturer's directions.

3.2.1.1Thioglycolate Broth

Add 28gram powder suspended in 1 L D.W., heated to completely dissolve the powder, 5ml of broth medium were transferred to tightly sealed screw caps and autoclaved for 15 minutes at 121°C.

3.2.1.2 Blood Agar

According to the manufacturer's instructions, it was made by dissolving 40g of blood agar base powder in 1000ml of D.W., heating to boiling, sterilizing for 15 minutes at 121°C under 15 atm of pressure, and then adding 10% fresh human blood after allowing the mixture to cool to 45–50°C (Narciso & Aschtgen, 2023).

3.2.1.3 MacConkey Agar

Add 51.5% of a gram were suspended in one liter of D.W., boiled to completely dissolve the powder, autoclaved for 15 minutes at 121 °C, cooled to 50 °C, and then poured into sterilizing plates (Singh et al.,2023).

3.2.1.4 Mitis Salivarius Agar

The medium was sterilized using an autoclave for 15 minutes at 121 °C before being cooled to 50 °C. Fifty grams of the medium were suspended in one liter of D.W (Ammar et al.,2022).

3.2.1.5 Muller-Hinton agar

Pour 38.0 grams of distilled water into 1000 ml. To completely dissolve the medium, heat it until it boils. By autoclaving for 15 minutes at 121°C and 15 atm open of pressure, sterilize. to (45–50)°C. Mix thoroughly, then transfer to sterilized Petri dishes (Intra et al.,2019).

3.2.1.6 Nutrient Agar

In 1000 ml of pure or distilled water, was dissolved 28 grams. To completely dissolve the medium, heat it until it boils. By autoclaving for 15 minutes at 121°F/15 pounds of pressure, sterilize. to 45 to 50 °C (Nikulin et al.,2023)

3.2.1.7 Nutrient Broth

Mix 13.0 grams with 1000 ml of distilled water. To completely dissolve the medium, use heat if necessary. By autoclaving for 15 minutes at 121°F/15 pounds of pressure, sterilize. to 45 to 50 °C (Constantia et al.,2023)

3.2.1.8 Trypton Soya Agar

fourty grams of medium were added to 1 liter of D.W., boiled to completely dissolve the powder, autoclaved for 15 minutes at 121 degrees, then cooled to 50 degrees, and then 5% sterile blood was supplied aseptically along with vitamin solution K1 and L-cysine (Trinh & Kim, 2023).

3.2.1.9 Brain Heart Infusion

In 1000 ml of purified distilled water,was dissolve 52.0 grams. Bring to a boil to Save everything on the medium. Autoclaving for 15 minutes at 15 pounds of pressure (pathop 121) sterilizes. to 45–50 °C (Trinh & Kim, 2023).

3.2.3 Sample Collection

Samples were gathered from 100 (50 patients and 50 controls) with varying degrees of periodontitis in the center of Al-Amarah City. The patients' ages ranged from 11 to 70 years old, and the samples included those with gingivitis and periodontitis with a 5 mm periodontal pocket depth. throughout the period of November 21, 2023, to February 6, 2023.

3.2.4Culture of Sample

Greatest common factor GCF samples were immediately added to 25 cc of Thioglycolate broth for inoculation. Within two hours, all collected samples were transported to the microbiology lab and incubated for 24 to 48 hours at 37°c. According to Hungate's (1969) method, one loop-complete from the developed bacteria in thioglycolate broth was inoculated on Tryptone soya agar slant and flushed through a sterile cupper needle with filtered Co2 and N2 to replace O2. The lid was then tightly closed and sealed with paraffin film. According to (Chetan et al.,2011), the infected medium were incubated anaerobically for 3 to 7 days at 37 °C in an anaerobic environment (anaerobic jar) created by an anaerogen gas pack (Oxoid Ltd., England).

3.2.5 Identification of Bacterial Isolates:

Bacterial identification was carried out using the characteristics of their culture, stained with Gram stain, and validated using the automated microbiological Vitek2 system using the gram negative ID kit (Biomerieux, France) (Skucaite et al., 2010).

3.2.6 Morphological characterization of bacteria

Gram's stain was used to color smears from newly formed colonies, which were then cultivated on blood agar and inspected under a microscope to see how the dye interacted with the colonies' structure and organization. Colonies of the isolates grown on solid medium (blood agar) was described according to their shape, pigmentation, edge and the change in the color media.

3.2.7 Biochemical Tests

3.2.7.1 Catalase Test

A loop of bacteria was placed on a clean slide to examine catalase production. One drop of 3% hydrogen peroxide was injected, and the absence of bubble formation suggests unfavorable outcomes (Chandra, 2023)

3.2.7.2 Oxidase Test

The oxidase test has numerous procedure variations. These include the filter paper test, the filter paper spot test, the direct plate method, and the test tube method, among others. The suggested periods and concentrations are used for all calculations. Pick a well-isolated colony using a loop from a fresh (18–24hour culture) bacterial plate, then rub it onto filter paper that has been treated (please see the Comments and Tips section for information on suggested media and loops (Begega et al.,2023).

3.2.8 Bacterial Processing Isolated Along System of Vitek 2

To transfer enough colonies of pure young culture and microorganism drooping sterile saline, bacterial isolate suspension of 30 anaerobic bacteria was made with an applicator stick or sterile brush. 3 ml (0.45 to 0.50% aqueous NaCl, 4.5 to 7.0 pH), in a transparent glass test tube measuring 12 x 75 mm with turbidity between 2.70 and 3.30. Cards used for identification are collected with microorganism suspensions using an apparatus in which a special cassette rack with an integrated vacuum suspension tube is placed. The ANC card is then placed next to the slot while the transfer tube is inserted into the corresponding suspension tube. 10 or 15 tests can be stored on a cassette. The transport of a filed cassette is done either manually or mechanically vacuum chamber station. Air is then blasted back into the station after vacuuming, and then the suspension of the organism is forced into micro-channels that fill all of the test wells. Cards that have been inoculated are removed using a device that disables the transferring tube and cardboard sealing before loading them onto the carousel's incubator. Carousel's incubator can accommodate 30 or 60 cards. A line at 35.5 1.0 °C is used to incubate all types of playing cards. Every type of card is transported to an optical system for readings once every 15 minutes, caroused, and then returned to the incubator until the next reading. Every fifteen minutes over the entire duration, statistics are collected. Exam reactions can be interpreted using multiple visible wavelengths thanks to an optical transmittance technology. Each exam reaction was monitored for turbidity or colored substrate metabolism products every 15 minutes during incubation. Additionally, a particular algorithm was used to get rid of measurements that would have been skewed by any little bubbles that might have existed. To identify bacterial

isolates, the automated compact microbiology Vitek 2 system is used in bacteriology (Paluch et al.,2023).

Figure (3-1): A,B, diagnostic kit of vtek2 system (AST-P580) C, show vitek 2 compact in lab

3.2.9 Antibiotic Susceptibility Test

The well diffusion assay is the best option because challenging to dry on paper discs. Before plating on Muller-Hinton agar, each isolate was suspended to a turbidity of 0.5 McFarland standards. An antibiotic disc was attached to each plate. At 37°C, the plates were incubated for 24 hours. After incubation, the inhibition zone was evaluated (Vocat et al.,2023)

3.2.10 Molecular study of the Isolates

3.2.10.1 DNA Extraction:

According to the Geneaid Kit's manufacturer, DNA has been extracted. Tested bacteria colonies were injected into five ml of nutrient broth and cultured at 37 °C for 24 to 48 hours. The steps:

1-Initial Sample Creation

Gram-negative microorganisms Fill a 1.5 ml microcentrifuge tube with bacterial cells (up to $\times 10^9$) before centrifuging. Centrifuge at 14–16,000 x g for 1 minute, then discard the supernatant. Add 180 μl of GT Buffer, then use a vortex to resuspension the cell pellet. Add 20 μl of Proteinase K, being sure to include ddH2O. Incubate for at least 10 minutes at 60 °C. Every three minutes during incubation, flip the tube over.

2-Lysis

Add 200 µL of GB Buffer to the sample and mix by vortexing for 10 seconds. Incubate at 70 °C for at least 10 minutes to ensure sample lysate is clear. During the incubation, turn the tube every 3 minutes. At this time, preheat the required elution buffer (200 µl per sample) to 70 °C (for step 5 DNA elution). Optional RNA removal step After 70 °C incubation, add 5 µL of RNase A (50 mg/mL) to the clear solution and vigorously shake the tube at room temperature for 5 min

3-DNA Binding

The sample lysate should immediately be mixed by vigorously shaking after 200 l of 100% ethanol has been added. Use a pipette to partially disperse any precipitate that does develop. A 2 ml Collection Tube should contain a GD Column. Place combination, along with any insoluble precipitate, in the GD Column, and centrifuge at 14–16,000 x g for two minutes. Put the GD Column in a fresh 2 ml Collection Tube after discarding the 2 ml Collection Tube holding the flow-through.

4- Wash

Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14-16,000 × g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. Add 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column. Centrifuge at 14-16,000 × g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

5-Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required,

repeat the DNA elution step to increase DNA recovery and the total elution volume to approximately 200 µl. Transfer the dried GD Column to a clean 1.5 ml microcentri ge tube. Add 100 µl of pre-heated Elution Buffer[1], TE Buffer2 or water[3] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

3.2.10.2 Identified the Bacterial DNA by Using Nanodrop

DNA was quantified using the NanoDrop-1000 spectrophotometer. The DNA concentrations were determined by measuring the absorbance at 260 nm wavelength (A260) and 280 nm wavelength (A280). Purity was determined by calculating the ratio of absorbance at 260 nm and the absorbance at 280 nm (A260/A280). The spectrophotometer was connected to a software installation system. The machine was initialized, blanked by using DNA nuclease-free water, elution buffer using two microlitres each respectively, the measurement of the DNA was read using the same volume and the pedestal was cleaned after each reading to prevent cross-contamination of the DNA products (Bunu et al.,2020).

3.2.10.3 Identified the Bacterial by Using PCR

The bacterial isolates was identified by using PCR to amplify universal 16S rRNA primers. And specific primers used to identification bacterial isolates p.gingivalis. Amplification of the PCR, detection of amplicons, and extraction of DNA from specimens are all crucial steps in the diagnosis PCR assay. Specifically, every step of the PCR testing process needs to be meticulously planned and carried out when testing particular clinical specimens that contain a low concentration of bacteria, Agarose Gel Electrophoresis (Normington et al., 1989).

Reagent

-1× Buffer of TBE

-Agarose

-Ethidium bromide

3.2.10.4 Agarose Preparation

Agarose gel electrophoresis (1%) was preparing by dissolving 1g in 100ml 1X TBE buffer, left to cool at 50°C and 5μl of ethidium bromide was added to agarose and poured on preparing tray. Comb was removed after hardening of agarose leaving wells (Sambrook and Russell, 2006).

3.2.10.5 16S ribosomal RNA

Isolated bacteria specimens were identified using oral cavity have been proved identify via utilizing technique of PCR techniques though amplification of 16S rRNA gene primers

Table (3-11): PCR Amplification Program for universal primers Used in the 16S rRNA gene. (Eeward,2005).

NO	Stage	Temperature	Time	No. of cycle
1	Denaturation as initial	95	3min	1
2	Denaturation	95	45sec	35
3	Annealing	56	1min	
4	Elongation	72	2min	
5	Final Elongation	72	5min	1

Table (3-12): PCR Amplification Program for specific primers Used in the study. (Mineoka et al.,2008)

NO	Stage	Temperature	Time	No. of cycle

1	Denaturation as initial	95	30 sec	1
2	Annealing	58	45sec	35
3	Extension as Final	72	15min	1

Table (3-13): PCR Amplification Program for universal primers for virulence genes (Sikkema et al.,2023)

NO	Stage	Temperature	Time	No. of cycle
1	Denaturation as initial	95	3min	1
2	Denaturation	95	45sec	35
3	Annealing	56	1min	
4	Elongation	72	2min	
5	Final Elongation	72	5min	1

Table (3-14): PCR Amplification Program for specific primers for virulence genes (Enersen et al.,2013).

NO	Stage	Temperature	Time	No. of cycle
1	Denaturation as initial	95 °C	5min	1
2	Denaturation	95 °C	30 Sec	35
3	Annealing	55 °C	30 Sec	
4	Extension	72 °C	30 Sec	
5	Extension as Final	72 °C	5 min	1

Reagents

used in PCR technique are shown in the table below.

Table (3-15): Mixture Reaction 50μl for PCR Amplification of 16S r RNA Gene

NO	Reagent	Volume
1	Master Mix (Bioneer)	25 μl
2	forward Primer	5 μl
3	Reverse Primer	5 μl
4	DNA Sample	5 μl
5	Nuclease water free	10 μl
Total volume 50 μl		

3.2.10.6 Gene Sequence Analysis

BLAST was used to compare the modified sequences to sequences that had been deposited in the NCBI "bacteria" database. The identity—the number of bases that were identical between the query and the subject sequence in the database—was taken into consideration when determining the best and second-best taxon matches. The following outcomes could be attained by combining BLAST analysis with partial 16S rRNA gene sequencing: standard phenotypic identification with a distance in Max score bits to the next best taxon match of greater than 15, or "probable" (best species match matched the gold standard identification). (Wolff et al., 2010).

Chapter Four

Results And Discussion

4. Results and Discussion

4.1 Distribution of periodontitis Patients According to Sex and Age

The distribution of periodontitis patients Sex is shown in (Table 1-3) which is a total of 100 sample (50 patients and 50 control) periodontitis patients 43 Females and 57 Males. The age categories for patients with periodontitis and non-periodontitis are distributed in the patients' distribution by age table (3-1). Patients under the age of 35 had the greatest detection rates (100%), and statistical differences across all age categories were significant ($P < 0.01$)

Table (4-1): Distribution of Periodontitis and Gingivitis According to the Sex and Age.

No.	Age (Years)	Age %percentage	Number of Patients				%percentage
			Female 43%		Male 57%		
			Number	%percentage	Number	%percentage	
1	≤ 10	5	3	6.98	2	3.51	5
2	11 - 20	18	11	25.58	7	12.28	18
3	21 - 30	33	11	25.58	22	38.60	33
4	31 - 40	27	10	23.25	17	29.82	27
5	41 - 50	10	5	11.62	5	8.77	10
6	51 - 60	5	2	4.65	3	5.26	5
7	61 - 70	2	1	2.33	1	1.75	2
43 Total				57 Total		Total 100	
Chi-square = 4.907 *(P < 0.01)							

Statistically significant is the difference in Sex of the same group depending on the covered percentage for the distribution of periodontal patients according to age and Sex. Samples were collected. A total of (100) samples belonging to seven age groups (≤ 10), (11-20), (21- 30), (31-40), (41-50), (51-60), (61-70) years from 21 November and ending on 6 February 2023.

The highest isolation rate was 100% in age groups (21-30), (31-40), (51-60) and (61 -70) to determine the bacterial isolates that cause periodontitis. While the other age groups were not significant in the age group (10 or less), (11-20), The findings in [Table 3-1], are concurrent with the results of (sun et al.,2020). In contrast, the findings of the present study indicated that women are at lower risk than men, who are more likely to acquire periodontitis and dental caries, which are the same outcomes as those stated in (Zhao et al., 2021).

They claimed that because men often practice poorer oral hygiene than women, they are more susceptible to developing periodontitis. Age, sex, the kind and duration of psychiatric disease and its treatment, smoking, and being a woman were among the documented caries-predisposing factors (Dordevic et al., 2016). In contrast to the current study, the percentage of females infected with gingivitis is higher than the percentage of males, and this is the opinion of the researcher (Ciobanu et al.,2022). These results were analyzed statistically using the Chi-Square system, and the ratio was (4.907), which indicates the presence of significant differences.

4.2 Bacteriological Analysis.

The microbial analysis 100 sample (50 patients and 50 control) periodontitis patients showed 36 sample Porphyromonas gingivalis have some diversity of morphological type. Gram stain test was used to primarily identify complete

microbial isolates from the oral cavity of periodontitis (36 samples) bacterial isolates were isolated of Gram-negative (G-Ve) coccobacilli. The majority of the bacterial isolates were moderately identified by Gram stain reaction through the microscopic test, this is cohesive with identification using vitek 2 system.

In this investigation, 100 bacterial isolates were gathered from 50 individuals with clinical periodontitis and 50 individuals with clinical non-periodontitis from Al - Amarah City in southern Iraq. Clinical samples of the periodontium and periodontal pocket were selected for collection from various oral cavities. Between the gums and teeth, pockets can form as a result of bacterial plaque accumulation. These pockets' resident bacteria can result in gingivitis and cavities at the tooth roots. They bleed often, especially when eating or brushing their teeth. Most of the time, people are pain-free. The plates were incubated at 37°C for 48 hours to allow the dental caries bacteria to be isolated using an enrichment selective procedure, which also involved cultivating the germs on blood agar and MacConkey agar using paper points. is concurrent with the results of (Mohammed,2021).

4.3 Morphological and Microscopic Diagnosis:

Thirty-five out of one hundred bacterial isolates from periodontitis samples were taken from the oral cavity After inoculating the samples from the carrier medium tubes into the liquid thioglycolate isolation tubes supported and incubated anaerobically in the presence of resazurin reagent. Significant positive growth was observed in turbidity formation in the middle. When inoculating dishes with blood medium and incubating under the condition in anaerobes, distinct colonies appeared with a white color

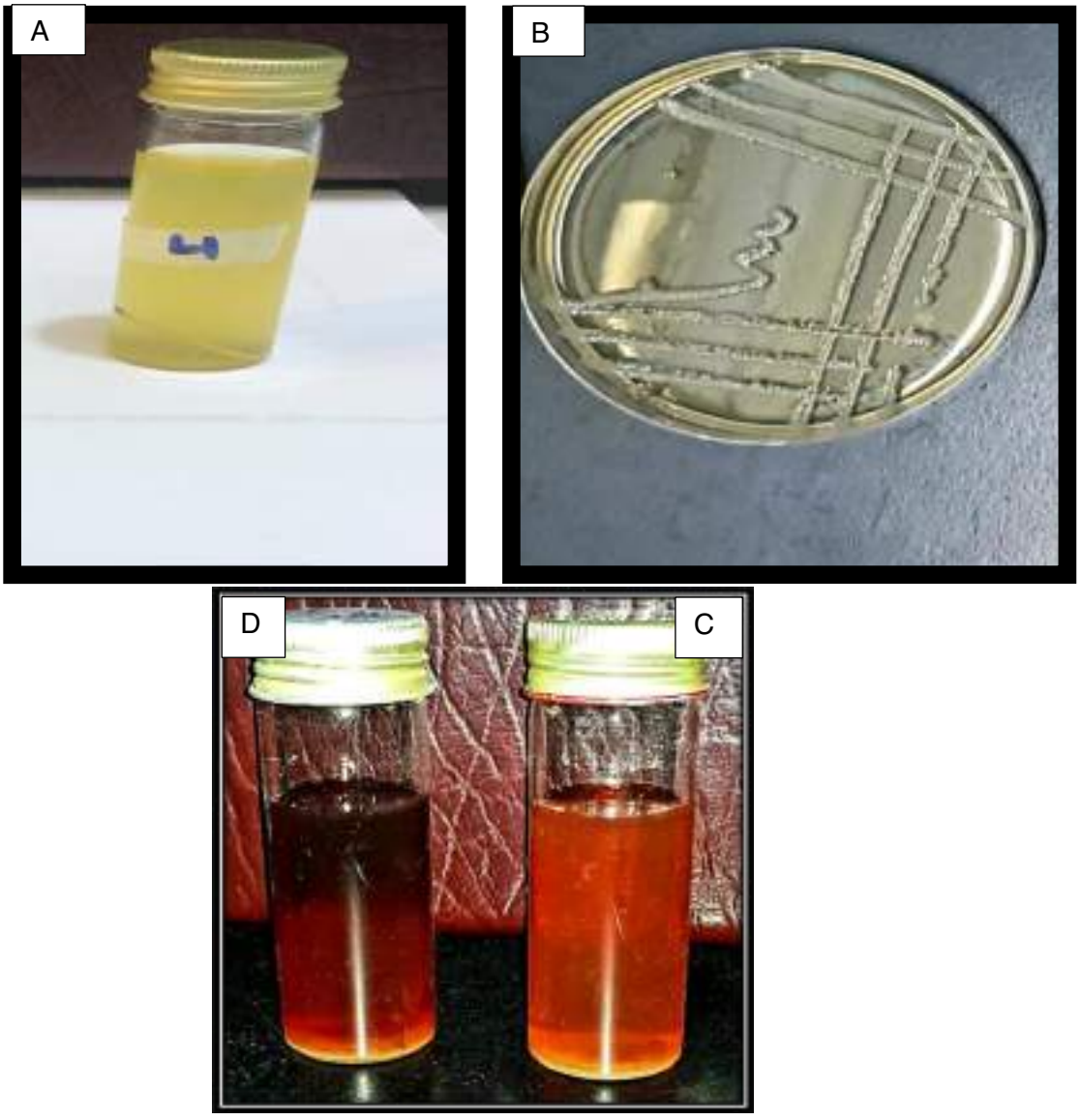


Figure (4-1): A Colony of Porphyromonas gingivalis on thioglycolate broth After 2 Days of at 37°c, B Colony of Porphyromonas gingivalis on thioglycolate Agar After 2 Days of at 37°C. Inocubation of Paper Points in Thioglycollate broth after Sample Collection from Periodontal Pocket, after 3 days, D. Inocubation of Paper Points in Thioglycollate broth after Sample Collection from Periodontal Pocket, after 7 days.

The purposes of primary isolation, the development of these germs, and the most important additions needed to support their growth. The efficiency of the thioglycolate medium has been shown as carrier media in transporting and

maintaining the vitality of Porphyromonas gingivalis in the mouth until it is delivered to the laboratory and cultured, as well as the efficiency of the types of primary isolation media and solid development media in supporting the growth of these bacteria Porphyromonas gingivalis colonies appear small, shiny, white color [Figure 4-1]. The same observations have been reported (Deppe et al.,2022).

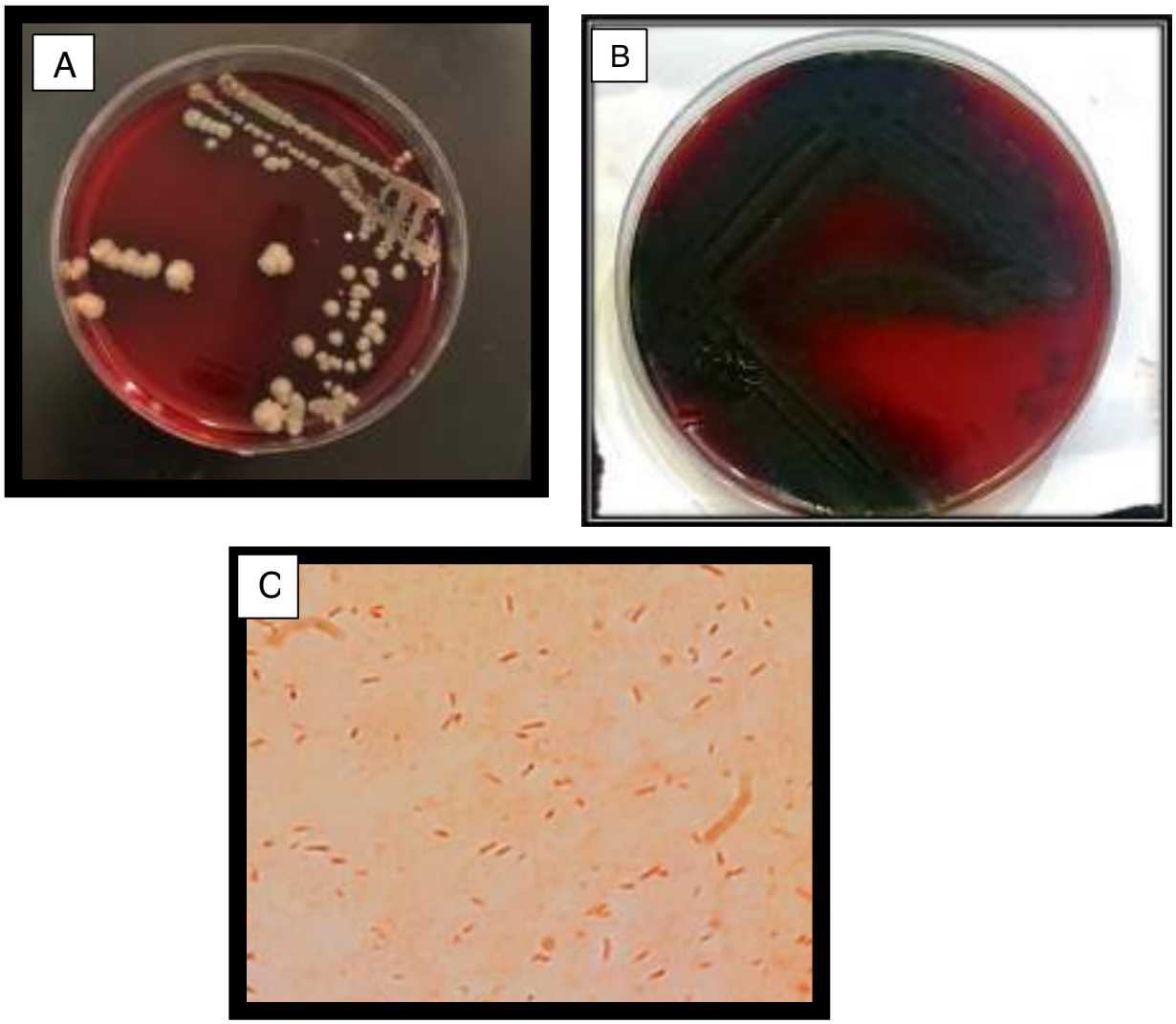


Figure (4-2): A Colony of Porphyromonas gingivalis on Blood Agar After 7 Days of Incubation 37°C, B Colony of Porphyromonas gingivalis on Blood Agar After 14 Days of Incubation 37°C, C. Important Oral Bacteria Porphyromonas gingivalis Observed by Microscopy 1000 x G-ve and coccobacilli

After which they were grown on blood agar at 37°C in an anaerobic chamber colonies appeared dark pigmentation, after 7 days of incubation p. gingivalis cultured on blood agar plates produced black and rod shap of colonies. Porphyromonas gingivalis has been determined that the buildup of hemin generated from erythrocytes on a surface and inside bacterial cells is what causes this black colony coloration. All microorganisms examined could develop on blood agar plates.

The aliquot to be utilized for culture was each supplemented with hemin and plated onto Blood Agar. For seven days, the plates were kept anaerobically in an anaerobic jar with a customized gas pack system. Appear in the blood agar as black-pigmented clumps. Hemin serves as a means of support for her movement, and as a result, she produces a black dye. This can explain why people who consume high amounts of iron are more prone to gingivitis.

Porphyromonas gingivalis bacteria produce a black pigment as a result of an accumulation of hemin, which it uses as a source of iron for its growth. and swelling of the gum and dental tissue. Plates were examined after the incubation period to see if any small, shiny, coccobacilli, black-pigmented, and mucoid colonies were present [Figure 4-2]. A study by Ingalagi et al (2022) reported that in colonies that are tiny, glossy, black-pigmented, and mucoid, with or without hemolysis, the value of identifying and quantifying Porphyromonas gingivalis and other periodontal infections using various methods in plaque samples.

After the differentiating medium between Gram-negative (G-ve) and Gram-positive (G +ve) bacteria was transferred into MacConkey agar Colonies appeared a pink color on MacConkey agar 7day under37°C in an anaerobic chamber

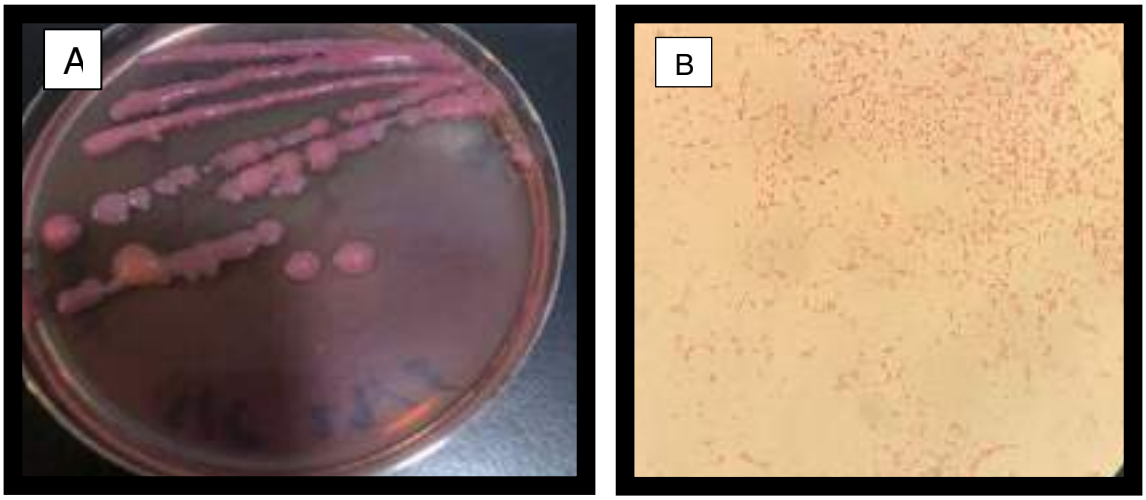


Figure (4-3): A Colony of Porphyromonas gingivalis on MacConkey agar After 7 Days of Incubation 37°C, B. Important Oral Bacteria Porphyromonas gingivalis Observed by Microscopy 1000 x, G-ve and coccobacilli.

bacteria were isolated and gram-positive and gram-negative lactose-fermenting bacteria were distinguished using MacConkey agar. This medium's selective action is a result of the presence of bile salts and crystal violet, both of which inhibit the majority of Gram-positive bacterial species. Porphyromonas gingivalis colonies appear small, shiny, pink color [Figure 4-3]. Which is the same results reported by Annor et al (2023). They reported that MacConkey Agar Lactose fermentation-positive strains grow red or pink. The red or pink color is due to acid production from lactose, absorption of the mild red color, and subsequent color change of the pigment.Then these isolates were grown on Mitis Salivarius Agar (MSA) medium colonies appeared a blue colonies after (48-72) hours under 37°C in an anaerobic chamber to cultivate microorganisms.

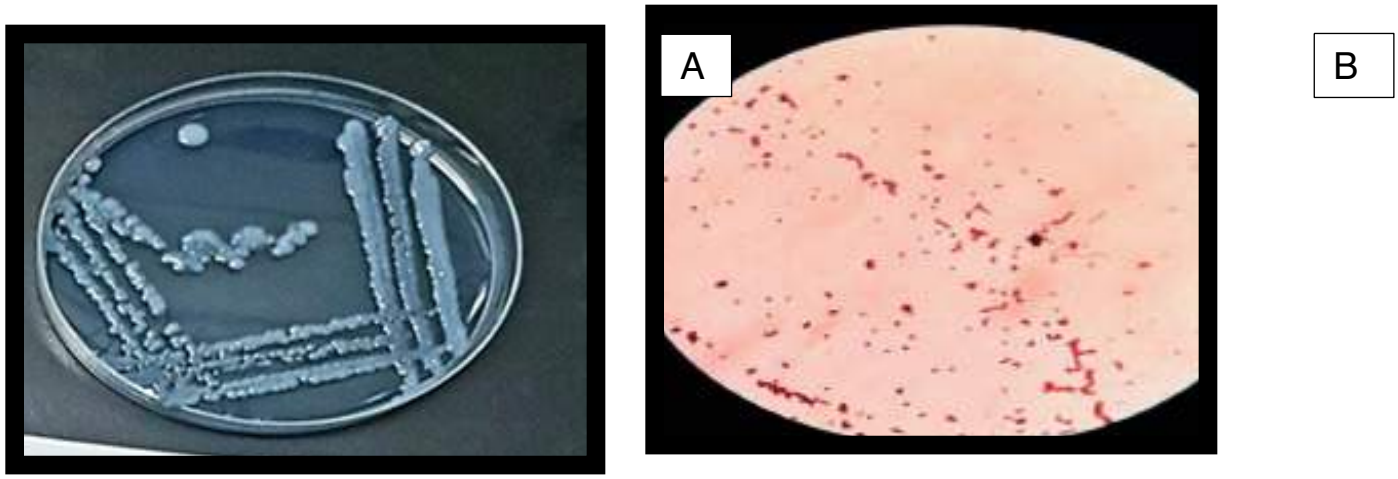


Figure (4-4): A Colony of Porphyromonas gingivalis on Mitis Salivarius After 7 Days of Incubation 37°C, B. Important Oral Bacteria Porphyromonas gingivalis Observed by Microscopy 1000 x

A general-purpose of culture medium for fastidious or nonfastidious microorganisms can be grown from trypton soy agar after 7day colony show the color of the colonies is yellow-green under 37°C in an anaerobic chamber.

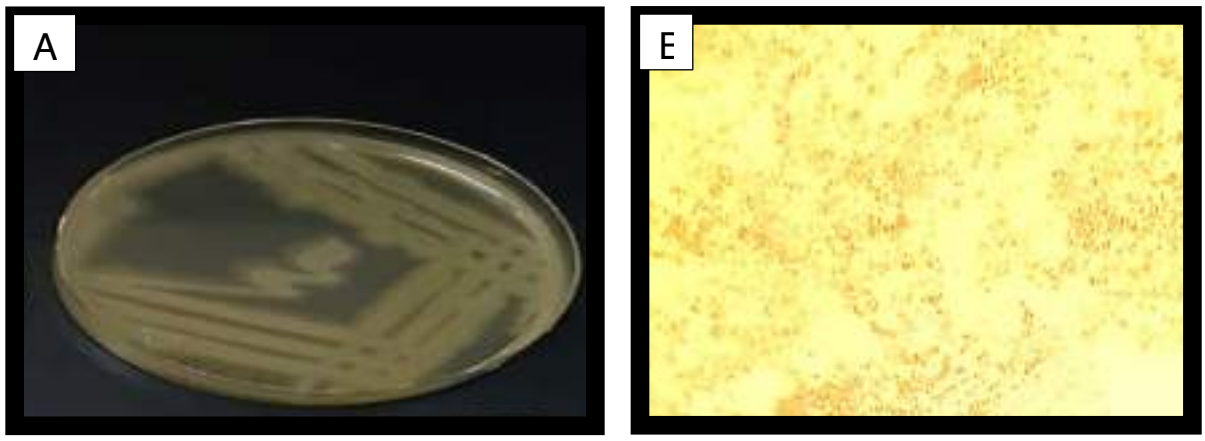


Figure (4-5): A Colony of Porphyromonas gingivalis on trypton soy agar After 7 Days of Incubation 37°C, B. Important Oral Bacteria Porphyromonas gingivalis Observed by Microscopy 1000 x G-ve and coccobacilli.

When obtaining pure cultures of microorganisms and observing colony shape, TSA is frequently used as the major growth medium. A variety of microorganisms

can grow in the medium with enough nutrients for their growth, enabling isolation and identification. Additionally, it serves as a medium for storing bacterial cultures as well as for additional biochemical testing of microorganisms. As a result, this media was employed in this research Plates were examined after the incubation period to see if any small, shiny, coccobacilli, yellow-green color, and mucoid colonies were present [Figure 4-5]. Which is the same results reported by Shibli et al (2021), On tryptone soy agar with yeast extract enhanced with 1% hemin, 5% menadione, and 5% sheep blood, Porphyromonas gingivalis was developed. Tryptone soy agar with yeast extract, all species were moved to tubes containing

It is a nutrient-rich media that can be used to cultivate a wide range of microscopic creatures. After 72 hours under the conditions described, the Porphyromonas gingivalis colony in the Brain Heart Infusion (BHI) agar shows as a white color BHI medium under 37 in anaerobic chamber.

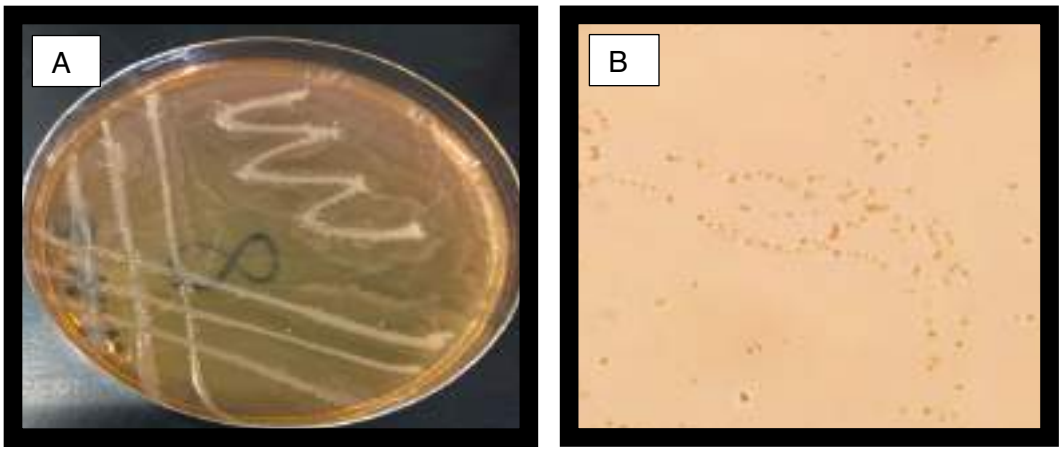


Figure (4-6): A Colony of Porphyromonas gingivalis on Brain Heart Infusion (BHI) agar after 7 days of incubation 37°C, B. Important Oral Bacteria Porphyromonas gingivalis observed by microscopy 1000 x G-ve and coccobacilli

BHI is widely utilized in both clinical and research settings to cultivate a wide range of microorganisms. In this study Plates were examined after the incubation

period to see if any small, shiny, coccobacilli, white color, and mucoid colonies were present [Figure 4-6]. In contrast to the current study, Porphyromonas gingivalis exhibited morphological similarities to the parent strain Porphyromonas gingivalis and continued to produce black pigment on blood-BHI agar plates. When growth rates were compared, it was discovered that the mutant divided every 4.7 h while the parent divided every 3.5 h. The fatty acid analysis of the culture supernatants from the parent strain and the mutant showed no changes (Park and McBride,1993).

Table (4-2): Morphological and media growth characteristics of porphyromonas gingivalis.

Media growth	Result of growth
Blood agar	dark pigmentation with non-hemolytic, coccobacilli
MacConkey agar	pink color colony, coccobacilli
thioglycolate agar	white color colony, coccobacilli
Mitis Salivarius agar	Blue color colony, coccobacilli
The Brain Heart Infusion	white color colony, coccobacilli
trypton soy agar after	yellow-green color colony, coccobacilli

4-4 Biochemical Tests

The research includes identifying and describing diverse bacterial strains using a variety of biochemical assays, such as the catalase test, oxidase test, indole test, and motility. Each biochemical test required the creation of a particular growing medium, the inoculation of bacteria, and the observation of a certain enzymatic or physiological reaction. In order to facilitate further bacterial investigation and characterization, these tests provide quick and accurate ways for differentiating various bacterial strains based on their distinctive properties. The bacterial isolates were initially identified by microscopic examination of colony characteristics and biochemical testing, as shown in table (4-4), and then the isolates' diagnoses were validated by the Vitek 2 system.

Since anaerobic bacteria lack the ability to manufacture the catalase enzyme, facultative anaerobic bacteria do so. The outcome of this investigation was negative, indicating that streptococci or obligate anaerobic bacteria do not form bubbles as a result of hydrogen peroxide's breakdown. The indole test is a biochemical process, used to identify the indole-producing organism from tryptophan. Tryptophan is an important amino acid found in most bacterial cell proteins. Porphyromonas gingivalis positive for indole test shown in [Figure 4-7]. Which is the same results reported by Ingalagi et al (2022), Compared to positive (greenish color) and negative (no color change) controls, the indole test results were positive (greenish-black color shift); When compared to a positive result (effervescence produced), the catalase test did not create any. This test helps to distinguish between various types of bacteria and can be used to evaluate whether isolated bacteria have the cytochrome c oxidase enzyme. Since Porphyromonas gingivalis fails this test and

does not turn purple (does not change color). the bacteria do not produce the enzyme cytochrome c oxidase show in [Figure 4-7]. similar outcomes to those that were reported (Khader et al.,2020).

Table (4-3): Microscopic and Biochemical tests for porphyromonas gingivalis isolates

Biochemical tests	Result of biochemical tests
Gram stain and shape on microscope	G -ve coccobacilli
Catalase test	_
oxidase test	_
Indole	+
Motility	_

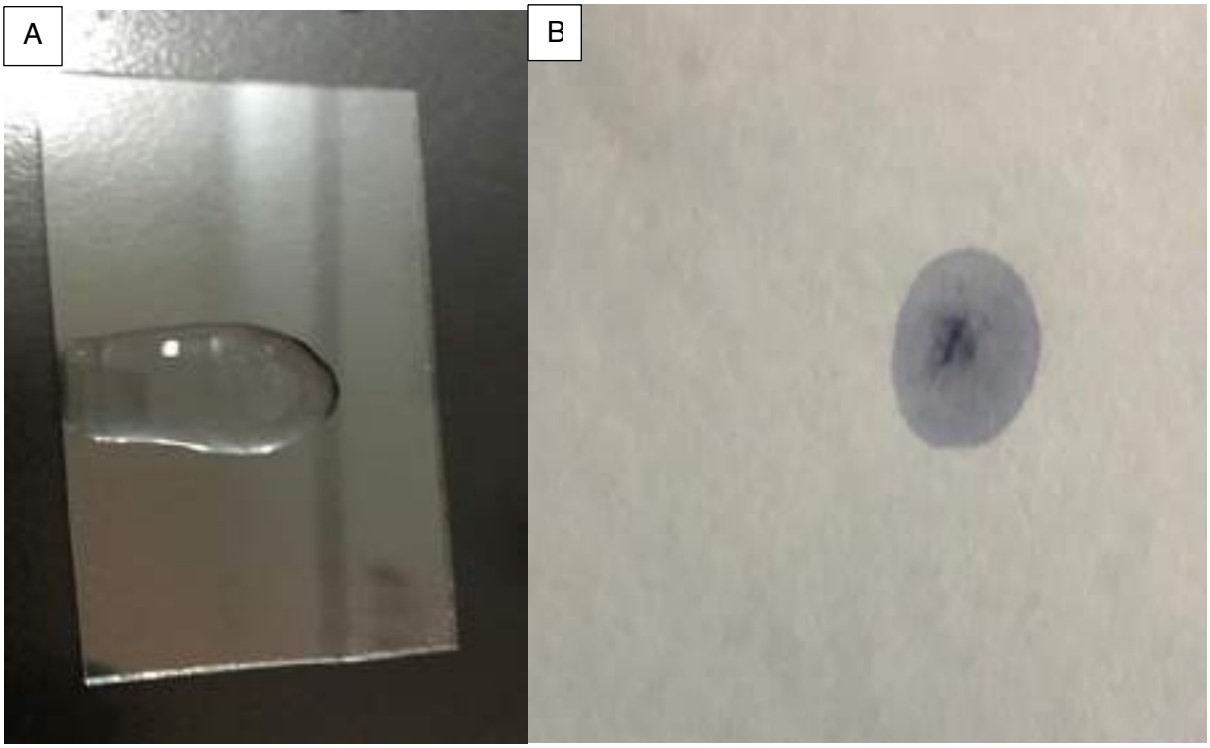


Figure (3 -7): Biochemical tests A, Catalase test -ve B, oxidase test -ve

4.5 Identification of Bacterial Isolates by Vitek2 System

In comparison to standard diagnostic techniques, the Vitek2 System is thought to be one of the best systems used to swiftly and accurately identify different species of bacteria. Before using the Vitek2 System, there are numerous processes of cultivating bacteria on culture media and isolating them in their purest form. After these steps, the germs are then detected using the system.

Vitek2 System was unable to diagnose this isolate despite the use of a special kit for Gram-negative and anaerobes. Vitek2 was used to investigate 26 pathological samples, however it was unable to identify any of them. And was used to investigate 26 control samples, however, it was unable to identify any of them. shown in (Table 3-4).

Table (4-4): Distribution of porphyromonas spp. In periodontitis and non-periodontitis patients

Isolate No.	The Date	Vitek 2 compact system
(1-12)	2023/3/29	unidentified
(13-25)	2023/4/11	unidentified
(26-38)	2023/4/16	unidentified
(39-52)	2023/6/18	unidentified

4.6 Antibiotic Susceptibility Test (AST)

To assess the antibiotics Cefoxitin (fox), Doxycycline (Do), Ciprofloxacin (CIP), and Nalidixic acid (NA), by Porphyromonas gingivalis in vitro, antibiotic susceptibility testing (AST) was carried out utilizing the disc diffusion method. Their antibacterial activity against specific pathogenic bacteria isolated from Periodontal patients in Al-Amarah City, Iraq, was evaluated using disk diffusion methods. The necessity for the development of innovative medicines to treat oral infections is demonstrated by the known side effects of already-prescribed antiseptics and the growing threat of antibiotic resistance. We recently carried out a C en of a repurposing library to find nov D dicines with strong action against the oral pathogen Porphyromonas gingivalis (Gerits et al.,2017). In this study, we deepened our examinations of porphyromonas gingivalis activity and discovered that antibiotic successfully prevents the growth of Gram-negative anaerobic isolates in vitro.

Table (4-5): Distribution of Antibiotics to porphyromonas gingivalis and the Antibiotics Inhibition Zone (mm)

Isolate No.	Antibiotics and Concentration µg disk	Antibiotics Inhibition Zone (mm)
10	Cefoxitin (fox) 30 µg	10
13	Cefoxitin (fox) 30 µg	10.3
2	Ciprofloxacin (CIP) 5µg	10
4	Ciprofloxacin (CIP) 5 µg	8.6

6	Ciprofloxacin (CIP) 5 µg	9
8	Ciprofloxacin (CIP) 5 µg	7.6
14	Ciprofloxacin (CIP) 5µg	8.3
18	Ciprofloxacin (CIP) 5µg	13.3
1	Doxycycline (Do) 30 µg	7.3
4	Doxycycline (Do) 30 µg	8.6
7	Doxycycline (Do) 30 µg	7
12	Doxycycline (Do) 30 µg	15
5	Nalidixic acid (NA) 30 µg	9.6
6	Nalidixic acid (NA) 30 µg	8
18	Nalidixic acid (NA) 30 µg	9

Nalidixic acid is a synthetic quinolone antibiotic called nalidixic acid that primarily acts as a bactericidal agent. Transformation alters a person without using bacteria to create a clone of them. in our study resistance to 20% of the isolates of Porphyromonas gingivalis (pallo et al.,2023).

Ciprofloxacin (CIP) is a bacterial antibiotic belonging to the fluoroquinolone family. It is effective against most Gram-negative and Gram-positive bacteria, so it is used to treat various types of bacterial infections. was resistant to 60% of Porphyromonas gingivalis isolates, which is consistent with many other studies and

suggests that this antibiotic is broad-spectrum. similar outcomes to those that were reported (AlHarthi et al.,2020).

Cefoxitin (fox) is a β-lactam antibiotic a second-generation cephalosporin antibiotic is cefoxitin. In addition to some gram-negative bacteria, it has an antibacterial action on gram-positive bacteria. It differs from other second-generation cephalosporins in that it protects against anaerobic bacteria, which thrive in anoxic circumstances. was 13% of the Porphyromonas gingivalis in our study resistant, similar results to those that were reported (Milazzo et al.,2002).

Doxycycline is a broad-spectrum antibacterial, belonging to the tetracycline family. It fights germs by preventing them from multiplying. In addition, it has an anti-inflammatory effect, which reduces the severity of the infection on the body. was resistant to 26.6 % of the Porphyromonas gingivalis isolates in our study, which is consistent with a several other investigations and implies that this antibiotic has a broad spectrum. similar outcomes to those that were reported (Chaves et al.,2000).

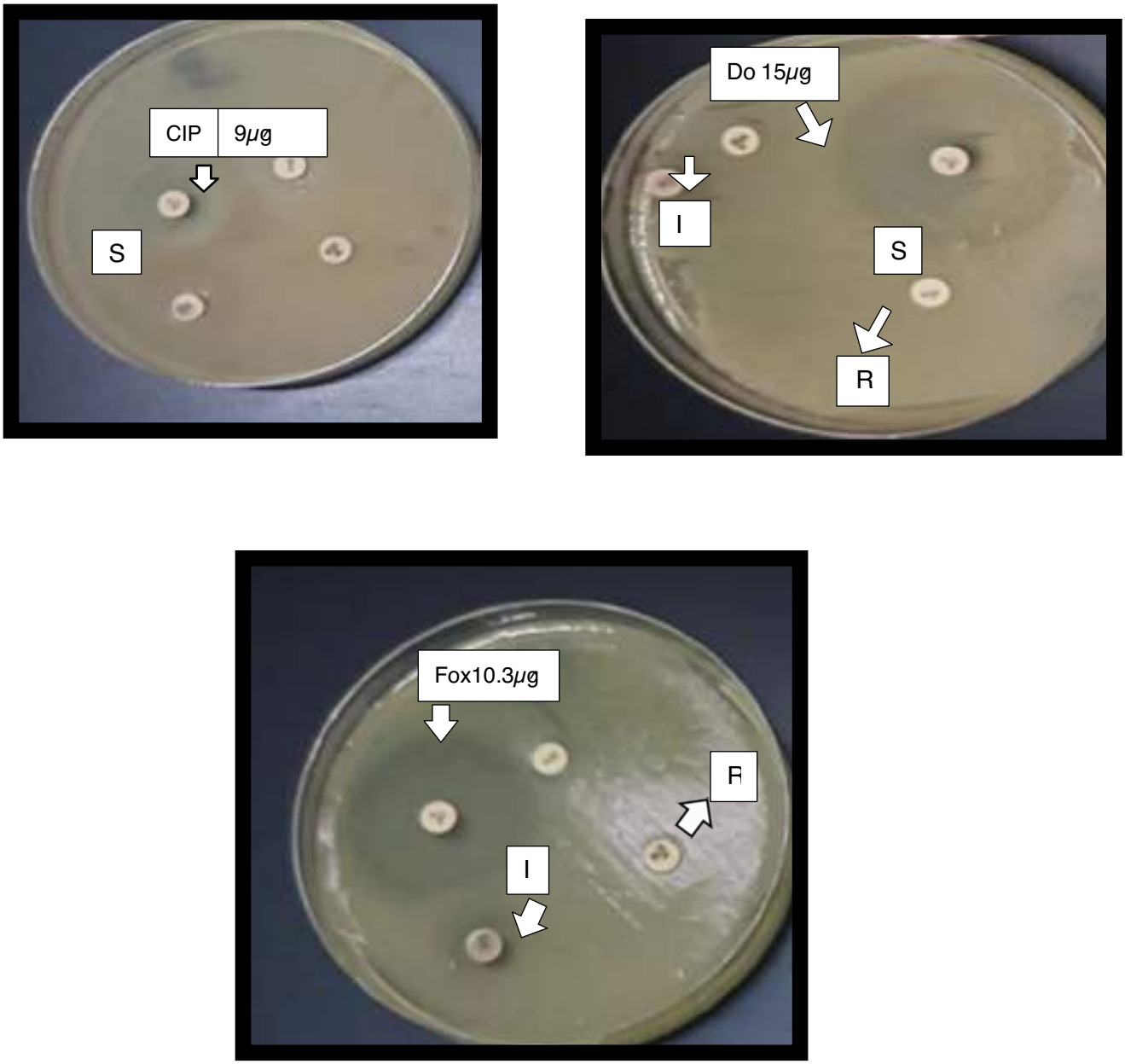


Figure (4-8): Effect of Some Antibiotics Against Porphyromonas gingivalis Pathogens Bacterial Isolated from Some periodontitis Patients in Al-Amarah City, R (Resistant) S (Sensitive)

4.7 Molecular study

4.7.1 DNA Extraction

Genomic DNA was extracted from isolated bacterial cells and detected using agarose gel Electrophoresis.

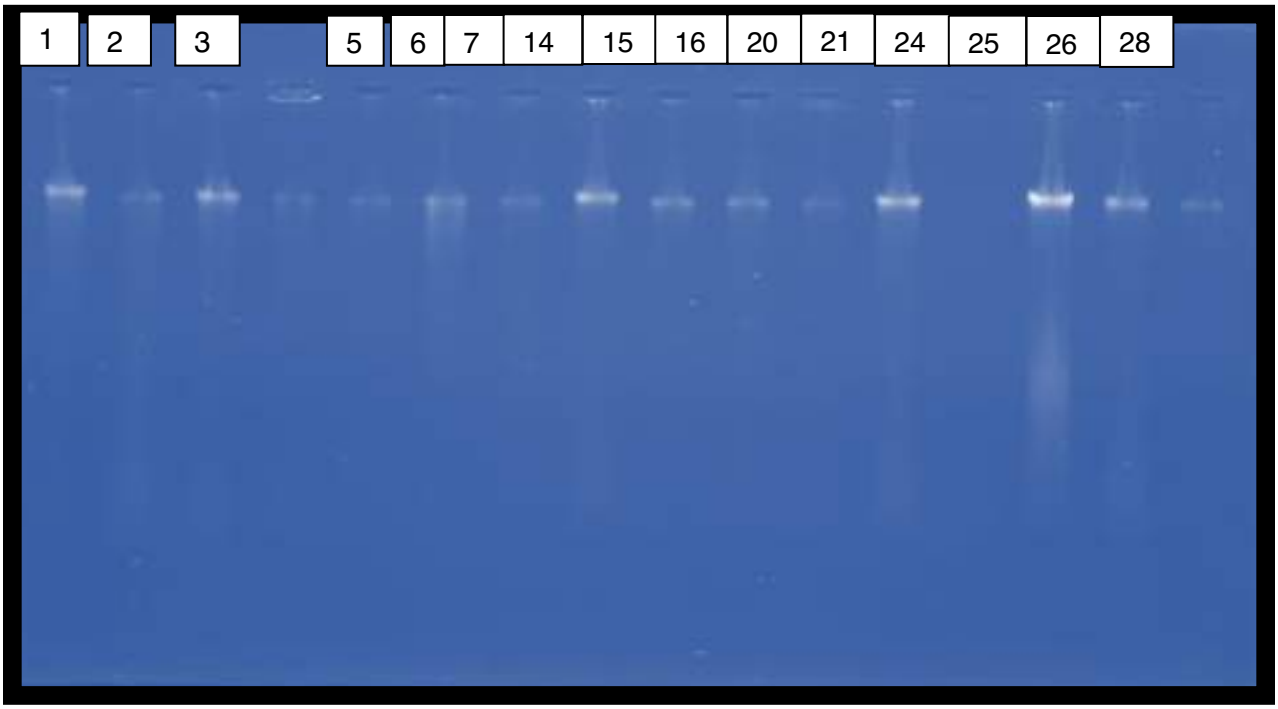


Figure (4-9): Band Patterns on Agarose Gel Electrophoresis for Bacterial Isolates.

4.7.2 Amplification of 16S rRNA Gene

In this study, two types of primers were used the universal primer obtain accurate results. The collected DNA was used in a PCR experiment to amplify 16SrRNA. All 6 sample bacterial isolates successfully amplified the 16S rRNA gene, yielding favorable results. Gel electrophoresis was used to identify the amplified 16S rRNA gene; the separate gene bands were distinguished by 1500 bp in comparison to the typical molecular DNA marker (50-2000 bp).

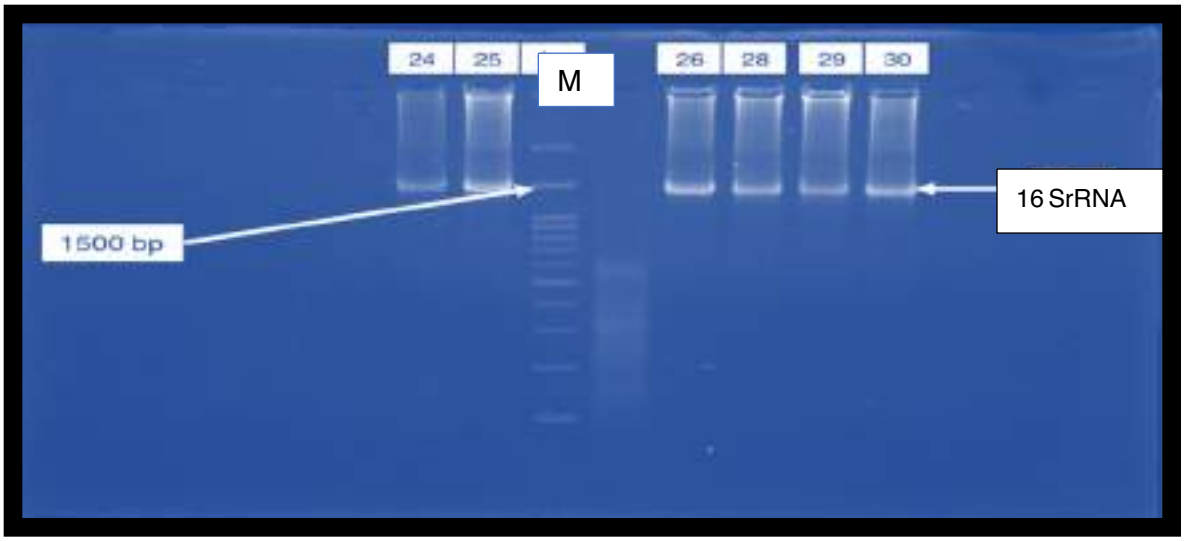


Figure (4-10): PCR Products Amplified of 16Sr RNA on Agarose Gel Electrophoresis, m: marker.

Because endodontic bacteria differ between individuals, the cause of periodontitis is heterogeneous. Endodontic samples can help discover species that are challenging to cultivate using molecular detection techniques like species-specific PCR assays. Nine hypervariable areas on the bacterial 16S rRNA show uniform sequence diversity among different bacterial species, and they can be used to identify bacteria. In refractory cases, the PCR approach is more sensitive than conventional culture procedures for microbiological identification (Tiwari et al.,2020).

The hundred bacterial isolates that were chosen based on distribution and frequency for the present study's identification of anaerobic bacteria were successfully identified by Vitek2 and 16S rRNA gene sequencing. These findings are consistent with a recent study by Al-Farhan (2018).

The Porphyromonas gingivalis primer set targeted region was discovered to be directly related to ability in Porphyromonas gingivalis in the current investigation. However, Porphyromonas gingivalis was amplified for the detection in the final identification.

4.7.3 Amplification of Specific Primers

The genomic DNA was use as DNA template was used in to PCR technique for amplification of specific primers. A particular primer was successful in amplifying all 36 identified bacteria. Gel electrophoresis was used to identify the amplification of specific primers. the separate Gene bands were distinguished by 200 bp in comparison to the typical molecular DNA marker (100-300 bp)

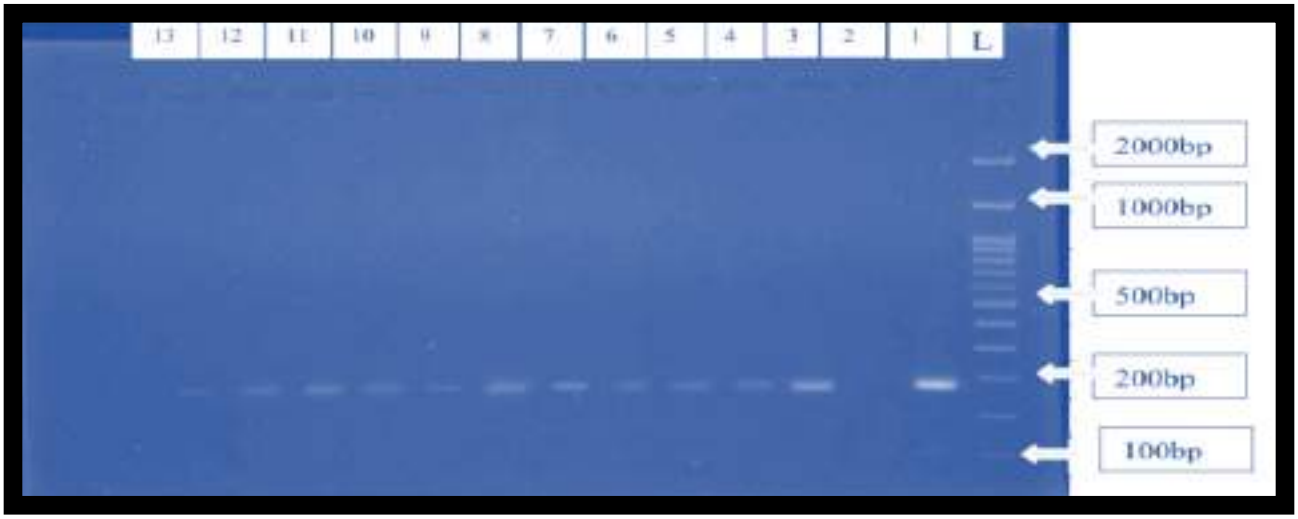


Figure (4-11): PCR Products Amplified of specific primers on Agarose Gel Electrophoresis, M: marker.

A specific primer gave good results in the detection Porphyromonas gingivalis bacterial isolates [Figure 4-13]. A study by Noguchi et al (2005) reported the method of identifying bacteria using bacterium-specific PCR primers, however, is a technique to detect just the DNA of the target bacterium and is not appropriate for qualitative examination of numerous unidentified bacterial species. According to several investigations, obligatory anaerobes are rarely found in the root canal of patients with periapical periodontal disease when utilizing traditional culture techniques. The PCR-based 16S rRNA gene assay is useful for identifying a wide range of anaerobic bacteria that are difficult to grow by standard culture methods.

4.7.4 16S DNA Gene Sequences

Table (4- 6): Bacteriological and molecule techniques applied to bacterial isolates are tested.

Isolate No.	Gram stain	Vitek 2 compact system	Morphology	16S r DNA gene squence
25	Gram – ve	unidenttified	coccobacilli	porphyromonas gingivalis
26	Gram – ve	unidenttified	coccobacilli	porphyromonas gingivalis
28	Gram – ve	unidenttified	coccobacilli	porphyromonas gingivalis
29	Gram – ve	unidenttified	coccobacilli	porphyromonas gingivalis
30	Gram – ve	unidenttified	coccobacilli	porphyromonas gingivalis

4.7.5 virulence genes:

4.7.5.1 Fimbria (fim)

In this study, two types of primers were used the universal primer and the special primer when using a special primer, all 26 of the newly identified microbes could be amplified. Gel electrophoresis was applied to identify the amplified primers. The individual gene bands can differ by (234bp).

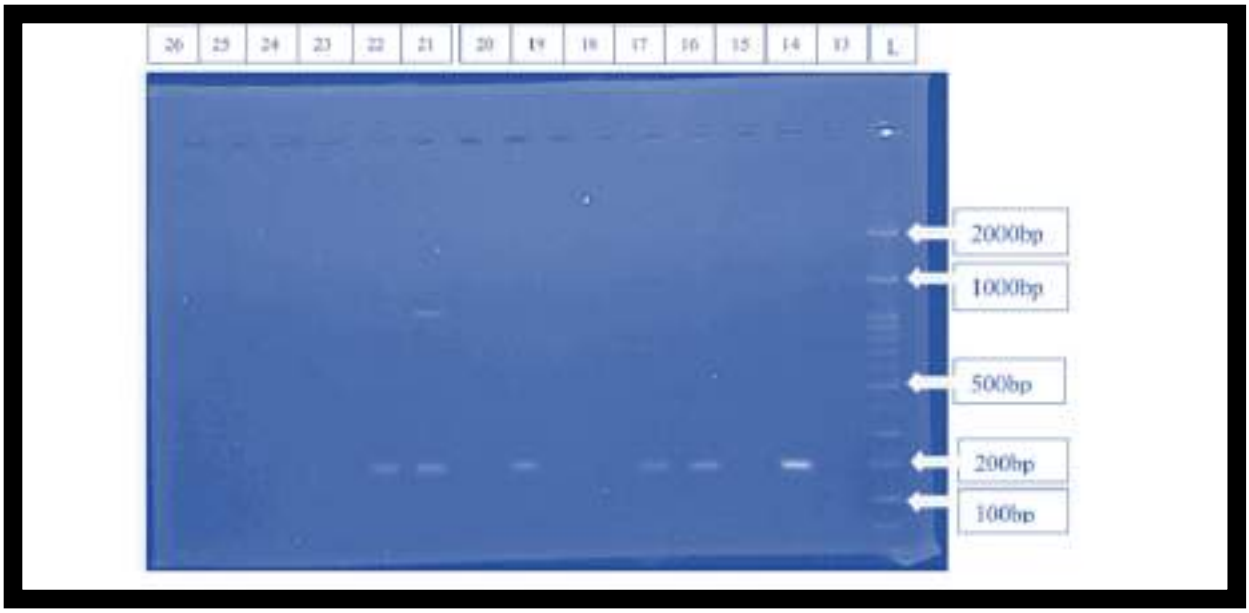


Figure (4-12): Fim A gene (294 bp.) of Porphyromonas gingivalis- specific primer set. Showing by gel-electrophoresis results of evaluating specific primer binding fim A gene region on the Porphyromonas gingivalis through Polymerase chain reaction (PCR)

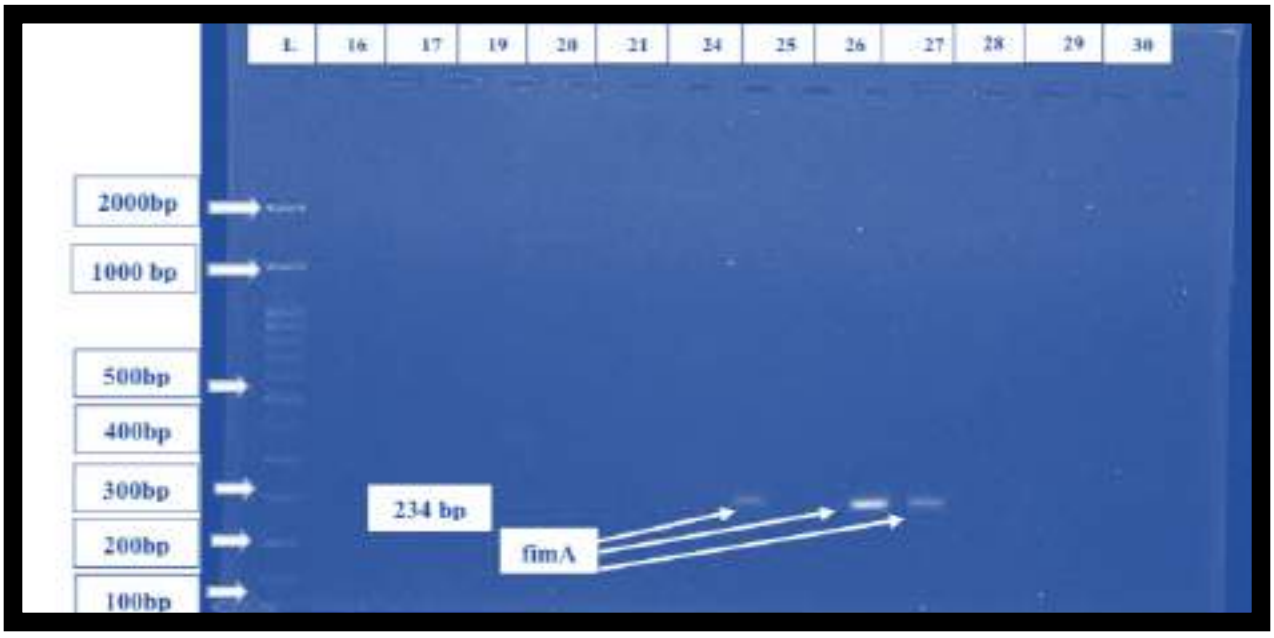


Figure (4-13): fim A gene (234 bp.) of Porphyromonas gingivalis- specific primer set. Showing by gel-electrophoresis results of evaluating specific primer binding fim A gene region on the Porphyromonas gingivalis through Polymerase chain reaction (PCR)

When using universal primers no band is obtained, and all 26 of the newly identified microbes could be amplified. Gel electrophoresis was applied to unidentify the amplified primers. Fig (4-15)

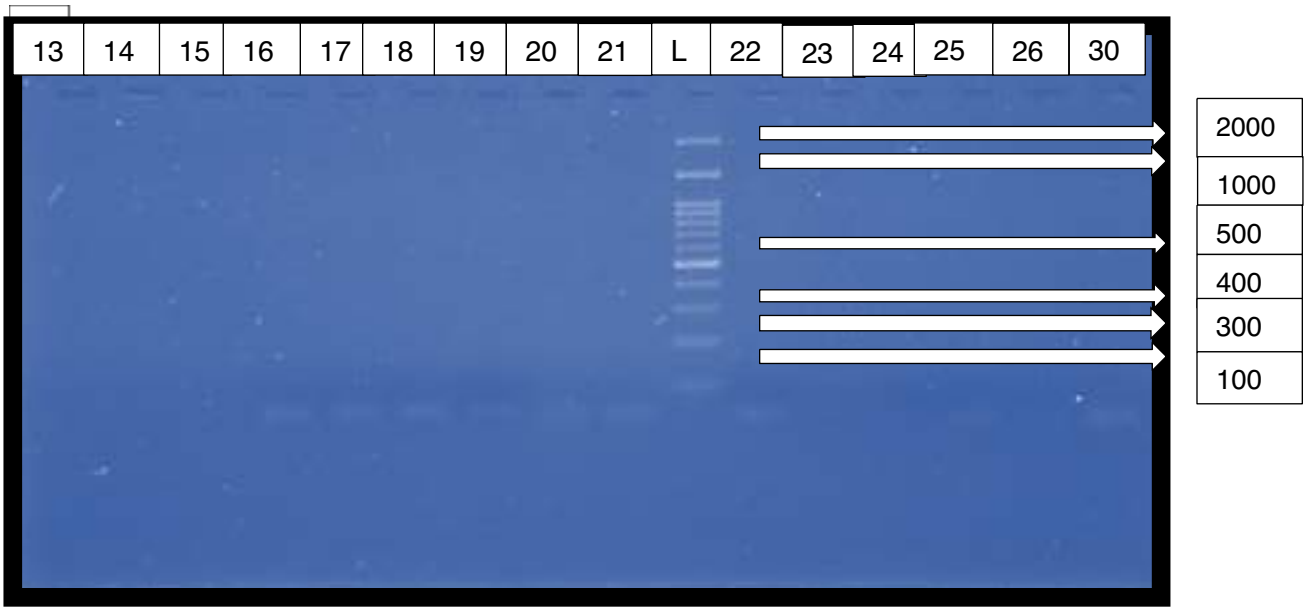


Fig (4-14): fim A gene of Porphyromonas gingivalis- universal primers set. Showing by gel-electrophoresis results of universal primers primer binding fim A gene region on the Porphyromonas gingivalis through Polymerase chain reaction (PCR).

The Porphyromonas gingivalis chromosome only contains one copy of the fimA gene, which encodes fimA. Six varieties (I, Ib, II, III, IV, and V) of the gene have been identified based on nucleotide sequence variation (Enersen et al.,2013). Fimbriae play a significant role in the colonization process of Porphyromonas gingivalis as they facilitate attachment to other oral bacteria and host cells. The fimbriae produced by Porphyromonas gingivalis are recent type V fimbriae that are also present in a variety of commensal and pathogenic Bacteroides species that make up the human microbiota (Alaei et al.,2019). According to the current population-based study, Porphyromonas gingivalis has diverse occurrence patterns at the level of its virulence genes, and a variety of factors influence its prevalence. Saliva samples showed a typical periodontal profile in general. A higher detection

rate for both pathogens was noted in the “Periodontal disease” (PD) group, with a higher abundance of Porphyromonas gingivalis, compared with that in the healthy subjects. in the current study a PCR test with primer sets that can reliably identify the presence of Porphyromonas gingivalis as well as the presence or absence of essential virulence factors, such as fimA, and shows how it can be used in the context of periodontal disease in adults from Iraq. Any of these virulence genes were strongly associated with periodontal damage patients (Gu et al.,2023). Systematic analysis of prophages carried by Porphyromonas gingivalis.

The fim A primer set targeted region was discovered to be directly related to ability in Porphyromonas gingivalis in the current investigation. However, fim A was amplified for the detection in the final identification. test shown in [Figure 4-14]. In this study, it was found that the percentage of presence of fimA was 34% and the amplification fim A gen 234. In the same study reported by Nakamura et al (2000), the fimA probe is a 200 or more bp short-terminal flanking sequence, as well as other Porphyromonas gingivalis -specific sequences, are present in addition to the fimA sequence.F 5′ -ATA ATG GAG AAC AGC AGG AA -3′ R 5′ -TCT TGC CAA CCA GTT CCA TTGC -3′. In the same results reported by Aabed et al (2023), The detection of their respective virulence genes, fimA and prtC and IktA and fap, was also substantially correlated with the increasing frequency of the target pathogens Porphyromonas gingivalis and A. actinomycetemcomitans. Increased prtC detection rate and increased Porphyromonas gingivalis carriage in the PD group showed a statistically significant correlation, while fimA showed a lesser correlation. In terms of gene expression, the healthy subjects showed a different pattern of occurrence, with fimA being expressed more than prtC.

In their study People of all ages, including small toddlers, can develop periodontitis. Those who get inflammation of the tissues sustain their teeth without first

experiencing an infection; this is more common in those between the ages of 31 and 70 shown in table (4-10). In the same study as those that were reported (Kim et al.,2016).

Table (4-7): Pathogenicity of porphyromonas gingivalis for the sex and age in the present study.

No.	Age (Years)	Number of Patients				pathogenicity
		Female 43%		Male 57%		
		Number	%percentage	Number	%percentage	present
1	10 or less	3	6.98	2	3.51	_
2	11 - 20	11	25.58	7	12.28	+
3	21 - 30	11	25.58	22	38.60	_
4	31 - 40	10	23.26	17	29.82	+
5	41 - 50	5	11.63	5	8.77	++
6	51 - 60	2	4.65	3	5.26	+++
7	61 - 70	1	2.33	1	1.75	+
43 Total		57 Total		Total 100		
Chi-square = 4.907 *(P < 0.01)						

One of the main causes of tooth loss in the pathological process of periodontitis is the breakdown of the periodontal tissues that support the teeth. The interaction between oral cavity bacteria and host cells is a complex process that is linked to the development and progression of this disease. The actions of this bacteria are influenced by a number of virulence factors, including lipopolysaccharide (LPS), fimbriae (FimA and Mfa1), cysteine proteases (Rgp and Kgp), and the capsule. These surface elements and secretory enzymes support effective colonization,

growth, and nutrition uptake as well as defense against host defense mechanisms. P. gingivalis has been linked in recent research to the start of several systemic diseases, such as rheumatoid arthritis, cardiovascular diseases, and neurological diseases. In this study, it was found that pathogenicity in males is higher than in females, due to immunity to smoking and alcohol, and it increases in older ages, due to weak immunity, and this is similar to the study. (De Vries et al.,2022).

Table (4-8): Virulence genes factor (FimA) for porphyromonas gingivalis.

Virulence genes factor	Percentage%
Universal primer	0
Specific primer type I	23
Specific primer type II	46.6

4.7.6 Phylogenetic tree

The Porphyromonas genus is composed of several organisms from both human and animal sources. Based on NCBI annotation, 16S rRNA gene sequences were recovered from the genomes of P. gingivalis strains for the purpose of 16S rRNA gene phylogeny. During the phylogenetic tree construction process, a copy of the 16S rRNA gene sequence from AP012203.1 Porphyromonas gingivalis TDC60 DNA complete genome was utilized as the out-group.

In conclusion, studying evolutionary relationships between the Porphyromonas genus could be important to explain the origin of the P. gingivalis phenotype. Comparing the core and persistent gene families and the accessory gene fractions revealed that housekeeping and central metabolism/transport functions were more represented in the core than in the Accessory gene set (Morales-Olavarría et al.,2023).

Fig (4-15) Neighbor-joining phylogenetic tree of strain P. gingivalis Bootstrap values (expressed as a percentage of 1000 replications) > 65% are shown at the branch points 16Sr RNA primer.

detail phylogenetic relationships between P. gingivalis and its closest homologs, we carried out additional analyses based both on amino acid and nucleotide alignments, including new sequences isolated from patients examined in this study. For this purpose, the entire gene was amplified using genomic DNA isolated from human samples. Subsequently, known nucleotide sequences. In periodontitis were compared with data obtained from sequencing analysis. All Porphyromonas sequences clearly separated from other Bacteroidia sequences with high and moderate support. One sequence with unspecified taxonomic affiliation described as Bacteroidetes oral taxon (Gmiterek et al.,2013)

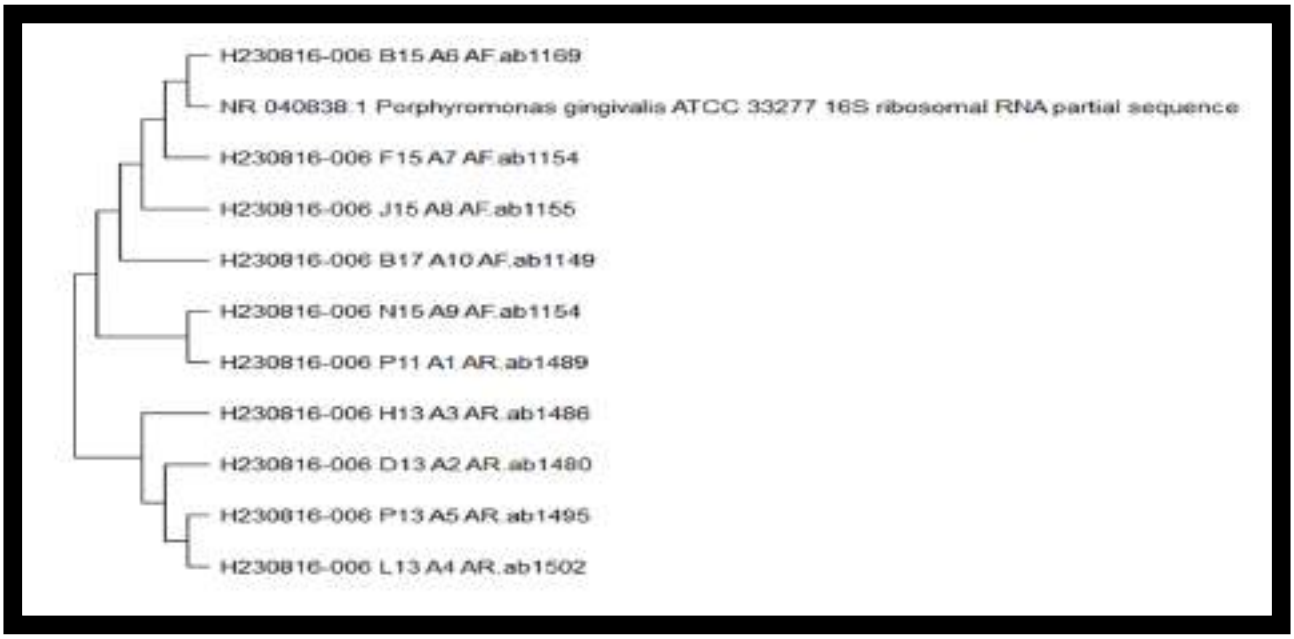


Fig (4-16): The general tree with the real branch length leading of P. gingivalis clade (marked by the gray rectangle).The tree with the branch length leading to P. gingivalis shortened 100 times (the dashed line) to show detailed relationships between P. gingivalis strains and isolates. Joined names of strains and isolates indicate their 100% sequence identity.

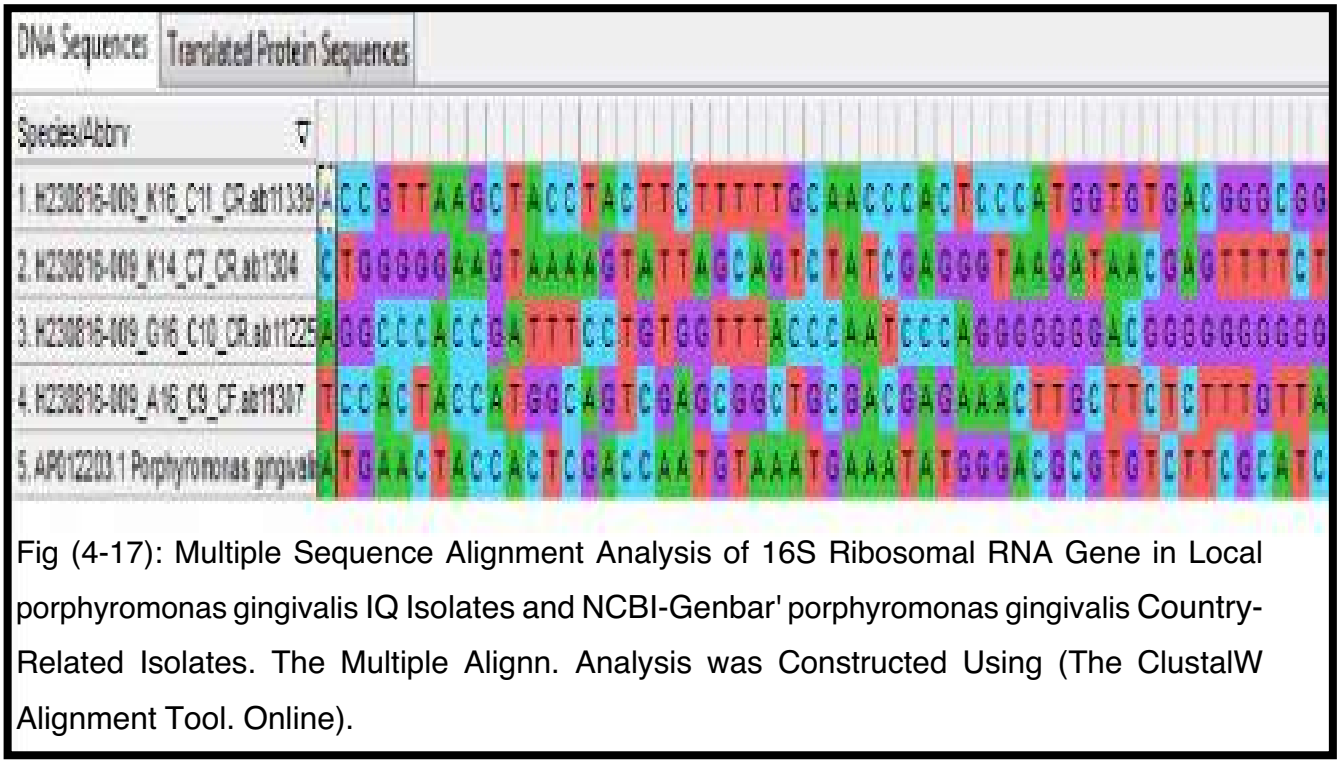


Fig (4-17): Multiple Sequence Alignment Analysis of 16S Ribosomal RNA Gene in Local porphyromonas gingivalis IQ Isolates and NCBI-Genbar' porphyromonas gingivalis Country-Related Isolates. The Multiple Alignn. Analysis was Constructed Using (The ClustalW Alignment Tool. Online).

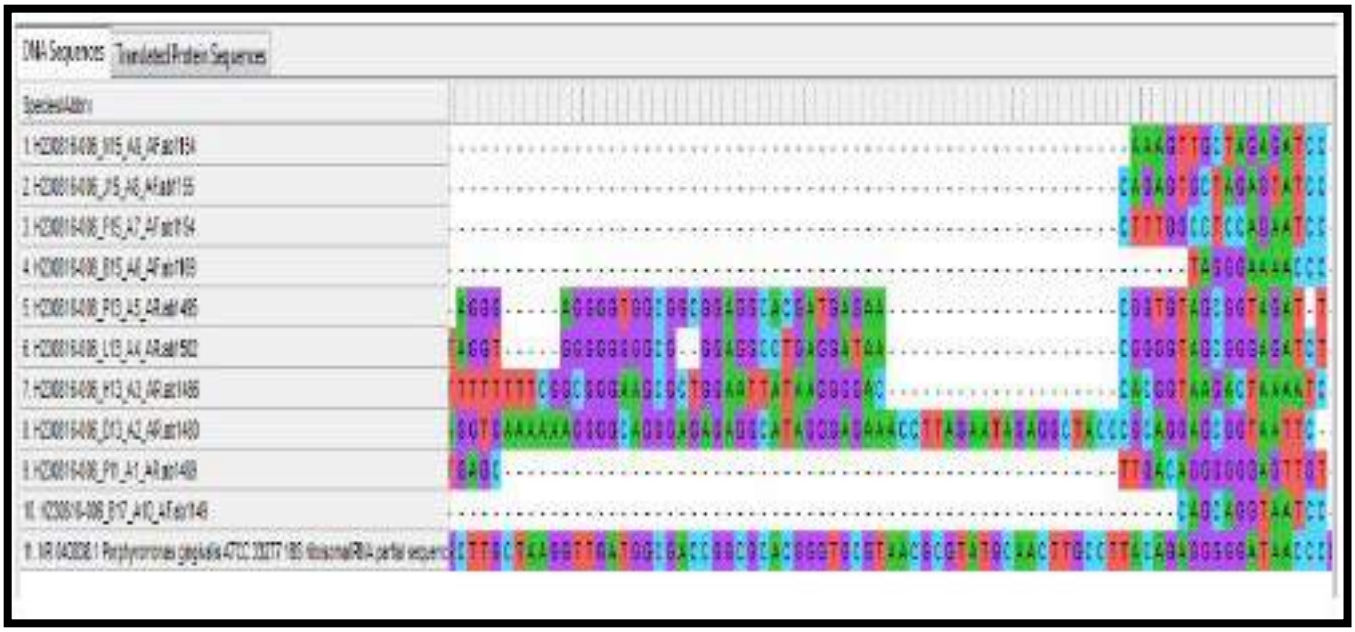


Fig (4-18): Multiple Sequence Alignment Analysis of specific Gene in Local porphyromonas gingivalis IQ Isolates and NCBI-Genbar' porphyromonas gingivalis Country-Related Isolates. The Multiple Alignn. Analysis was Constructed Using (The ClustalW Alignment Tool. Online).

Chapter five

Conclusions & Recommendations

Conclusions:

The findings of this study are as follows:

1- The most important cause of gingivitis is bacterial isolation Porphyromonas gingivalis.

2- The age groups from 31-70 year

3- or more are the most affected by gingivitis and periodontitis.

4- It is discovered that the genes of Fimbriae, particularly the two types, are among the most significant factors that promote the disease of gingivitis and periodontitis, according to the molecular analysis.

Recommendations

1- Exploring the factors affecting cell invasion by other periodontal pathogens (Porphyromonas gingivalis) and the host cell response to these bacterial stimuli would be worthwhile.

2- The bacterial isolate Porphyromonas gingivalis can be isolated from various places in the body, as it causes endocarditis, Alzheimer's disease, and rheumatoid arthritis.

-3 Study of other virulence factors of pathogenic bacterial isolates from the oral cavity of gingivitis patients, lipopolysaccharides, gingipains, hemolysin, and hemagglutinins are the virulence components of P. gingivalis

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Appendixes

Questionnaire form

The question	The answer
Name	
No of sample	
Age	
Sex	
Propping depth	
Drugs	
Clinical diagnosis	
Number of times brush the teeth	
Sample collection site	

Figure (1): A. Inoculation of Paper Points in Thioglycolate Broth After Sample Collection and Incubation B. Control

Figure (2): Anaerobic Bacterial Isolates from periodontal disease Patients

Figure (3): Anaero Gen 2.5 L Gas Pack

Figure (4): Thermocycler PCR Machine

Figure (5): Eppendorf appears in the centrifuge

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:34 CDT

Patient Name:
Location:
Lab ID: 944

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:

Identification Information	Analysis Time:	5.80 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 6201500720550		
ID Analysis Messages				

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	(-)
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	-	22	dMAL	-
28	SAC	+	30	ARB	(-)	33	NAG	+	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	(-)	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:30 CDT

Patient Name:
Location:
Lab ID: 1010

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Collected:

Source:

Comments:

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7737732730750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	(-)	41	AARA	(+)	42	AGALI	(-)	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:30 CDT

Patient Name:
Location:
Lab ID: 1009

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism		Unidentified Organism		
	Bionumber:	7537733830750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	(-)	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUi	+	36	URE	+	37	BGURi	-
39	BGALi	+	41	AARA	+	42	AGALi	-	43	BMAN	+	44	ARG	(-)	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGi	-	56	AMANi	-	57	AIFUC	-
59	PHOS	+	60	lARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:35 CDT

Patient Name:
Location:
Lab ID: 941

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.02 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 2603000620440		
ID Analysis Messages				

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	(+)	62	OPS	-	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:35 CDT

Patient Name:
Location:
Lab ID: 942

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	Bionumber:	**Unidentified Organism** 7717710730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:34 CDT

Patient Name:
Location:
Lab ID: 943

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.78 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617710730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	(-)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	58	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:45 CDT

Patient Name:
Location:
Lab ID: 966

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.78 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3727500630750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	(+)	10	PyrA	+
11	dCEL	-	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	-	33	NAG	+	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	(-)	42	AGALI	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:45 CDT

Patient Name:
Location:
Lab ID: 967

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information:	**Analysis Time:**	5.77 hours	**Status:**	Final
Selected Organism		**Unidentified Organism**		
	Bionumber:	7617710730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	-
39	BGALi	-	41	AARA	(-)	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANi	-	57	AlFUC	(-)
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:37 CDT

Patient Name:
Location:
Lab ID: 940

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.02 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7717710730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	-
39	BGALi	-	41	AARA	(-)	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGi	-	58	AMANi	-	57	AlFUC	-
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 961

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.97 hours	**Status:** Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7407710730550	
ID Analysis Messages			

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	(-)	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 962

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.95 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7603710330570		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	(+)	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	(-)	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	(-)
39	BGALI	(-)	41	AARA	(-)	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	-
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGI	-	56	AMANI	-	57	AlFUC	(-)
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(+)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 963

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.95 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617730730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	(-)
39	BGALI	-	41	AARA	(-)	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	(+)
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGi	-	56	AMANi	(-)	57	AlFUC	(-)
59	PHOS	+	60	lARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	(+)
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 958

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.98 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617712330570		
ID Analysis Messages				

Biochemical Details																
4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	(-)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALi	-	41	AARA	(+)	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	-
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGi	-	56	AMANI	-	57	AIFUC	(-)
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(+)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 959

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.98 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7707710734510		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	(-)	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGI	-	56	AMANI	-	57	AIFUC	+
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	(-)
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 960

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.97 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 3717700630750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	(+)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AlFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	CPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:45 CDT

Patient Name:
Location:
Lab ID: 965

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.93 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7527710730750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	(-)	10	PyrA	+
11	dCEL	(-)	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	58	AMANI	-	57	AlFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:43 CDT

Patient Name:
Location:
Lab ID: 956

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 6403610720510		
ID Analysis Messages				

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	(-)	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	-	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	-	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	-
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 957

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7727712330550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PhsA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	-
39	BGALi	-	41	AARA	+	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	(-)
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGi	-	56	AMANi	-	57	AlFUC	(-)
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:26 CDT

Patient Name:
Location:
Lab ID: 1002

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3607703670550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUi	-	36	URE	-	37	BGURi	-
39	BGALi	+	41	AARA	+	42	AGALi	-	43	BMAN	(-)	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	(+)	55	BNAGi	-	56	AMANi	-	57	AIFUC	(-)
59	PHOS	+	60	IARA	-	61	dRIB2	(+)	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 276

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.82 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3337757772770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	-
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	+
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	(+)	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	(-)	56	AMANI	+	57	AIFUC	-
59	PHOS	+	60	tARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 16, 2023 09:54 CDT

Patient Name:
Location:
Lab ID: 277

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7717717770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 272

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3717753773770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	(+)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	+
39	BGALI	+	41	AARA	+	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	(+)	56	AMANI	(+)	57	AIFUC	(-)
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 273

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism		**Unidentified Organism**		
	Bionumber:	3717717770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	(+)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 274

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.03 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7717737770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	(-)	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGUR	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:25 CDT

Patient Name:
Location:
Lab ID: 1001

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.02 hours	Status:	Final
Selected Organism	Bionumber:	**Unidentified Organism** 7717753732750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUi	+	36	URE	-	37	BGURi	(+)
39	BGALi	+	41	AARA	+	42	AGALi	-	43	BMAN	(+)	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	(-)	55	BNAGi	(-)	56	AMANi	(+)	57	AIFUC	(-)
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:25 CDT

Patient Name:
Location:
Lab ID: 1000

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.03 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7737777570750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	(+)	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	(+)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	(-)	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:27 CDT

Patient Name
Location
Lab ID 1004

Patient ID
Physician
Isolate Number: 1

Organism Quantity
Selected Organism : Unidentified Organism

Source

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.98 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7737713777770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	-
39	BGALi	+	41	AARA	+	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGi	+	56	AMANI	+	57	AIFUC	+
59	PHOS	+	60	lARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer

Microbiology Chart Report

Printed Apr 16, 2023 08:27 CDT

Patient Name:
Location:
Lab ID: 1004

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.98 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7737713777770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	+	56	AMANI	+	57	AIFUC	+
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer

Microbiology Chart Report

Printed Apr 18, 2023 08:27 CDT

Patient Name:
Location:
Lab ID: 1003

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	8.00 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3727713630550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	8	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	-
39	BGAU	+	41	AARA	+	42	AGALI	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANi	-	57	AIFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 271

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.02 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7737777770770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURi	+
39	BGALi	+	41	AARA	+	42	AGALi	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGi	(-)	56	AMANi	(-)	57	AIFUC	-
59	PHOS	+	60	XARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(+)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 271

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.02 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7737777770770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	8	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	+
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	(-)	56	AMANI	(-)	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(+)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 11, 2023 10:36 CDT

Patient Name:
Location:
Lab ID: 939

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

[redacted]	

Identification Information	Analysis Time:	5.78 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 6203100720540		
ID Analysis Messages				

Biochemical Details

4	dGAL	-	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	-
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	+	30	ARB	-	33	NAG	-	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	-	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AlFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	-	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Mar 29, 2023 08:44 CDT

Patient Name:
Location:
Lab ID: 964

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.93 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7737712334570		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	(+)	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	38	URE	-	37	BGURI	-
39	BGALI	-	41	AARA	+	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	(-)
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	(+)
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(+)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:49 CDT

Patient Name:
Location:
Lab ID: 264

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism		**Unidentified Organism**		
	Bionumber:	7737737772752		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGUR	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	+	57	AlFUC	(-)
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:48 CDT

Patient Name:
Location:
Lab ID: 261

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.82 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617732730752		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	(-)	41	AARA	(+)	42	AGALI	-	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	lARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:45 CDT

Patient Name:
Location:
Lab ID: 259

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information			
Analysis Time:	5.80 hours	**Status:**	Final
Selected Organism	**Unidentified Organism**		
Bionumber:	7717737770752		
ID Analysis Messages			

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	(+)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	16	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	(+)	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:52 CDT

Patient Name:
Location:
Lab ID: 268

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.87 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 7717737774772		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALi	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGi	-	56	AMANI	(-)	57	AIFUC	+
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:49 CDT

Patient Name:
Location:
Lab ID: 266

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:

Identification Information			
Selected Organism	**Analysis Time:**	5.85 hours	**Status:** Final
	Bionumber:	**Unidentified Organism** 7757737770752	
ID Analysis Messages			

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	(-)	15	APPA	(+)	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGUR	-
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:49 CDT

Patient Name:
Location:
Lab ID: 265

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:

Identification Information	Analysis Time:	5.85 hours	Status:	Final
Selected Organism		Unidentified Organism		
	Bionumber:	7717737770752		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	(+)	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	+		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:55 CDT

Patient Name:
Location:
Lab ID: 280

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.95 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3737737770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	lARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:52 CDT

Patient Name:
Location:
Lab ID: 270

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.02 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 3617733730750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	(+)	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	(-)	43	BMAN	+	44	ARG	+	45	PVATE	(+)
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:52 CDT

Patient Name:
Location:
Lab ID: 269

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.03 hours	Status:	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 3603523670150		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	(+)
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	+	30	ARB	-	33	NAG	+	34	BGLUi	-	36	URE	+	37	BGURi	-
39	BGALi	+	41	AARA	+	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGi	-	56	AMANi	-	57	AIFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	-	62	OPS	+	63	AARAf	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:57 CDT

Patient Name:
Location:
Lab ID: 283

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.92 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3737737770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURi	-
39	BGALi	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 15, 2023 09:56 CDT

Patient Name:
Location:
Lab ID: 282

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7737737770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	46	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	(-)	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:55 CDT

Patient Name:
Location:
Lab ID: 281

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.93 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617737730770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUi	+	36	URE	+	37	BGURi	-
39	BGALi	+	41	AARA	(+)	42	AGALi	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AIFUC	-
59	PHOS	+	60	lARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:54 CDT

Patient Name:
Location:
Lab ID: 278

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7617730730750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	(+)	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	-
39	BGALI	-	41	AARA	(-)	42	AGALI	(-)	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AlFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Jun 18, 2023 09:55 CDT

Patient Name:
Location:
Lab ID: 279

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.95 hours	Status:	Final
Selected Organism		Unidentified Organism		
	Bionumber:	3617717770750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURI	(-)
39	BGALI	+	41	AARA	+	42	AGALI	+	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer

Microbiology Chart Report

Printed Jun 18, 2023 09:53 CDT

Patient Name:
Location:
Lab ID: 275

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.82 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3337757772770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	-
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	-	37	BGURi	+
39	BGALi	+	41	AARA	+	42	AGALi	(+)	43	BMAN	+	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	+	55	BNAGi	-	56	AMANi	(+)	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 18, 2023 08:28 CDT

Patient Name:
Location:
Lab ID: 1006

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	6.03 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7737731730750		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	+	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	+	37	BGURI	(-)
39	BGALI	(+)	41	AARA	(-)	42	AGALI	-	43	BMAN	(+)	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	(-)	56	AMANI	(-)	57	AIFUC	-
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:28 CDT

Patient Name:
Location:
Lab ID: 1005

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.98 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 7727507754770		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	(+)	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	-	33	NAG	+	34	BGLUI	-	36	URE	-	37	BGURI	-
39	BGALI	+	41	AARA	+	42	AGALI	(+)	43	BMAN	+	44	ARG	(+)	45	PVATE	+
51	MTE	+	53	ESC	(-)	54	BdFUC	+	55	BNAGI	-	56	AMANI	(-)	57	AIFUC	+
59	PHOS	+	60	IARA	+	61	dRIB2	+	62	OPS	+	63	AARAF	+	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:30 CDT

Patient Name:
Location:
Lab ID: 1011

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source:

Collected:

Comments:	

Identification Information	Analysis Time:	5.80 hours	Status:	Final
Selected Organism	Bionumber:	Unidentified Organism 3703500630450		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	-	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	(-)	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	-
28	SAC	+	30	ARB	-	33	NAG	+	34	BGLUi	-	36	URE	-	37	BGURi	-
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGi	-	56	AMANI	-	57	AIFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	-	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

bioMérieux Customer:

Microbiology Chart Report

Printed Apr 16, 2023 08:29 CDT

Patient Name:
Location:
Lab ID: 1007

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Collected:

Source:

Comments:	

Identification Information	Analysis Time:	6.02 hours	Status:	Final
Selected Organism	Biorumber:	Unidentified Organism 7727772730550		
ID Analysis Messages				

Biochemical Details

4	dGAL	+	5	LeuA	+	6	ELLM	+	7	PheA	+	8	ProA	+	10	PyrA	+
11	dCEL	-	13	TyrA	+	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUI	+	36	URE	(+)	37	BGURI	(+)
39	BGALI	-	41	AARA	(+)	42	AGALI	-	43	BMAN	(+)	44	ARG	+	45	PVATE	+
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGI	-	56	AMANI	-	57	AIFUC	-
59	PHOS	+	60	IARA	-	61	dRIB2	+	62	OPS	+	63	AARAF	(-)	64	dXYL	+
	GRAM	-		MORPH	?		AERO	-									

خلاصة

Porphyromonas gingivalis وهي بكتيريا سالبة لصبغة غرام، لا هوائية إجبارية، وغير متحركة، وغير مكونة للأبواغ، يمكن أن تسبب عدوى محلية في أنسجة اللثة حول السن تجري الأنواع البكتيرية التابعة للاتحاد انسجاما منتظمًا مع بعضها البعض؛ مكونين المعقد الأحمر. تهدف هذه الدراسة إلى عزل وتشخيص البكتيريا المسببة للأمراض اللاهوائية Porphyromonas gingivalis من تجويف الفم لبعض مرضى التهاب اللثة وأمراض اللثة في مركز محافظة ميسان للفئات العمرية ما بين 9-70 سنة من المرضى المترددين على مركز طب الأسنان التخصصي، مستشفى الطفل التعليمي. ومركز دجلة في مدينة العمارة جنوب وسط العراق. تم ذلك باستخدام الاختبارات التقليدية وجهاز Vietk 2 والكيمياء الحيوية، ومن ثم تم تحديدها بالطرق الجزيئية عن طريق الجين التشخيصي S16 rRNAبواسطة التفاعل المتسلسل (PCR) والبادئات المحددة المستخدمة في هذه الدراسة لتشخيص عزلات بكتيريا Porphyromonas gingivalis و تم عمل الشجرة التطورية لعزلات البكتيريا المدروسة بالاعتماد على بنك الجينات.NCBI

تم جمع العينات في الفترة من 21 نوفمبر إلى 6 فبراير 2023. تم جمع ما مجموعه 100 عينة من البكتيريا اللاهوائية المعزولة تعود إلى 50 مريضاً يعانون من التهاب اللثة وأمراض اللثة في مركز محافظة ميسان لسبع فئات عمرية تتراوح بين ≥ 10-70 سنة. (≤ 10، 11-20، 21-30، 31-40، 41 -50، 51-60، 61- 70 سنة). منها 43 أنثى (43%) وذكور (57%) وكانت أعلى نسبة عزل 100% للذكور في الفئتين العمريتين (21-30) و(31-40) للتعرف على العزلات البكتيرية المسببة لالتهاب اللثة وأمراض اللثة. أشارت الدراسة الحالية إلى أن العزلات البكتيرية المسببة للأمراض Porphyromonas gingivslis كانت الأكثر شيوعاً.

الضراوة هي قدرة الكائن الحي على إصابة المضيف والتسبب في المرض. يعمل غشاء البلازما Porphyromonas gingivalis كواجهة ديناميكية بين مسببات الأمراض الفموية والمناطق المحيطة بها. تشتمل عوامل الضراوة على outer membrane vesicles (OMVs), lipopolysaccharides (LPS), gingipains, hemolysins, and hemagglutinins.

Fimbriae، وهي هياكل خيطية صغيرة موجودة على سطح معظم سلالات P. gingivalis تتكاثر خارج الغشاء الخارجي وتساعد في تكوين الأغشية الحيوية، وارتباط البكتيريا بالخلايا المضيفة، والغزو.

وأخيرا حساسية المضادات الحيوية لبكتيريا Porphyromonas gingivalis التي تم عزلها من بعض المرضى في مدينة ميسان. هذه تعزل سلالات البورفيروموناس. اللثة، تم اختبارها ضد بعض المضادات الحيوية مثل سيفوكسيتين (فوكس 30 ميكروجرام)، دوكسيسيكلين (DO 30 ميكروجرام)، سيبروفلوكساسين (CIP 30 ميكروجرام)، حمض الناليديكسيك (NA 30 ميكروجرام). أظهرت سلالات Porphyromonas gingivalis مقاومة إجمالية (100%) لجميع الأجسام المضادة المستخدمة في التجربة.

في هذه الدراسة تم تشخيص جين الضراوة. جينعلى Fim A نوعين (234 bp) و(294 bp) باستعمال Specific Primers لجين الضراوة للعزلة الجرثومية Porphyromonas gingivalis تظهر بواسطة الترحيل الكهربائي ان حجم الجين (234bp-294pb). وباستعمال universal primers جين Fim A على Porphyromonas gingivalis من خلال تفاعل البلمرة المتسلسل (PCR)وجينFim A Porphyromonas gingivalis .لم تظهر اي حزم خلال الترحيل الكهربائي الهلامي للبادئات العالمية في منطقة الجينات على Porphyromonas gingivalis من خلال تفاعل البوليميراز المتسلسل.(PCR)

أخيرًا شجرة النشوء والتطور (Mega) ذات طول الفرع الحقيقي المؤدي إلى P. gingivalis (المميز بالمستطيل الرماديالشجرة ذات طول الفرع المؤدي إلى p.gingivalis اقصر 100 مرة (الخط المتقطع) لإظهار العلاقات التفصيلية بين عزلات و سلالات Porphyromonas gingivalis. تشير الأسماء المرتبطة للسلالات والعزلات إلى هوية تسلسلها بنسبة 100 %. تظهر شجرة النشوء والتطور المجاورة لسلالة P. gingivalis(يتم التعبير عنها كنسبة مئوية من 1000 نسخة متماثلة)> 65 عند النقاط الفرعية 16 Sr RNA.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

دراسة جزيئية لبعض عوامل الضراوة للعزلة Porphyromonas gingivalis المعزولة من بعض مرضى التهاب اللثة في مدينة ميسان/العراق

رسالة مقدمة إلى

مجلس كلية العلوم / جامعة ميسان

كجزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

زهراء فرقد فاروق

بكالوريوس علوم الحياة / جامعة ميسان

(٢٠٢٠)

بإشراف

أ. م. د. محمد عباس عبد علي جبار

أ.د. سامي خلف

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