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Department of Biology

Molecular Study of Antibiotic Resistance Pattern of *Actinomyces* spp. Isolated from Root Canal Dental Diseases Patients in Misan Governorate Center

A Thesis

Submitted to the College of Science, University of Misan, in Partial Fulfillment of the Requirements for M.Sc. Degree in Biology

By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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Dedication

Designer of the human civilization which was established on the Unity of Almighty Allah...

To the redeemer of human volition and thought...

To the Seal of the Prophets and the Master of all beings; the Holy Prophet Mohammed peace be upon him and his Household...

I dedicate this survey on the Seal of his Successors, the Reviver of his Faith, and the resuscitator of his Mission: **Imam al-Mahdi**, peace be upon him who shall fill the earth with justice and righteousness after it will be filled with injustice and prejudice. I hope that this humble work will meet your satisfaction.

Nahida

Abstract

The present study designed to isolate and identify pathogenic *Actinomyces* spp. from the oral cavities of patients with root canal infections at the Misan Governorate Center, specifically targeting the age group between 13 and 75 years who visited the Specialized Dental Center and Dental Clinics in Al-Amara City, southern Iraq. All specimens were identified by traditional methods (Gram stain, biochemical tests, and the Vitek 2 compact system) and molecular methods, including PCR technology using universal and specific primers, and DNA gene sequencing based on the NCBI gene bank.

Samples were collected from November 25, 2024, to March 5, 2025. A total of 50 samples were collected from 50 patients suffering from diseases resulting from tooth root infection in Misan Governorate Center for seven age groups ranging from 13-75 (Less than 20, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79) including 26 females (52%) and 24 males (48%). The highest percentage of isolation was in the age groups (30-39) and (50-59).

Finally, the antibiotic resistance of these isolates were studied. The resistance of these isolates to the following antibiotics were tested using the Kirby-Bauer method: Amoxyclav, Ampicillin, Cephalexin, Azithromycin, Erythromycin, Clindamycin, Doxycycline, Tetracycline, Vancomycin, Cefoxitin, Levofloxacin, and Imipenem. The results showed that two isolates are multi-resistant to antibiotics (MDR), as it exhibited resistance to each of the following antibiotics: Amoxyclav, Ampicillin, Erythromycin, Clindamycin, Cephalexin, Cefoxitin, Imipenem, Vancomycin, and Azithromycin.

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List of Abbreviation

Abbreviation	Key
µl	Microliter
%	Percent
16S r DNA	16S ribosomal Deoxyribonucleic Acid
ANC ID Kit	Anaerobe Card Identification Kit
AMC	Amoxyclav
AMP	Ampicillin
AZM	Azithromycin
Bp	Base Pair
˚C	Degree Celsius
CD	Clindamycin
CL	Cephalexin
CX	Cefoxitin
DO	Doxycycline
DNA	Deoxyribonucleic Acid
E	Erythromycin
F	Forward
G	Gram
IMP	Imipenem

LEV	Levofloxacin
L	Liter
MI	Milliliter
MDR	Multi-drug resistance
Mcg/disc	Microgram per disc
NCBI	National Center for Biotechnology Information
PH	Power of Hydrogen
PCR	Polymerase Chain Reaction
R	Reverse
Sp	Species
TBE	Buffer-10x (Tris borate EDTA)
TE	Tetracycline
VA	Vancomycin
+Ve	Positive

CHAPTER ONE

Introduction

1.1 Introduction

The oral cavity harbors an exceptionally varied microbial population, largely because of its role in consuming and breaking down food. It is distinguished from other areas of the body by the presence of specialized mucosal surfaces, teeth, saliva, and gingival crevicular fluid. (Sobieszczanski *et al*., 2023; Marsh & Martin, 2009)

The relationship between the mouth microflora and the host in a state of dynamic equilibrium. As a result of changes in oral biology resulting from exogenous sources, e.g., courses of antibiotics, ingestion of fermentable carbohydrates, or endogenous sources, e.g., changes in the integrity of host defenses after drug therapy, as well as tooth extraction and other trauma, this relationship can be disturbed, and diseases can occur. (Santacroce *et al*., 2023; Li *et al*., 2022)

Periodontitis, alveolar bone loss, endodontic infection, and tonsillitis are oral infectious diseases caused by the oral microflora. It has been estimated that more than 700 microorganisms species inhabit the human mouth. The oral cavity microflora includes two main types of bacteria: Gram-positive and Gram-negative. (Sudhakara *et al*., 2018; Freire *et al*., 2021)

Actinomyces species are found in the mouth as part of the oral cavity microflora, they able attach on oral tissues, making them resistant to cleansing processes such as salivary flow, classified as Gram-positive, facultative anaerobic, non-spore forming, and filamentous organisms. (Dioguardi *et al*., 2020; Steiniger & Willinger, 2016) The genus *Actinomyces* is a primary colonizer involved in biofilm formation, along

with *Streptococcus*, and linked to a broad spectrum of diseases caused by microbes living in biofilms, include dental caries and periodontal disease. As part of a polymicrobial infection, *Actinomyces* is commonly found in dental pulp infections. These bacteria are also involved in extra-radicular infections. (Könönen & wade, 2015; Sarkonen, 2007)

In contrast to the oral cavity, the root canal system is devoid of microflora. Once microorganisms enter the root canal system (due to mechanical or thermal trauma), harmful effects occur, ranging from reversible pulpitis to pulp tissue necrosis, which eventually leads to the formation of the apical periodontitis and periapical lesion. (Persoon & Ozok, 2017)

When microbes invade the root canal system, they can trigger apical periodontitis, an inflammatory condition affecting tissues around the tooth root. (Siqueira *et al*., 2024) This disease progresses through acute and chronic phases. During the acute stage, neutrophils predominantly infiltrate the area and bone begins breaking down; patients typically experience a persistent, pulsating discomfort that worsens when biting or tapping the tooth. As the condition transitions to its chronic phase, these neutrophils give way to other inflammatory cells. Pain generally diminishes during this later stage, though sudden flare-ups can still happen. (Zajac *et al*., 2021)

1.2 Aims of the Study

1. The isolation and identification of *Actinomyces* spp. by some biochemical tests, Vitek 2 compact system.
2. Detection of *Actinomyces* spp. by specific primer sequence by PCR and 16S rDNA sequencing.
3. Study of antibiotic resistance patterns of *Actinomyces* spp isolated from the root canal system.

CHAPTER TWO

Literature Review

2.1 Literatures Review

2.1.1 Microbial ecology of the oral cavity

The oral cavity is a heterogeneous environment for microbial colonization. It is characterized by the presence of mucosal surfaces (such as lips, cheeks, palate, and tongue) and teeth, which due to their biological properties, support the growth of distinct microbial community. (Fine & Schreiner, 2023; Akimbekov *et al*., 2022). Saliva profoundly influences the oral environment. Its PH, which ranges between 6.75 and 7.25, enables many microorganisms to grow, and its ionic composition also stimulates its properties to buffering and ability to remineralize tooth enamel (Oktanauli *et al*., 2023; Gozali & Sianita, 2022). Saliva's main organic constituents, proteins and glycoproteins including amylase, mucin, immunoglobulin (predominantly sIgA), lactoferrin, lysozyme, and sialoperoxidase (Shinde *et al*., 2024); influence oral microbial communities through:1) Adsorption on oral surfaces, especially teeth, thus forming a layer that enables microorganisms to adhere to it, 2) Provides essential nutrients (carbohydrates and proteins) to the resident oral microflora without causing harmful PH drops, 3) They mask bacterial antigens by binding to the surface of the bacteria, making them look like the host, 4) They act as components of host defenses and thus prevent the adhesion and growth of certain exogenous microorganisms, 5) Clumping microbes together, which allows them to be cleared from the mouth through swallowing (Batabyal *et al*., 2012) .

During an individual's lifetime, the characteristics of some key oral habitats change. In the earliest months after birth, for instance, the oral cavity contains mostly mucosal tissues that support microbial establishment. As children age, teeth emerge as hard, permanent surfaces, creating a distinct environment within the body where microbes can settle and persist. Tooth eruption also provides another habitat for microbial colonization, the gingival crevice, and an additional source of nutrients (gingival crevice fluid). (Ali & Tanwir, 2012)

Teeth provide a unique surface that allows large microorganisms population (dental plaque) to build up in the form of biofilms. (Abebe, 2021)

In addition to geographical differences, the structure of the oral microflora among individuals is affected by age, gender, diet, and disease. (Tuominen & Rautava, 2021)

2.1.2 Normal population of the oral cavity

More than seven hundred species of microorganisms, such as, bacteria, viruses, and fungi, inhabit human mouth. The types, as well as abundance of these microorganisms vary depending on age of person, diet, and individual hygiene. (Alghamdi, 2022)

In healthy conditions, oral microorganisms and their host maintain a balanced relationship that serves an important protective function-blocking external bacteria from establishing themselves and potentially affecting the body's overall health. Oral diseases, such as tooth decay, gum disease, and periodontal disease, are caused by disruptions in this relationship, which can be triggered by smoking and the consumption of

foods containing various chemicals, thereby affecting the oral microorganism composition. Therefore, understanding oral microorganism is important for understanding the mechanisms of oral diseases.(Miranda *et al*., 2025)

In the oral cavity, bacteria constitute the bulk of the microbial communities. They are mainly composed of: *Staphylococcus* spp., *Streptococcus* spp., *Actinomyces* spp., *Veillonella* spp., *Fusobacterium* spp. , *Porphromonas* spp., *Prevotella* spp., *Treponema* spp., *Nisseria* spp., *Haemophilus* spp., *Eubacteria* spp., *Lactobacterium* spp., *Capnocytophaga* spp., *Eikenella* spp., *Leptotrichia* spp., *Peptostreptococcus* spp., and *Propionibacterium* spp. (Kitamoto *et al*., 2020; Avila *et al*., 2009)

In healthy individuals, oral fungi are the most complex and diverse group of fungi . *Candida* species constitute the major component of the oral fungal community, although many other species exist. However, oral fungi are not fully understood. (Defta *et al*., 2024) *Candida* numbers increase more in people undergoing chemotherapy, antibiotics, or corticosteroids , as well as in people with diabetes and HIV. (Quindós *et al*., 2019)

The oral cavity roughly 6 billion bacteria alongside possibly 35 times more viruses. Bacteriophages make up most of these viral populations and likely help shape how bacterial communities develop and interact within the oral environment. (Thakkar *et al*., 2022; Edlund *et al*., 2015)

Other members include protozoa parasites, of which studies have revealed two types *Trichomonas tenax* and *Entamoeba gingivalis*. (Gharavi *et al*., 2006)

2.1.3 Root canal system

The pulp consists of soft connective tissue situated at the tooth's core and is connected to the periodontal membrane through a small apical foramen, through which it receives its nerve and vascular supply. It is frequently termed the pulp cavity or root canal system. The root canal system consists of two parts: the coronal part; called pulp chamber, and the radicular part; called root canal, both of which are surrounded by coronal and radicular dentin, as in [Figure 2-1]. (Ahmed *et al*., 2024; Darling, 1959)

In contrast to other parts of the oral cavity, the root canal system does not contain microflora; therefore, bacteria inhabiting infected root canals act as opportunistic pathogens, functioning either as harmless commensals or disease-causing agents. (Teles *et al*., 2013)

Due to several factors, such as caries, cracks and fractures, microorganisms and their toxins can easily reach the pulp chamber, thus triggering an inflammatory response of the pulp that eventually leads to necrosis of pulp. The inflammation can spread to alveolar bone surrounding the tooth, causing periapical disease. Oral sepsis is one of the most serious consequences of pulpitis and may pose a hazard to life. Consequently, infections spreading from maxillary teeth may result in meningitis, purulent sinusitis, orbital cellulitis, brain abscess, and cavernous sinus thrombosis. In contrast, the spread of infection from the

mandibular teeth can lead to mediastinitis, parapharyngeal abscess, pericarditis, emphysema, and jugular thrombophlebitis. (Yu & Abbott, 2007)

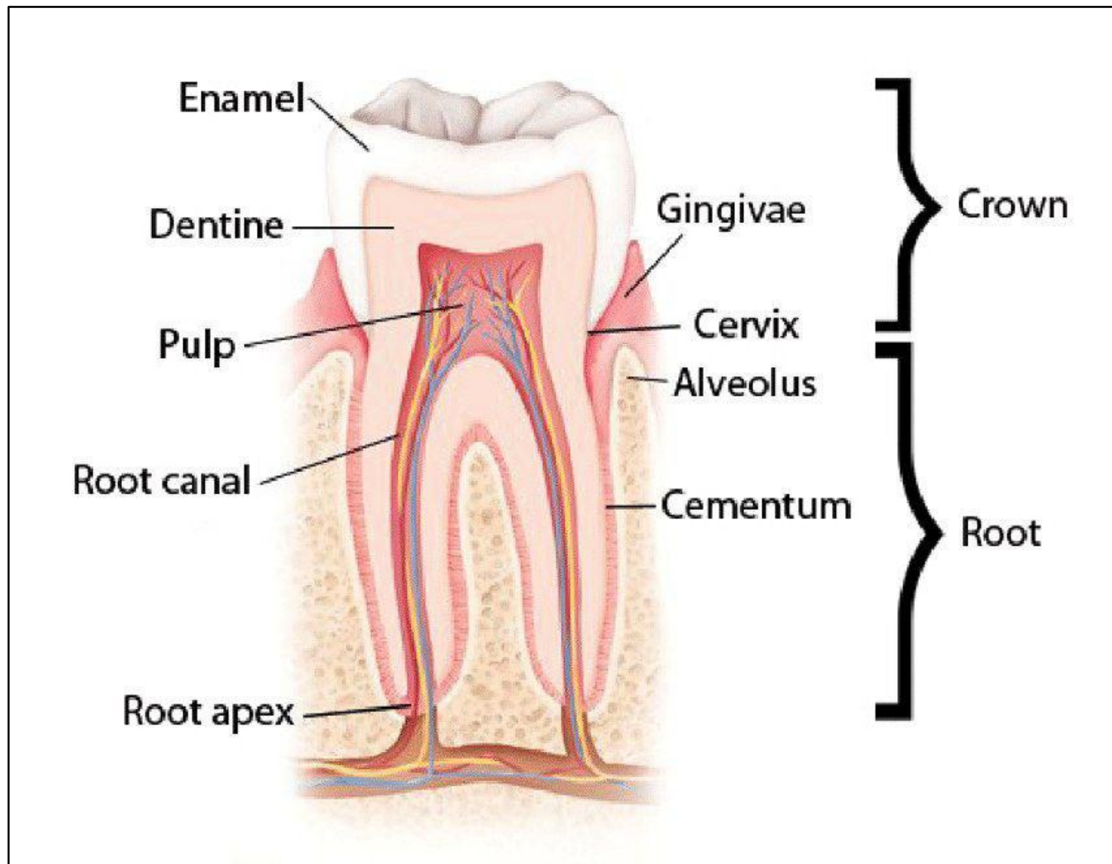


Figure (2-1): Diagram showing components of the root canal system. (Lemmers, 2017)

2.1.4 *Actinomyces* spp.

Actinomyces spp, Gram-positive, Microaerophilic-facultative anaerobe, slow-growing, saprophytic, filamentous, branching bacilli, nonspore forming, non-acid fast, found in the mouth, pharynx, gastrointestinal tract, and female genitourinary tract as components of the normal commensal bacteria in the human. (McHugh *et al*., 2017; Kaya, 2011)

Actinomyces, in the oral cavity, constitute a large proportion of the native oral microbiota and an essential group involved in early colonization for dental plaque formation. In general, the genus *Actinomyces* is associated with root caries, periodontal disease (particularly in inactive sites), and endodontic infections. The role of individual *Actinomyces* species in the root caries process remains unclear, although *Actinomyces naeslundii, A. gerencseriae* were the most isolated species. (Sarkonen, 2007; Eshraghi, 2006)

Actinomyces act as opportunistic pathogens causing actinomycosis, a slowly progressive, indolent granulomatous disease affecting all organs and tissues. Actinomycosis classified as cervicofacial, abdominopelvic, and thoracic. *A. gerencseriae* and others, are the most common species causing this disease. Researchers have suggested that *Actinomyces* invade compromised or dead tissue as their pathogenic strategy for penetrating and multiplying within deeper anatomical structures. *Actinomyces* are associated with systemic diseases, including central nervous system, cardiovascular system, and gastrointestinal tract. (Li *et al*., 2018)

Traditionally, anaerobic Gram-positive bacilli from the *Actinomyces* genus have been identified through phenotypic testing-methods detecting enzymes like oxidase, urease, catalase, along with sugar fermentation patterns, indole production, and commercial biochemical kits including the ANC identification card. These approaches frequently cause misidentification at species and strain levels, though, and the ANC card only covers seven *Actinomyces* species. Modern identification now relies on molecular diagnostic techniques: 16S rRNA sequencing, polymerase chain reaction (PCR), and16S ribosomal DNA restriction analysis with targeted primer. MALDI-TOF MS has appeared as another quick and precise way for identifying anaerobic bacteria like *Actinomyces*, providing reliable genus-level identification rapidly. (Gajdács & Urbán, 2020; Wolff *et al*., 2022)

2.1.4.1 Taxonomy of *Actinomyces* spp.

In bacterial taxonomy, domain represents the top-level classification, with subsequent ranks including phylum, class, subclass, order, suborder, genus, and species. Members within families exhibit phenotypic traits that remain evolutionarily stable. *Actinomyces* falls within the Actinomycetaceae family, alongside other genera like Actinobaculum, Mobiluncus, Varibaculum, and Arcanobacterium, as in [Table 2-1]. (Sarkonen, 2007)

Table (2-1): Taxonomic ranks (Barka *et al*., 2016)

Taxonomic ranks	Example
Domain	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales
Suborder	Actinomycineae
Family	Actinomycetaceae
Genus	*Actinomyces*
Species	*Actinomyces odontolyticus*

2.1.5 Root canal dental diseases

2.1.5.1 Pulpitis

It is an inflammation of the tooth pulp. Besides caries, which is the most common cause of pulpitis, the inflammation also result from thermal or chemical irritation or mechanical damage to the tooth. (Brook, 2004) Pulpitis is divided into two types: reversible and irreversible. In reversible pulpitis, the tooth is more sensitive to harmful stimuli such as food heat, and heals very slowly. The individual experiences localized pain and can identify the affected tooth. If left untreated, reversible pulpitis progresses to irreversible pulpitis, in which the individual experiences severe, persistent pain that is not localized, which eventually leads to pulp necrosis, a terminal disease. (Pohl *et al*., 2024; Ali & Mulay, 2015)

2.1.5.2 Pulp necrosis

There are several reasons for the loss of vitality of the soft pulp in a tooth, including trauma or severe bacterial infection. This condition is called pulp necrosis. Irritating pain in the tooth or surrounding area is the first sign of pulp necrosis. (Abdulwahab *et al*., 2021) Depending on the extent of the damage, the pain can range from minor to serious, with swelling and discomfort when chewing. Affected teeth exhibit a persistent discoloration ranging from pink to gray without returning to their standard color, as in [Figure 2-2]. (Zajac *et al*., 2021)

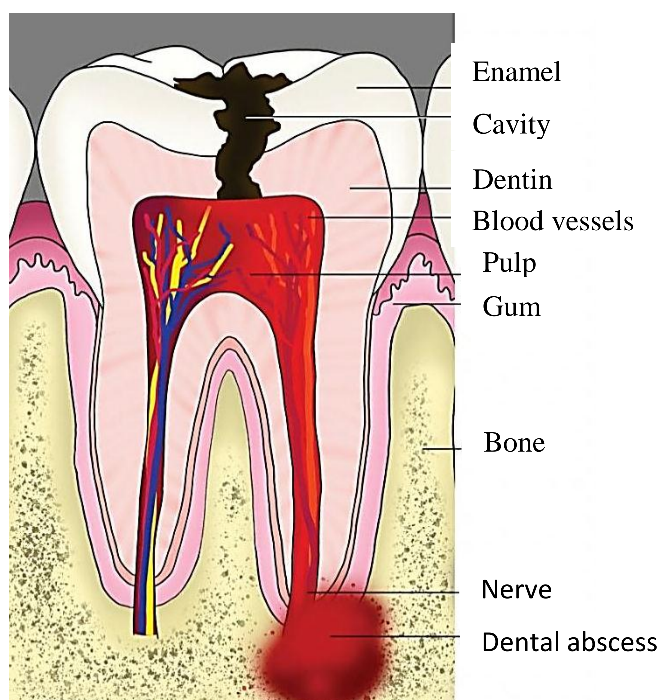


Figure (2-2): Bacterial invasion to the root canal system and the occurrence of a dental abscess. (StatPearls, 2024)

2.1.5.3 Periapical diseases

In reaction to appearance of microorganisms and irritants inside system of root canal, an inflammatory process occurs around the apex of the tooth, also referred to as apical periodontitis. (Abbott, 2004 a)

Classification of periapical diseases

- Apical periodontitis.
- Periapical abscess.
- Radicular cysts. (WHO, 1995).

2.1.5.3.1 Apical periodontitis

Apical periodontitis is an inflammatory disease affecting the periapical tissues and present in acute or chronic forms, with the potential to progress into a periapical abscess. Acute apical periodontitis is commonly caused by bacterial infection and result in the formation of an acute periapical or alveolar abscess, although it can also arise from chemical or physical trauma. Clinically, the affected tooth appear slightly elevated in its socket due to inflammatory edema within the periodontal ligament, and patients often experience severe pain upon biting or palpation. Histopathological, features include vascular dilation and infiltration of polymorphonuclear leukocytes. Chronic apical periodontitis, also referred to as a periapical granuloma, typically develops as a consequence of untreated acute apical periodontitis and asymptomatic or associated with mild discomfort during mastication or percussion, accompanied by a sensation of slight tooth elongation. A periapical abscess develop from acute or, more frequently, chronic apical periodontitis and is characterized by localized pus accumulation in the periapical region. Acute abscesses present with tenderness to pressure, progressive pain, tooth extrusion, possible tissue swelling, and associated with systemic manifestations such as fever and lymphadenitis, whereas chronic abscesses usually exhibit minimal clinical features due to limited suppuration and a low tendency to spread to surrounding tissues (Sivapathasundharam, 2016).

2.1.5.3.2 Periapical abscess

A periapical abscess arises from acute periodontitis or, more commonly, from chronic periodontitis. It is a localized accumulation of pus in the area surrounding a tooth and acute or chronic. When a chronic periapical lesion suddenly flares up, it's called a phoenix abscess. An acute abscess is characterized in its early stages by tenderness of the tooth, which is often susceptible to pressure. Over time, the tooth becomes extremely painful and protrudes slightly from its socket. There tissue swelling. These symptoms may be correlate with severe systemic manifestations, like fever and lymphadenitis. A chronic abscess, on the other hand, presents with no clinical features because it is essentially an area of mild suppuration with little tendency to spread from the surrounding area. (Burczyńska *et al*., 2017)

2.1.5.3.3 Radicular cysts

A radicular cyst develops as an inflammatory lesion from epithelial tissue remnants in the periodontal ligament and ranks among the most frequently occurring jaw lesions. These cysts appear at tooth apices or laterally along the tooth surface where lateral root canals exist. They cause bone breakdown and can grow substantially large, becoming problematic once infection sets in. Two distinct types exist: true radicular cysts, where epithelial lining completely encloses a cavity, and apical radicular cysts-epithelially lined cavities that remain connected to root canals, now termed periapical pocket cysts. True apical cysts constitute more than half of all cystic pathologies, whereas apical pocket cysts represent the residual cases, as in [Figure 2-3]. (Nainani & Sidhu, 2014)

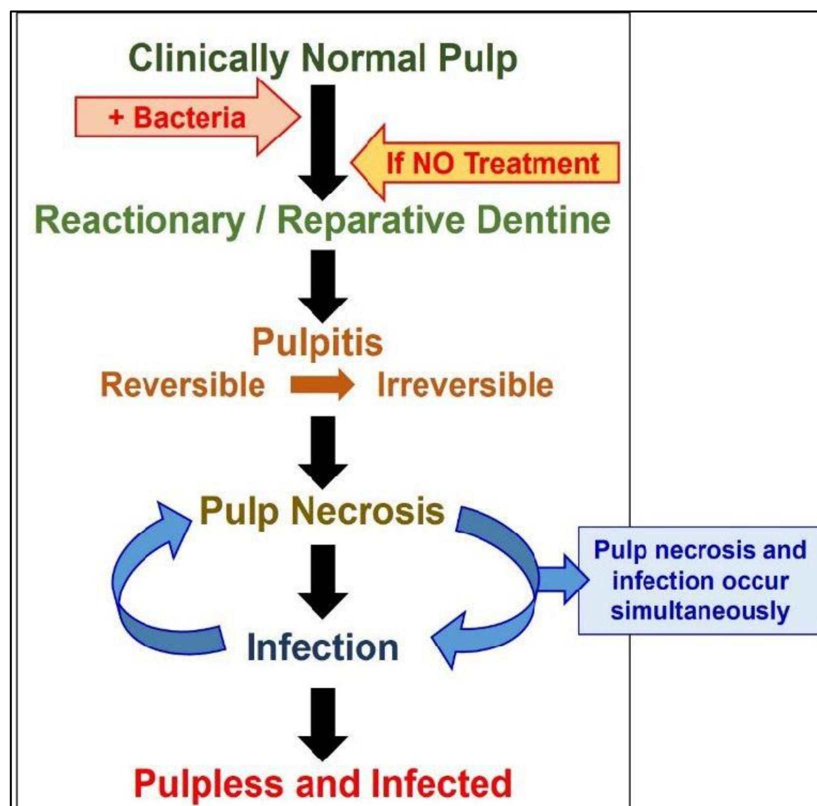


Figure (2-3): Diagram the progression of disease within the root canal system and pulp. (Abbott, 2024 b)

2.1.6 Antimicrobial resistance

Antimicrobial resistance, particularly to bacterial antimicrobials, represents a global health concern. The antibiotic resistance concept refers to the ability of microorganisms, such as bacteria, to develop resistance to the action of antimicrobials over time, leading to the appearance of drug-resistant pathogens. In 2021, it was estimated 4.71 million mortalities worldwide were related with resistance to antimicrobials. According to these estimates, by 2050, resistance to antimicrobials kill 10 million person every year if adequate measures are not taken to reduce it. (Ho *et al*., 2025; Naghavi *et al*., 2024)

The World Health Organization has placed antimicrobial resistance within the top 10 worldwide health challenges (WHO, 2019). Multiple factors drive antimicrobial resistance, particularly excessive antimicrobial use, improper prescription practices, and misapplication for wrong treatment purposes. (Tang *et al*., 2023)

CHAPTER THREE

Materials
&
Methods

3.1 Materials

3.1.1 Equipment and Materials

Table (3-1): Instruments and Equipment in the current study.

No.	Equipment	Supplier	Origin
1.	Autoclave	Hiarayam	Japan
2.	Anaerobic Jar	Oxoid	England
3.	Burner	Himedia	India
4.	Conical flask	General	USA
5.	Cylinder	HBG	England
6.	Cotton swap	Al-Rawbi	China
7.	Disposable petri dishes	Al-Hani	USA
8.	Disposable face masks	Dr. Moxa	USA
9.	Electrophoresis system	Cleaver	England
10.	Eppendorf centrifuge	Eppendorf	Germany
11.	Eppendrof tubes	Apogent	USA
12.	Ethanol 70%	Tiebah	Iraq
13.	Forceps	Himedia	India
14.	Gloves	Broche	Malaysia
15.	Hood	Daihan Labtech	Korea
16.	Incubator	Fisher Scientific	USA

17.	Light Microscope	Olympus	Philippine
18.	Magnetic stirre hot plate	Heidolph	Germany
19.	Mini vortex	Apogent	USA
20.	Micropipettes	Dragon	China
21.	Microscopic slides	Superestar	India
22.	Microscopic cover slides	Citogias	China
23.	McFarland meter	BioMerieux	France
24.	Oven	Memmet	Germany
25.	PCR thermal cycler	Biometra	Germany
26.	Plain tubes	Afco	Jordan
27.	Plastic ruck	Meheco	China
28.	Paper point	Tribest	China
29.	Para film	Jenway	Germany
30.	Sensitive balance	Denver	Germany
31.	Standard wire loop	John Botten	England
32.	Screw cap vials	Pyrex	England
33.	Test tube	ALS	Canada
34.	Tips	Plasti Lab	Lebanon
35.	UV light transilluminator	Cleaver	England
36.	Vitek 2 Compact System	BioMerieux	France
37.	Water bath	Memmet	Germany

3.1.2 Culture media and Chemicals

3.1.2.1 Culture media

Table (3-2): Culture media that used in Lab.

No.	Media	Supplier	Origin
1.	Actinomyces agar	Himedia	India
2.	Blood agar base	Himedia	India
3.	Muller-Hinton agar	Himedia	India
4.	Nutrient agar	Himedia	India
5.	Nutrient broth	Himedia	India
6.	Potato dextrose agar	Himedia	India
7.	Thioglycollate broth	Himedia	India
8.	Tryptone water	Himedia	India

3.1.2.2 Chemicals

Table (3-3): All chemicals used in the study.

No.	Chemicals	Supplier	Origin
1.	AnaeroGen™ 3.5 L Gas back	Oxoid LTD	England
2.	Absolute ethanol	Scharlan	Spain
3.	Agarose	Promega	USA
4.	Catalase test	Himedia	India

5.	DNA marker (100-1000 bp)	Takara	China
6.	Kovacs' reagent	Lobachemie	India
7.	Mini g DNA Bacteria kit	Geneaid	Korea
8.	Master mix	Bioneer	Korea
9.	Nuclease free water	Bioneer	Korea
10.	Oxidase test	Himedia	India
11.	TBE buffer 5x	Promega	USA
12.	Vitek 2 ANC kit	BioMerieux	France

3.1.3 Stains

Table (3-4): Stains used in the study.

No.	Stains	Supplier	Origin
1.	Ethedium bromide	Promega	USA
2.	Gram stain	Titan Biotech LTD	India

3.1.4 Antibiotic discs

Table (3-5): All antibiotic discs used in the study.

No.	Antibiotic	Symbol	Concentration	Origin
1.	Amoxyclav	AMC	30 mcg/disc	India
2.	Ampicillin	AMP	10 mcg/disc	India
3.	Azithromycin	AZM	15 mcg/disc	India
4.	Clindamycin	CD	2 mcg/disc	India
5.	Cephalexin	CL	30 mcg/disc	Turkey
6.	Cefoxitin	CX	30 mcg/disc	India
7.	Doxycycline	DO	30 mcg/disc	India
8.	Erythromycin	E	15 mcg/disc	India
9.	Imipenem	IMP	10 mcg/disc	India
10.	Levofloxacin	LEV	5 mcg/disc	Turkey
11.	Tetracycline	TE	30 mcg/disc	India
12.	Vancomycin	VA	30 mcg/disc	Turkey

3.1.5 Primers used in PCR amplification in this study

Amplification of isolates using universal and specific primers for all samples, as in [Table 3-6 and 3-7].

Table (3-6): Sequence of universal primer that use in this study.

No.	Primer		Sequence	Length (bp)	Amplicon size (bp)
1.	16S r DNA	F	5'-AGAGTTTGATCCTGGCTCAG-3'	20	1500
2.	16S r DNA	R	5'-GGTTACCTTGTTACGACTT-3'	19	1500

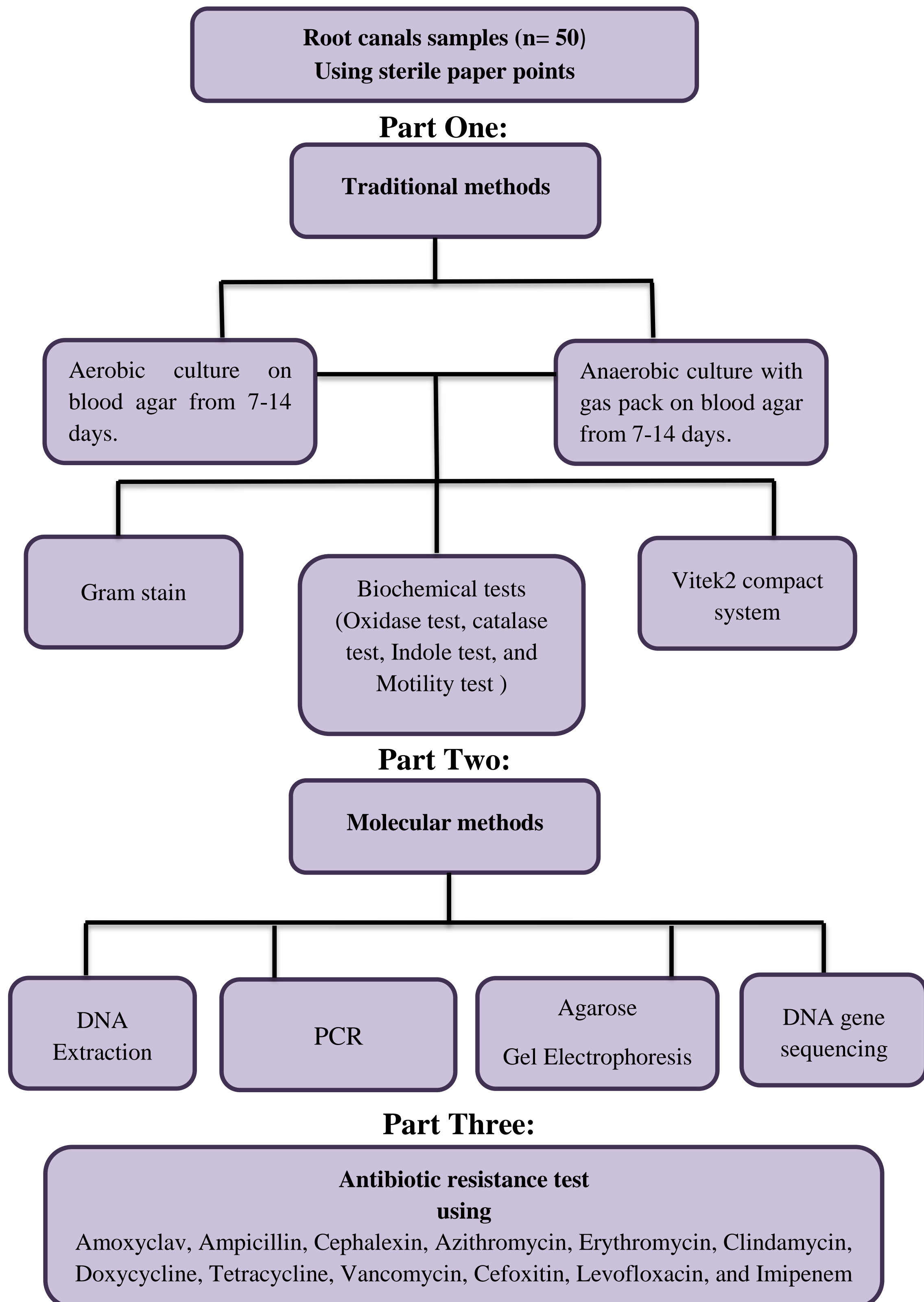
(Raji *et al*., 2008)

Table (3-7): Sequence of specific primer that use in this study.

No.	Primer		Sequence	Length (bp)	Amplicon size (bp)
1.	SCACT19	F	5'-CCGTACTCCCCAGGCGGGG-3'	19	500
2.	SCACT20	R	5'-CGCGGCCTATCAGCTTGTTG-3'	20	500

(Stach *et al*., 2003)

Steps to isolate and diagnose of *Actinomyces* spp.



3.2 Methods

3.2.1 Preparation of the media

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

3.2.1.1 Thioglycollate broth

The powder (28 gram) was suspended in 1 L distilled water (DW), heated to full dissolving. Broth medium (5 ml) was then dispensed into screw-cap tubes with tight seals. Sterilization occurred through autoclaving for 15 minutes at 121°C.

3.2.1.2 Blood agar

Forty grams of powder was dissolved in 1 L DW and heated until boiling. The mixture was sterilized for 15 minutes at 121°C under 15 lbs pressure. Once cooled to 45-50°C, it was supplemented with 10% fresh human blood and distributed into sterile plates. (Forbes *et al.*, 2007)

3.2.1.3 Nutrient agar

Twenty-eight grams of agar was suspended in 1 L DW, heated until fully dissolved, sterilized at 121°C for 15 minutes. After cooling to 50°C, the medium was poured into sterile plates. (Abd Ali & Al-waeli, 2025)

3.2.1.4 Nutrient broth

Thirteen grams of agar was putted in 1 L DW and heated until fully dissolved. Broth medium (5 ml) was then dispensed into tightly capped screw-cap tubes and sterilized at 121°C for 15 minutes.

3.2.1.5 Potato dextrose agar

Thirty-nine grams of agar was putted in 1 L DW, heated until fully dissolved, then sterilized at 121°C for 15 minutes. After cooling to 50°C, the medium was dispensed into sterile plates. (de Farias *et al*., 2010)

3.2.1.6 Actinomyces agar

The powder (77.22 grams) was suspended in 1 L DW and heated to boiling to fully dissolving. Autoclaved at 121°C for 15 minutes under 15 lbs pressure. (Atlas, 2004)

3.2.1.7 Tryptone water

Twenty-five grams of agar powder was suspended in 1 L DW and heated. The medium was then dispensed into tubes, sterilized by autoclaving.

3.2.1.8 Muller-Hinton agar

Thirty-eight grams of medium was suspended in 1 L DW and heated. Autoclaved, then cooled and dispensed into plates.

3.2.2 Samples collection

Specimens were obtained from 50 patients aged 13-75, attending the Specialized Dental Center and Dental Clinics in Amarah City Center. Between November 25, 2024, and March 5, 2025. Using sterile paper points, specimens were collected from the oral cavity of 50 cases, specifically the root canal of the tooth.

3.2.3 Culture of samples

All specimens of Root canal were inoculated directly in 25 ml of thioglycollate and nutrient broth (separate tubes). Then transported to the microbiology laboratory within 2 hours, incubated at 37°C for 72 hours. One complete ring of bacteria developed in thiogllycolate broth, and the nutrient broth was inoculated onto blood agar. The inoculated media were incubated both aerobically and anaerobically (using an anaerobic jar) created by anaerogen gas pack (Oxoid Ltd, England) at 37°C for 7–14 days.

3.2.4 Identification of bacterial isolates

Identification of bacterial was carried out using the characteristics of their culture, stained with Gram stain, and validated using the automated microbiological Vitek 2 Compact system using the ANC ID kit (Biomerieux, France).

3.2.4.1 Morphological characterization of bacteria

Gram stain was used to stain smears of newly formed colonies. These were then cultured on blood agar and examined under a microscope to determine how the stain interacted with the colony structure and organization. Colonies of isolates cultured on blood agar were characterized according to their morphology, pigmentation, edge, and the change in color in the medium. These were then cultured on *Actinomyces* agar medium and Potato dextrose agar medium.

3.2.4.2 Biochemical tests

3.2.4.2.1 Catalase test

A loopful of colony was placed on a clean slide to examine catalase production. One drop of 3% hydrogen peroxide was injected, and bubble formation suggests favorable outcomes. (Chandra, 2023; Reiner, 2016)

3.2.4.2.2 Oxidase test

Several methods exist for testing for oxidase, such as the filter paper assay, spot-on-filter paper technique, agar plate direct inoculation approach, test tube-based protocol, among additional procedures. An individual, distinctly separated colony is picked utilizing an inoculation loop from a recently prepared bacterial culture plate (72-hour culture), rubbed well on a small filter paper, and color changes are observed for 60-90 seconds. A positive result indicates a purple color, while a negative result indicates no color change. (Chavan *et al*., 2022)

3.2.4.2.3 Indole test

Tryptophan represents a vital amino acid that undergoes oxidation through bacterial tryptophanase enzyme activity. Bacterial species synthesizing tryptophanase possess the capability to decompose tryptophan into three distinct products: pyruvic acid, ammonia, and indole. Detection of indole occurs through its reaction with Kovacs' reagent, a compound consisting of 4-dimethylaminobenzaldehyde, hydrochloric acid, and butanol. A positive reaction manifests as a cherry-red ring formation at the medium's surface, whereas the absence of coloration signifies a negative outcome. (Sarma and Bhattacharjee, 2020)

3.2.4.2.4 Motility test

The motility test is used to determine the ability of microorganisms to move. The test is performed using a semi-solid motility medium containing a low concentration of agar (0.3–0.5%), which allows motile organisms to migrate through the medium. A sterile straight needle is used to pick a well-isolated colony, and the medium is inoculated by a single vertical stab to approximately 1 cm above the bottom of the tube. Care is taken to avoid lateral movement during inoculation to prevent false-positive results. The inoculated tubes are incubated at 37°C for 72 hours. Motility is indicated by diffuse or turbid growth spreading away from the stab line, whereas growth restricted to the line of inoculation indicates a non-motile organism. (Palma *et al*., 2022)

3.2.4.3 Identification of bacteria isolates by Vitek 2 compact system

Following the manufacturer's protocol, bacterial suspensions were formulated using 72-hour pre-purified cultures in test tubes filled with sterile 0.45% sodium chloride solution, adjusted to 0.5 MacFarland turbidity standard, measured using a Turbidimeter for accuracy. Subsequently, the ANC card alongside suspension-containing tubes were introduced into a Vitek 2 compact automated analyzer and subjected to incubation for roughly 6 hours. (Li *et al*., 2014)

3.2.5 Molecular study of isolates

3.2.5.1 DNA Extraction

According to the manufacturer's instructions for the Geneaid Kit, DNA has been extracted, After injection the cultured bacterial colonies into five ml of nutrient broth and Thioglycollate broth, cultured at 37 °C for 72 hours. (Faroq, 2024)

The steps:

Initial Sample Creation

Bacterial cells (up to 1 x 10°) were introduced into a 1.5 ml microcentrifuge tube. A 1-minute centrifugation at 14-16,000 x g was executed, after which the supernatant was discarded. The requisite volume of Gram+ Buffer (200 µl/sample) was dispensed into a 15 ml centrifuge tube. Lysozyme (0.8 mg/200 µl) was incorporated into the Gram+ Buffer (inside the 15 ml centrifuge tube), with vortexing employed to ensure total Lysozyme solubilization. Following this, 200 µl of Gram+ Buffer (containing dissolved Lysozyme) was added to the sample in the 1.5 ml microcentrifuge tube, and pellet resuspension was accomplished via vortexing or pipetting. A 30-minute incubation at 37°C was carried out, during which tube inversion was implemented at 10-minute increments. Proteinase K (20 µl, prepared with ddH2O) was subsequently introduced, followed by vortex mixing. An incubation at 60°C was then conducted for no less than 10 minutes, with tube inversion carried out at 3-minute increments. Following completion, the protocol progressed to step 2: Lysis.

Lysis

The sample received 200 µl of GB Buffer, followed by 10 seconds of vortex agitation. A 70°C incubation period lasting at least 10 minutes was implemented to achieve total lysate transparency. During this thermal treatment, the tube underwent inversion every 3 minutes. Concurrently, the required Elution Buffer (200 µl per sample) was warmed to 70°C in preparation for the upcoming DNA elution procedure (step 5).

Optional RNA Removal Step

Upon completion of the 70°C incubation, RNase A (5 µl at 50 mg/ml concentration) was loaded to the clarified lysate, followed by vigorous vortexing. Incubation was subsequently conducted at room temperature for 5 minutes.

DNA Binding

Absolute ethanol (200 µl) was loaded to sample lysate, and immediate mixing was performed through vigorous shaking. In cases where precipitate formation occurred, maximum disruption was achieved using a pipette. A GD Column was inserted into a 2 ml Collection Tube. The entire mixture (including all insoluble precipitate) was loaded onto the GD Column, after which centrifugation at 14-16,000 x g was conducted for 2 minutes. The 2 ml Collection Tube holding the flow-through was then eliminated, and the GD Column was transferred into a new 2 ml Collection Tube.

Wash

The GD Column received 400 µl of W1 Buffer. A 30-second centrifugation at 14-16,000 x g was implemented, after which the flow-through was eliminated. The GD Column was placed back into the 2 ml Collection Tube. Wash Buffer (600 µl, ethanol-supplemented) was then dispensed onto the GD Column. Another 30-second centrifugation at 14-16,000 x g was carried out, with subsequent flow-through removal. The GD Column was reinserted into the 2 ml Collection Tube. A final centrifugation lasting 3 minutes at 14-16,000 x g was performed to ensure thorough drying of the column matrix.

Elution

The typical elution volume has been set at 100 µl. When processing fewer specimens, reducing the elution volume (30-50 µl) is recommended to increase DNA concentration. For situations demanding greater DNA yields, repeating the elution process is advised to optimize DNA retrieval, bringing the cumulative elution volume to roughly 200 µl. The desiccated GD Column was relocated to a fresh 1.5 ml microcentrifuge tube. Warmed Elution Buffer', TE Buffer, or water (100 µl) was delivered to the column matrix center. A minimum standing period of 3 minutes was allowed to ensure complete absorption of the Elution Buffer, TE Buffer, or water. Centrifugation was performed at 14-16,000 x g for 30 seconds to elute the purified DNA.

3.2.5.2 Identified the bacteria by using PCR

The bacterial isolates were identified by using PCR to amplify universal 16S rDNA primers.

F 5'-GTACAGTTGCTTCAGGACGTATC-3

R 5'-GGT TAC CTT GTT ACG AC TT-3'

In addition to specific primers used for the identification of bacterial isolates, *Actinomyces* spp.

F 5'-CCGTACTCCCCAGGCGGGG-3'

R 5'-CGCGGCCTATCAGCTTGTTG-3'

Reagent

1x Buffer of TBE

Agarose

Ethidium bromide

3.2.5.2.1 Agarose preparation

Agarose gel electrophoresis (1%) was formulated through dissolution of Ig agarose in 100 mL of IX TBE buffer, permitted to cooling to 50°C, followed by incorporation of 5 µl. ethidium bromide into the solution of agarose, which was subsequently dispensed into the casting tray. Following agarose solidification, comb was extracted, resulting in well formation. (Asad *et al*., 2023)

3.2.5.2.2 PCR amplification for universal and specific primers

Isolated bacterial specimens from the oral cavity have been identified using the PCR technique, which involves amplifying the 16S rDNA gene primers, as in [Table 3-8 and 3-9].

Table (3-8): PCR Amplification program for universal primers used in this study.

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 (3-9): PCR Amplification program for specific primers used in this study.

No.	Stage	Temperature	Time	No. of cycle
1.	Denaturation as initial	95	5min	1
2.	Denaturation	95	30sec	30
3.	Annealing	68	30sec	
4.	Elongation	72	30sec	
5.	Final elongation	72	5min	1

Table (3-10): Mixture reaction 50µl for PCR Amplification.

No.	Reagent	Volume
1.	Master mix	25µl
2.	Forward primer	5µl
3.	Reverse primer	5µl
4.	DNA sample	5µl
5.	Nuclease water free	10µl

3.2.5.3 Gene sequence analysis

DNA samples with primers (forward and reverse) were sent to Macrogen company (Seoul, South Korea), and the resulting sequences were compared with sequences that previously registered in the National Center for Biotechnology Information (NCBI) database by using BLAST to determine the sequence and number of nitrogenous bases. (Sayers *et al*., 2019)

3.2.6 Antibiotic resistance test

Antibiotic resistance of bacteria was evaluated employing the Kirby-Bauer disc diffusion technique. Pre-cultured colonies aged 72 hours were inoculated using test tubes with containing 1 ml of physiological saline. The turbidity of suspension was standardized using 0.5% McFarland's standard. A sterile cotton applicator was subsequently submerged into the bacterial suspension tube, rotated, and compressed against the tube's interior wall to eliminate surplus liquid. The applicator was then streaked

across Mueller-Hinton agar plates multiple times in varying orientations to ensure uniform bacterial distribution. Antibiotic discs were subsequently positioned onto the agar surface using sterilized forceps at equidistant intervals and lightly pressed. The plates were then maintained under anaerobic circumstance using Anaerogen 3.5 L bags at 37°C for three successive days. (Shwailiya *et al*., 2024; Bergqvist, 2024; Hudzicki, 2009)

3.2.7 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) program was used to analyze the data statistically, and data were expressed as frequencies and percentages. The Chi-square (χ^2) test was used to examine the association between categorical variables and to determine statistically significant differences among the groups at a significance level of ($P<0.05$).

CHAPTER FOUR

Results

&

Discussion

4.1 Results & Discussion

4.1.1 Sample collection

In this study, 50 bacterial samples were isolated from 50 patients with Root canal infections in Amarah city, southern Iraq. Clinical samples were collected from root canals using paper points. Root canal infections can spread to the surrounding tissues, causing periapical inflammation and potentially leading to other life-threatening diseases. Specimens were cultured on enriched and selective media, including blood agar media and *Actinomyces* isolation media, and incubated at 37°C for 7-14 days. This results aligns with the finding of (AlSaady, 2021).

4.1.2 Distribution of Root canal dental disease patients according to sex and age

Distribution of Root canal dental disease patients sex and age is shown in Table(4-1), which is a total of 50 Root canal dental disease patients, of which 26 (52%) were females and 24 (48%) were males, as in [Figure 4-1]. The age groups (30-39) and (50-59) had the highest isolation rates for identifying bacterial isolates causing root canal diseases. No statistically significant variations were detected among all age groups ($P > 0.05$), as in [Table 4-1].

Table (4-1): Distribution of patients with root canal dental pathologies based to the sex and age.

No.	Age (Years)	Number of root canal dental diseases patients				Total patients
		Female		Male		
		Number	% Pct.	Number	% Pct.	
1.	Less 20	4	15.4%	1	4.2%	5
2.	20-29	6	23.1%	2	8.3%	8
3.	30-39	4	15.4%	4	16.7%	8
4.	40-49	3	11.5%	2	8.3%	5
5.	50-59	8	30.8%	7	29.2%	15
6.	60-69	0	0%	5	20.8%	5
7.	70-79	1	3.84%	3	12.5%	4
26 Total			24 Total		50 Total	
Chi-Square= 0.11, P > 0.05						

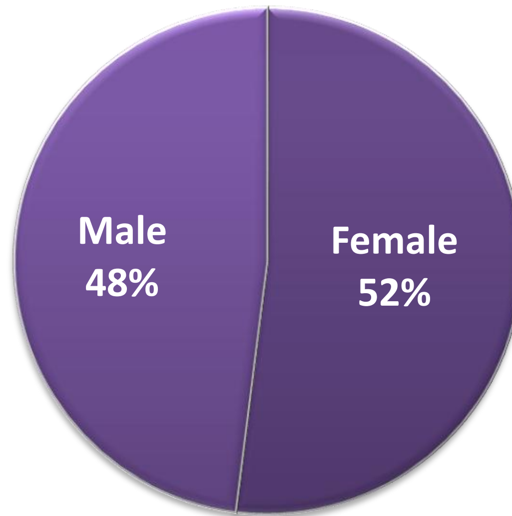


Figure (4-1): Female and Male sex distribution in Root canal dental diseases patients.

The present study demonstrated a slight predominance of females (52%) compared to males (48%) among patients diagnosed with root canal dental diseases, as in [Figure 4-1]. This higher prevalence among females attributed to several biological and physiological factors. Hormonal fluctuations experienced by females throughout different life stages, including puberty, pregnancy, and menopause, can influence pulpal blood flow, vascular permeability, and inflammatory response, thereby increasing susceptibility to pulpal and periapical infections. In addition, pregnancy-related changes such as alterations in salivary composition, decreased salivary pH, increased carbohydrate intake, and episodes of nausea and vomiting may contribute to a higher risk of dental caries and subsequent pulp involvement. This findings is concurrent with the results of (Yang *et al*., 2024)

A total of 50 samples belong to seven age groups (Less than 20), (20-29), (30-39), (40-49), (50-59), (60-69), and (70-79), the highest frequencies of root canal disease were observed in the 30–39 and 50–59 age groups. In the 30–39 age group, disease is often linked to untreated or poorly managed dental caries, failed restorations, repeated dental procedures, and prolonged exposure to cariogenic habits. For the 50–59 age group, increased prevalence reflects cumulative dental pathology, age-related changes in pulp tissue, reduced local immunity, gingival recession, xerostomia, and systemic conditions such as diabetes, all of which increase susceptibility to pulpal and periapical infections. These findings are consistent with previous studies reporting higher prevalence of endodontic diseases among middle-aged and older adults (Balado & Camacho-Aparicio, 2023).

4.1.3 Identification of bacterial isolates

4.1.3.1 Morphological characteristics of colonies and microscopic diagnosis

Morphological features are widely used to distinguish between genera, as the shape of bacteria growing on solid media can provide valuable and rapid clues for identification. Some common laboratory media, including nutrient agar, blood agar, and selective *Actinomyces* agar, have been shown to support the growth of *Actinomyces* spp. (Moncheva *et al.*, 2002). All 50 isolated root canal samples were initially inoculated using blood agar medium under strict aerobic and anaerobic conditions for 7-14 days at 37°C.

The plates were regularly monitored to check bacterial growth. After the growth period, it was noted that the colonies varied in number, color, and texture. After this, colonies were taken and examined microscopically using Gram stain. The results showed that 34 (68%) isolates (24 isolated under anaerobic conditions and 10 isolated under aerobic conditions) were Gram-positive with filamentous and rod-shaped forms, as in [Figure 4-5]. These results are consistent with (Cruz *et al*., 2015)

*Actinomyce*s spp. colonies on blood agar appeared creamy, dark brown in color, and were creamy in texture, smooth, convex, and circular in shape, while some were leathery, rough, beta-haemolytic and filamentous in shape, as in [Figure 4-2], These variations reflect multiple factors, including differences between species or strains of *Actinomyces* spp., metabolic activity, pigment production, composition of the culture medium, incubation conditions, and the growth phase of the colonies. These results agree with (Sarkonen *et al*., 2001)

When grown on selective *Actinomyces* agar, which is considered suitable for the growth of *Actinomyces* spp., as it supports their growth well, colonies appeared to be creamy white in color, and they were creamy in texture, smooth, convex, and circular in shape. Some of them were rough, leathery, and filamentous in shape, as in [Figure 4-3], The selective medium enhanced the typical morphological features of *Actinomyces* spp. by inhibiting competing flora, allowing clearer observation of characteristic growth patterns. These findings agree with Somya & Ramalingappa (2022), who reported similar colony morphologies on selective media.

Due to their structural resemblance to fungi, *Actinomyces* spp. were also cultured on Potato Dextrose Agar. Growth produced cream-colored, rough, leathery, and filamentous colonies, as in [Figure 4-4] and [Table 4-2]. Blyskal *et al*. (2017) documented similar observations, emphasizing the phenotypic resemblance of *Actinomyces* spp. to fungi.

The morphological and microscopic findings confirm the phenotypic diversity of *Actinomyces* spp. isolated from infected root canals. These results highlight the importance of integrating colony characteristics with Gram staining for accurate identification, while also demonstrating the influence of different culture media on the expression of morphological traits.

Table (4-2): Media growth characteristics of *Actinomyces* spp.

Media growth	Results of growth
Blood agar	Creamy, dark brown, Smooth, Circular, Convex, and leathery, rough, filamentous.
Actinomyces agar	White to creamy, Smooth, Circular, Convex, and leathery, rough, filamentous.
Potato dextrose agar	Creamy, leathery, rough, filamentous.

Figure (4-2): Colony of *Actinomyces* spp. on blood agar after 4 days of incubation 37˚C.

Figure (4-3): Colony of *Actinomyces* spp. on *Actinomyces* agar after 4 days of incubation 37˚C.

Figure (4-4): Colony of *Actinomyces* spp. on potato dextrose agar after 4 days of incubation 37˚C.

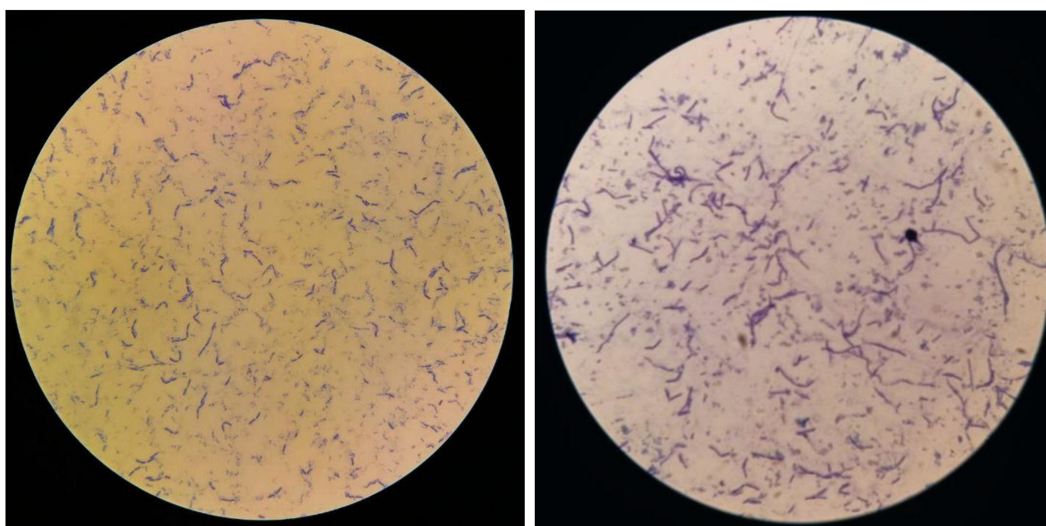


Figure (4-5): *Actinomyces* spp. observed by microscopy 1000x .

4.1.3.2 Biochemical tests

In this study, biochemical tests were performed on all 34 isolates to identify and characterize them, including catalase, oxidase, indole, and motility tests. Each test required the preparation of a specific growth medium, followed by bacterial inoculation and observation of distinct enzymatic or physiological reactions. These tests provide a rapid and accurate means of differentiating bacterial strains based on their distinctive properties, thereby facilitating further investigation and characterization.

All isolates showed a positive catalase reaction, as evidenced by bubble formation upon the addition of hydrogen peroxide, as in [Figure 4-6] and [Table 4-3]. This finding indicates the ability of these bacteria to decompose hydrogen peroxide into water and oxygen, reflecting their capacity to tolerate oxidative stress. The presence of catalase activity is consistent with the facultative nature of many *Actinomyces* species. Similar results were reported by Njeru *et al*. (2019), who also documented catalase positivity among *Actinomyces* spp. isolates.

In contrast, all isolates were oxidase-negative, indicating the absence of the cytochrome c oxidase enzyme, as in [Figure 4-6] and [Table 4-3]. During the test, no color change to purple was observed upon the addition of the oxidase reagent, confirming that these bacteria do not produce the enzyme. Similar results have been reported by Hari (2019).

Regarding the indole test, all isolates yielded negative results, demonstrating their inability to degrade tryptophan to produce indole, as in [Figure 4-6] and [Table 4-3]. This observation is in agreement with the findings of Singh *et al*. (2012), who similarly reported indole-negative reactions among *Actinomyces* species.

The motility test revealed that all isolates were non-motile, as in [Figure 4-6] and [Table 4-3]. This observation is consistent with the typical non-motile nature of *Actinomyces* spp., which generally lack flagella or other active motility structures. Similar findings have been reported by Sah & Dhakal (2023).

Table (4-3): Microscopic and biochemical tests for *Actinomyces* spp.

Biochemical tests	**Results**
Gram stain	G + ve
Catalase test	+
Oxidase test	_
Indole test	_
Motility	_

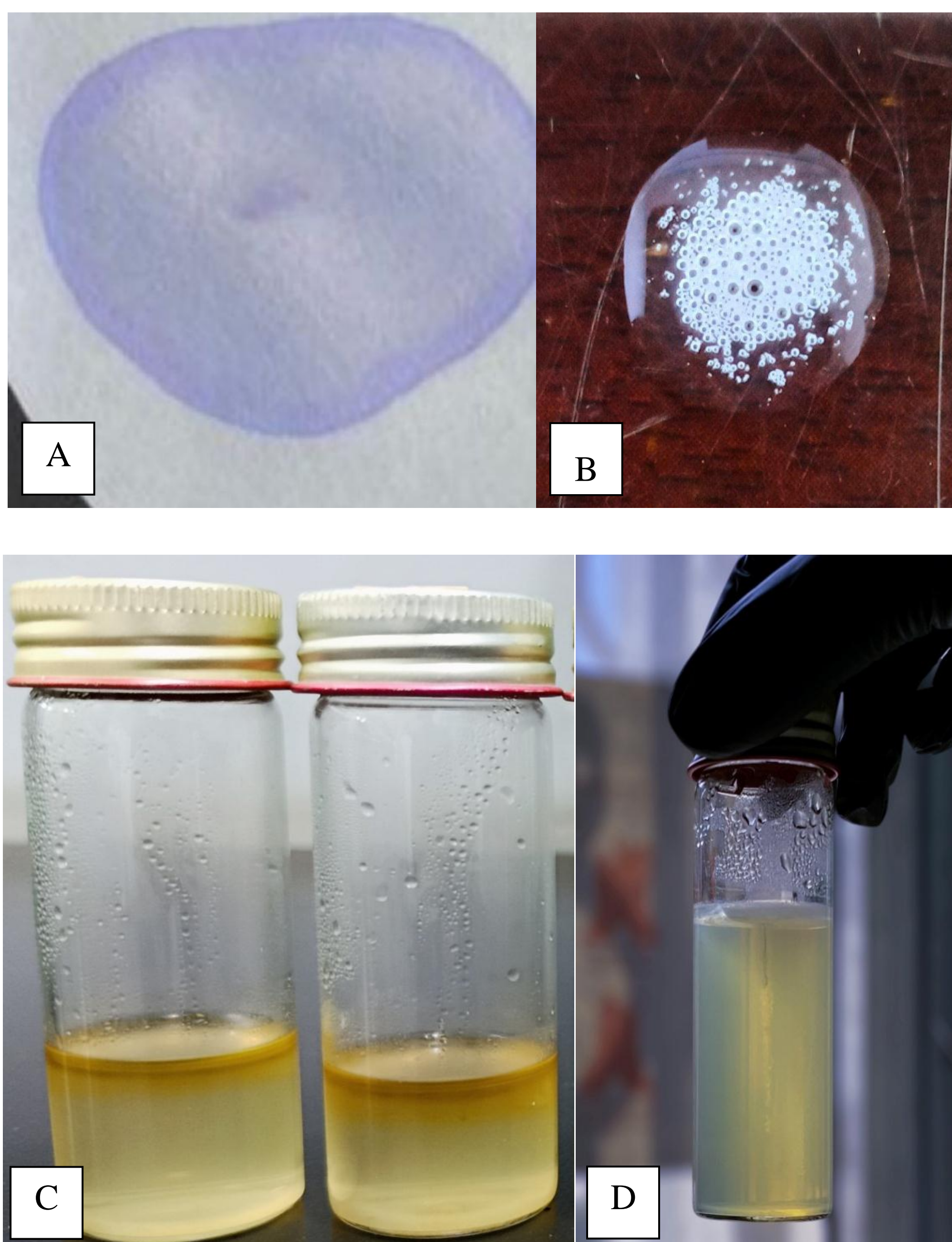


Figure (4-6): Results of biochemical tests A, Oxidase test - ve B, Catalase test + ve C, Indole test - ve D, Motility test - ve.

4.1.3.3 Diagnosis using the Vitek 2 compact system

In this study, the Vitek 2 compact system was employed to identify all 34 isolates, representing 68% of the total samples. The system utilized diagnostic ANC cards to perform biochemical profiling for identification. Among these, six isolates (18%) were identified as belonging to the genus *Actinomyces*, with identification probabilities ranging from 90–94%, while some isolates showed lower confidence levels of 50%, as in [Table 4-5]. Higher probability values indicate reliable identification; however, reduced confidence in certain isolates reflect phenotypic similarities among closely related species or limitations in the system database. In addition, ten isolates (29%) remained unidentified by the system, due to database limitations, particularly for uncommon or less frequently encountered species, as in [Table 4-4]. The remaining 18 isolates (53%) were identified as other bacterial species. These findings are consistent with Lee *et al*. (2011), who evaluated the Vitek 2 ANC card for identification of anaerobic clinical isolates and reported variability in identification accuracy depending on the bacterial species.

Table (4-4): Identification of *Actinomyces* spp. by Vitek 2 compact system

<table>
<tr><th>No. of sample</th><th>Gram positive</th><th colspan="2">Vitek 2 compact system</th></tr>
<tr><td rowspan="3">50</td><td rowspan="3">34 (68%)</td><td>Actinomyces spp.</td><td>6 (18%)</td></tr>
<tr><td>Unidentified</td><td>10 (29%)</td></tr>
<tr><td>Other</td><td>18 (53%)</td></tr>
<tr><td colspan="2">Total 50 (100%)</td><td colspan="2">Total 34 (100%)</td></tr>
</table>

4.1.4 Molecular identification

4.1.4.1 DNA Extraction of bacterial isolates

The results of genomic DNA extraction, as visualized by agarose gel electrophoresis in [Figure 4-7], demonstrated high-integrity and distinct bands for all 16 isolated bacterial. The manual extraction using a specialized kit (Geneaid, Korea) yielded sufficient concentration and high purity, with no visible signs of DNA degradation or protein contamination. These findings confirm the efficiency of the extraction protocol, providing a reliable genetic template for the subsequent molecular applications in this study.

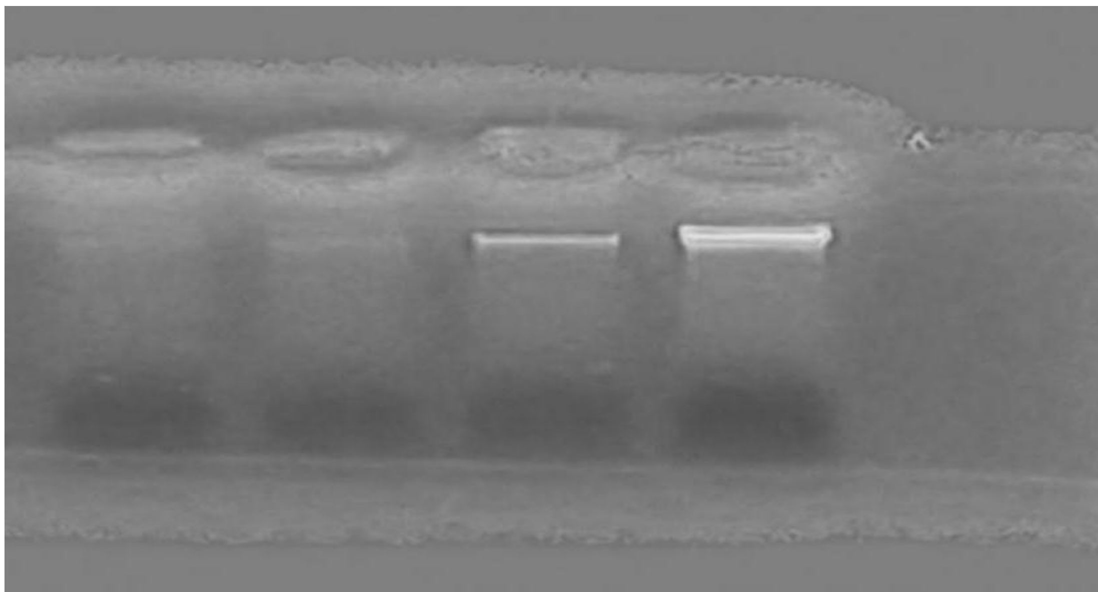


Figure (4-7): Band patterns on agarose gel electrophoresis for bacterial isolates.

4.1.4.2 Amplification of universal primer

In the current study, 16S rDNA gene sequencing was employed to detect bacterial isolates. After DNA extraction, polymerase chain reaction (PCR) analysis was performed on all 16 isolates using 16S rDNA. However, following amplification, all isolates yielded negative results. This unexpected outcome may be attributed to limitations associated with the universal primers used. Similar findings were reported by Mao *et al*. (2012), who demonstrated that certain universal primers fail to amplify some species belonging to the phylum Actinobacteria, including members of the *Actinomycetes* group. Such amplification failure result from primer–template mismatches, particularly near the 3′ terminus of the primer, in addition to sequence variability among different bacterial species.

4.1.4.3 Amplification of specific primer

Genus-specific primers successfully amplified all 16 bacterial isolates, yielding clear and distinct PCR products, as in [Figure 4-8]. This confirms their high specificity toward the target genus and supports their use as a reliable molecular tool for accurate identification. These results provide precise confirmation of the bacterial isolates, complementing the phenotypic and biochemical findings of this study. This is consistent with the findings of Rizkiantino *et al*. (2023), who reported the effectiveness of genus-specific primers for bacterial identification.

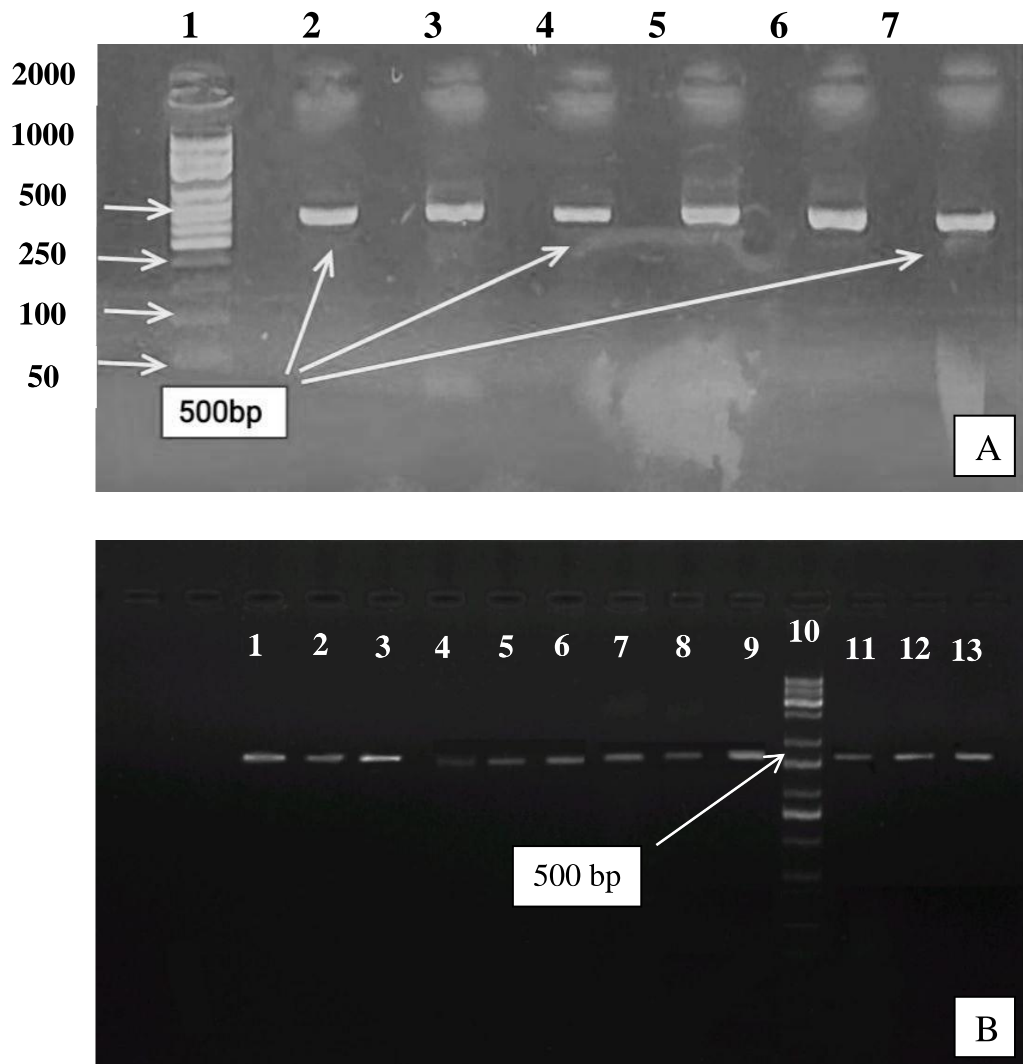


Figure (4-8): A, B, Gel electrophoresis of specific primers binding region on the *Actinomyces* spp. isolates through PCR. 1and 10, Ladder

4.1.4.4 16S DNA Gene Sequences

In this study, 16S rDNA sequencing was used to identify bacterial isolates at the molecular level, enhancing diagnostic accuracy compared to conventional biochemical methods. This approach is widely recognized for reliably identifying fastidious, slow-growing bacteria, and for detecting rare or newly described species. Based on the current findings, 16S rDNA sequencing successfully confirmed the identity of the bacterial isolates and showed concordance with the biochemical results, as in [Table 4-5]. These observations are consistent with Janda & Abbott (2007), who reported that 16S rDNA gene sequencing enhances bacterial identification in clinical settings and serves as a valuable adjunct to conventional phenotypic methods.

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1

Range 1: 3 to 483

Score	Expect	Identities	Gaps	Strand	Frame
706 bits(382)	0.0()	450/483(93%)	3/483(0%)	Plus/Minus	

```
Query  1    TCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACA  60
Sbjct  483  TCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCAAA  424
Query  61   TCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCC  120
Sbjct  423  TATCTACGCATTTCACCGCTACACTTGGAATTCCACTTTCCTCTTCTGCACTCAAGTTCC  364
Query  121  CCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC  180
Sbjct  363  CCAGTTTCCAATGACCTTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACC  304
Query  181  GCCTGCGCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGC  240
Sbjct  303  ACCTGCGCGCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGC  244
Query  241  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTA  300
Sbjct  243  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTAC-GAGCAG  185
Query  301  TT-CGAACGGTACTTGTTCTTCCCTAACAACAGAGTTTACGATCCGAAAACCTTCATCA  359
Sbjct  184  TTACTCTC-ATACTTGTTCTTCCCTAACAACAGAACTTTACGACCCGAAGGCCTTCTTCG  126
Query  360  CTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT  419
Sbjct  125  TTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT  66
Query  420  CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGC  479
Sbjct  65   CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGCCTGGC  6
Query  480  TAC  482
Sbjct  5    TAC  3
```

Figure (4-9): Data of the identification of the using *Actinomyces* spp. using NCBI Blast nucleotide sequence

Actinomycetes bacterium strain KAC7 16S ribosomal RNA gene, partial sequence
Sequence ID: **PP550672.1** Length: 639 Number of Matches: 1
Range 1: 50 to 630

Score	Expect	Identities	Gaps	Strand	Frame
1009 bits(1118)	0.0()	578/584(99%)	5/584(0%)	Plus/Plus	

```
Query  1    CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCATTC  60
Sbjct  50   CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATC-TTC  108
Query  61   GCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG  120
Sbjct  109  -CGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG  167
Query  121  TAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA  180
Sbjct  168  TAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA  227
Query  181  CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA  240
Sbjct  228  CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA  287
Query  241  GCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGA  300
Sbjct  288  GCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGA  347
Query  301  AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGG  360
Sbjct  348  AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGG  407
Query  361  AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCG  420
Sbjct  408  AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCG  467
Query  421  AAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGA  480
Sbjct  468  AAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGA  527
Query  481  TTAGATACCCTGGTAGTCCACGCCGTAGACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC  540
Sbjct  528  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC  587
Query  541  CCTTTAGTGCTGCAGCAA-CGCATTTAAGCACCCCGC-Tggggg  582
Sbjct  588  CCTTTAGTGCTGCAGCAAACGCATT-AAGCACCCCGCCTGGGGG  630
```

Figure (4-10): Data of the identification of the using *Actinomyces* spp. using NCBI Blast nucleotide sequence

Actinomycetes bacterium strain PPB 16S ribosomal RNA gene, partial sequence
Sequence ID: **PP704658.1** Length: 1487 Number of Matches: 1
Range 1: 383 to 632

Score	Expect	Identities	Gaps	Strand	Frame
407 bits(450)	6e-109()	240/250(96%)	0/250(0%)	Plus/Plus	

```
Query  1    CGTGAGTGATGAAGGTTTTCGGATCTGTAAACTCTGTTGTTAGGGAAGAAAAAGTACCGT  60
Sbjct  383  CGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGT  442
Query  61   TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG  120
Sbjct  443  TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG  502
Query  121  CAGCCGCGGTAATACGTAAGTTGCAGGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG  180
Sbjct  503  CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG  562
Query  181  CAGGCGATTTCTTGGGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAA  240
Sbjct  563  CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAA  622
Query  241  ACTGGGGAAC  250
Sbjct  623  ACTGGGGAAC  632
```

Figure (4-11): Data of the identification of the using *Actinomyces* spp. using NCBI Blast nucleotide sequence

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1
Range 1: 90 to 464

Score	Expect	Identities	Gaps	Strand	Frame
427 bits(231)	5e-120()	328/376(87%)	1/376(0%)	Plus/Minus	

```
Query  25   ACAGACAAGAAAGTCCCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGC  84
Sbjct  464  ACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCAAATATCTACGCATTTCACCGC  405

Query  85   TACAGGAGAAATTCCACTCTCCTCTTCTGCCCTCAAGTGTCGGAGTTTCCAATGAAcccc  144
Sbjct  404  TACACTTGGAATTCCACTTTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCTTC  345

Query  145  cccGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGC  204
Sbjct  344  CACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGC  285

Query  205  CCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCGCGGCACGTAGTT  264
Sbjct  284  CCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT-GGCACGTAGTT  226

Query  265  CCCCGGGGGTTCCTTGTTAAGTACCGTCCAGGAGTGAGCAGTTACTCTCGCACTTGCCCT  324
Sbjct  225  AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACGAGCAGTTACTCTCATACTTGTTCT  166

Query  325  TCCCTAACAACAAATCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGT  384
Sbjct  165  TCCCTAACAACAGAACTTTACGACCCGAAGGCCTTCTTCGTTCACGCGGCGTTGCTCCGT  106

Query  385  CAGACTTTCGTCCATT  400
Sbjct  105  CAGACTTTCGTCCATT  90
```

Figure (4-12): Data of the identification of the using *Actinomyces* spp. using NCBI Blast nucleotide sequence

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1

Range 1: 30 to 433

Score	Expect	Identities	Gaps	Strand	Frame
372 bits(201)	2e-103()	340/407(84%)	10/407(2%)	Plus/Plus	

```
Query  1    ACTCTGGGA-TGAGACACGGGCCAAACTCCTACTGGAGG-AG-GGTGGGGAA-CTTCCGC  56
Sbjct  30   ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC  89

Query  57   AATGGACGAAAGTGTGAAGGAGCAACGCCTCGTGAGTGATGAAGGTTTTCGGATCCTAAA  116
Sbjct  90   AATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAACGAAGAAGGCCTTCGGGTCGTAAA  149

Query  117  GCTCTGTTGTTAGGGAAGAAAAAGTACCGTTCGAAT-AGGGC-GGTACCTTGACGGTACC  174
Sbjct  150  GTTCTGTTGTTAGGGAAGAACAAGTA---TGAGAGTAACTGCTCGTACCTTGACGGTACC  206

Query  175  TAAACGGAAAGCCACGGGTTGCTACGTGCCTTTC-CCGCGGTAATTCGTAGGTGGCAAGC  233
Sbjct  207  TAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC  266

Query  234  GTTGTCCGGAATTATTGGGTTTAAAGGGCTCGCAGCCACCTTCTTATGTCTGATGTGAAA  293
Sbjct  267  GTTGTCCGGATTTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAA  326

Query  294  GCCCCCGGCTCAACCGGGGGCTTTCTTTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAG  353
Sbjct  327  GCCCACGGCTCAACCGTGGAAGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAA  386

Query  354  AGTGGAATTCCCCGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAA  400
Sbjct  387  AGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAA  433
```

Figure (4-13): Data of the identification of the using *Actinomyces* spp. using NCBI Blast nucleotide sequence

Table (4-5): Evaluation of conventional identification techniques and molecular approaches applied to bacterial isolates.

NO.	Gram stain	Vitek2 compact system	Morphology	16S rDNA gene sequence
1.	G +ve	*Actinomyces odontolyticus* 93%	Bacilli	*Actinomycetes* 98%
2.	G +ve	Unidentified	Filamentous	*Actinomycetes* 96%
3.	G +ve	Unidentified	Bacilli	*Actinomycetes* 95%
4.	G +ve	Unidentified	Filamentous	*Actinomyces* sp 93%
5.	G +ve	Unidentified	Bacilli	*Actinomyces* sp 87%
6.	G +ve	Unidentified	Bacilli	*Actinomyces* sp 83%

7.	G +ve	Unidentified	Bacilli	*Actinomyces* sp 82%
8.	G +ve	*Actinomyces odontolyticus* 93 %	Bacilli	*Actinomycetales* 80%
9.	G +ve	Unidentified	Bacilli	*Actinomyces* sp 78%
10.	G +ve	*Actinomyces meyeri* 50%	Bacilli	*Actinomyces* sp 73%
11.	G +ve	*Actinomyces odontolyticus* 94 %	Bacilli	*Actinomycetes* 71%
12.	G +ve	Unidentified	Bacilli	*Actinomyces* sp 70%
13.	G +ve	Unidentified	Filamentous	*Uncultured bacterium* 96%
14.	G +ve	*Actinomyces odontolyticus* 90%	Bacilli	*Uncultured bacterium* 96%

15.	G +ve	Unidentified	Filamentous	*Uncultured bacterium* 96%
16.	G +ve	*Actinomyces meyeri* 50%	Bacilli	*Uncultured bacterium* 96%

4.1.5 Antibiotic resistance test

Antibiotic resistance testing was performed using the Kirby–Bauer disk diffusion method to determine the resistance patterns of the *Actinomyces* isolates. Out of the sixteen isolates, two (13%) exhibited multidrug resistance (MDR).

The first isolate was the most resistant, resisting 8 out of 12 antibiotics (67%), including Amoxiclav, Ampicillin, Erythromycin, Clindamycin, Cephalexin, Cefoxitin, Imipenem, and Vancomycin, while the second isolate was resistant to 4 out of 12 antibiotics (33.3%), including Azithromycin, Clindamycin, Cephalexin, and Cefoxitin, as in [Table 4-6] and [Figure 4-14].

Findings from the present study corresponded with those of the researcher Rahdar *et al*. (2021), as most isolates showed sensitivity to the Imipenem antibiotic, while other isolates exhibited heterogeneous resistance patterns. As for the Amoxyclav antibiotic, the results were varied, as some isolates were responsive to this antibiotic, while others showed resistance to it; however, all isolates were highly resistant to the Cefoxitin antibiotic.

As for the following antibiotics Erythromycin, Cephalexin, Vancomycin and Ampicillin the results showed consistency with what was indicated by the researchers Nakouit & Hobbs (2012), as most of the isolates appeared resistant to Ampicillin and others were sensitive to it, and all the isolates appeared resistant to Cephalexin and some isolates were resistant to both Erythromycin and Vancomycin.

In research conducted by Zhao *et al*. (2017), bacteria exhibited very high resistance to both Clindamycin and Azithromycin, which is consistent with the findings of the current study. Similarly, Steininger & Willinger (2016) reported that nearly all tested species showed resistance to Clindamycin.

The significant resistance observed in *Actinomyces* isolates reflects their inherent biological and molecular mechanisms that enable survival in the presence of antibiotics. This resistance is primarily mediated through modification of cellular targets, which reduces the effectiveness of drugs on essential bacterial processes, and through the production of enzymes that inactivate antibiotics before they reach their target sites. Additionally, efflux pumps and decreased cell membrane permeability can limit the entry of antibiotics into the bacterial cell. This explains the heterogeneous resistance patterns observed in the current study (Peterson & Kaur, 2018).

Table (4-6): Multi-drug resistance patterns of the *Actinomyces* spp.

No.	Antibiotic disc	Iso. 1	Iso. 2
1.	AMC	R	S
2.	AMP	R	S
3.	AZM	S	R
4.	E	R	S
5.	DO	S	S
6.	TE	S	S
7.	CD	R	R
8.	LEV	S	S
9.	CL	R	R
10.	CX	R	R
11.	IMP	R	S
12.	VA	R	S

AMC: Amoxyclav, AMP: Ampicillin, AZM: Azithromycin, E: Erythromycin, DO: Doxycycline, TE: Tetracycline, CD: Clindamycin, LEV: Levofloxacin, CL: Cephalexin, CX: Cefoxitin, IMP: Imipenem, VA: Vancomycin, R: Resistant, S: sensitive.

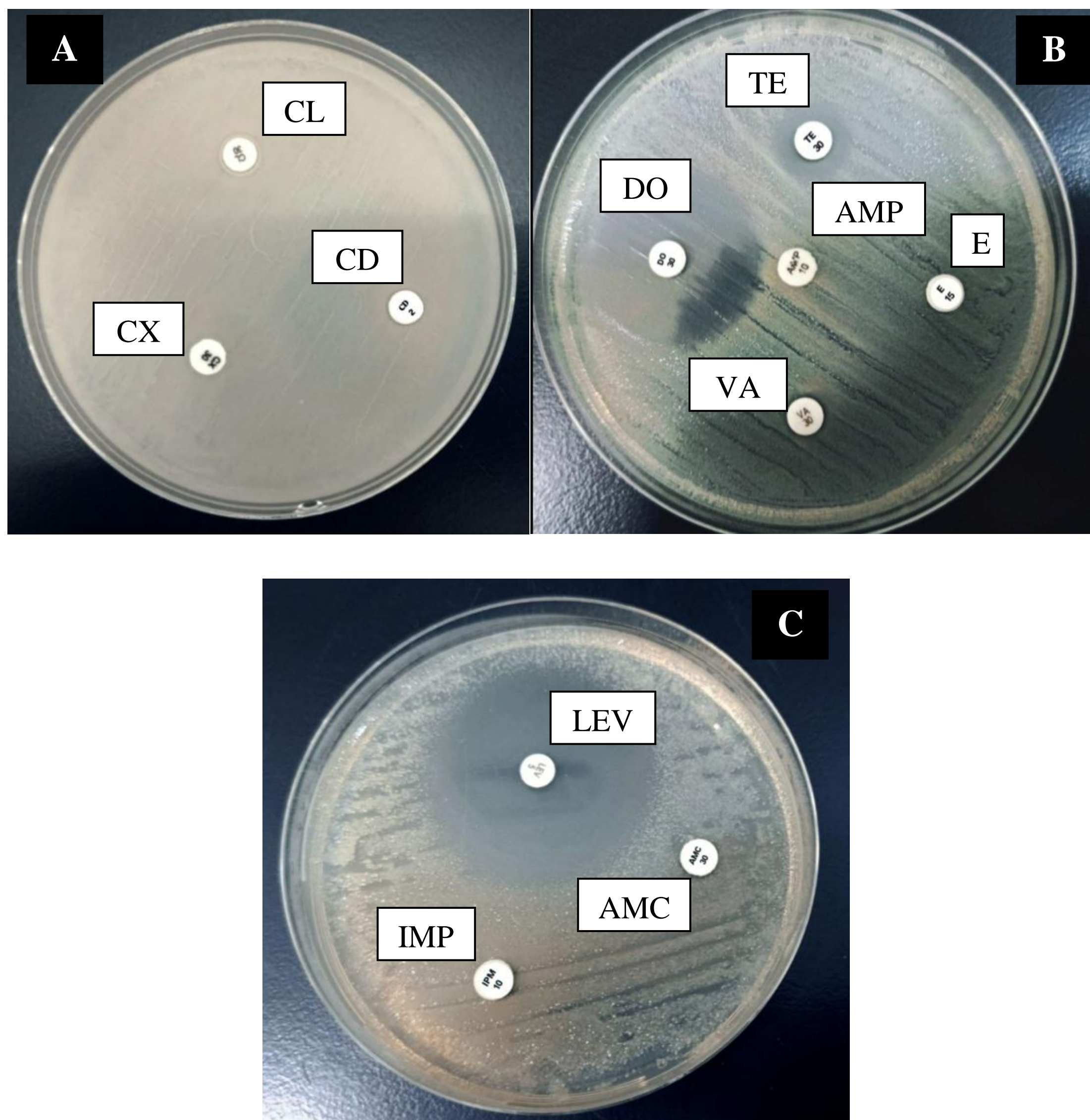


Figure (4-14): Resistance of *Actinomyces* spp. to antibiotic discs on Muller-Hinton agar A, Cephalexin, Cefoxitin, Clindamycin B, Tetracycline, Doxycycline, Ampicillin, Erythromycin, Vancomycin, C, Imipenem, Amoxyclav, Levofloxacin.

CHAPTER FIVE

Conclusions

&

Recommendations

5.1 Conclusions

- *Actinomyces* spp. play an essential role in the development of diseases resulting from tooth root inflammation.
- It was observed that individuals within the 30–39 and 50–59 age groups showed higher susceptibility to these diseases. Additionally, females exhibited a higher infection rate compared to males.
- In the current study, *Actinomyces* spp. exhibited resistance to Clindamycin, Cephalexin, and Cefoxitin.

5.2 Recommendations

- Preferably use of species-level primers to identify *Actinomyces* isolates by PCR.
- We recommend the necessity of considering the possibility of isolating *Actinomyces* spp. from various anatomical sites, particularly in cases of chronic lung, abdominal, and pelvic infections.
- We recommend Doxycycline as a first-line treatment for multidrug-resistant *Actinomyces* isolates obtained from patients with root canal infections, as the isolates have demonstrated sensitivity to this antibiotic.
- Preferably avoid random use of antibiotics without a prescription.

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Appendix

Name:

NO. of sample :

Date:

Age:

Sex:

Propping depth:

Drugs:

Clinical diagnosis:

Sample collection site :

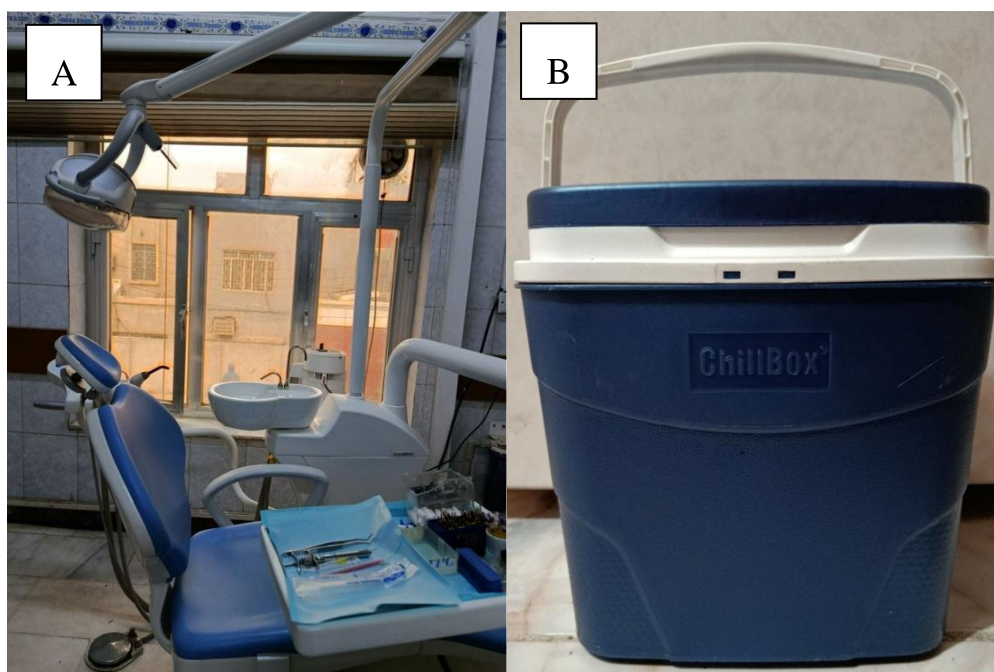

Figure (1): A, Sample collection at the Specialized Dental Center B, Sample collection case.

Figure (2): Workplace: Microbiology Laboratory, College of Science, University of Misan.

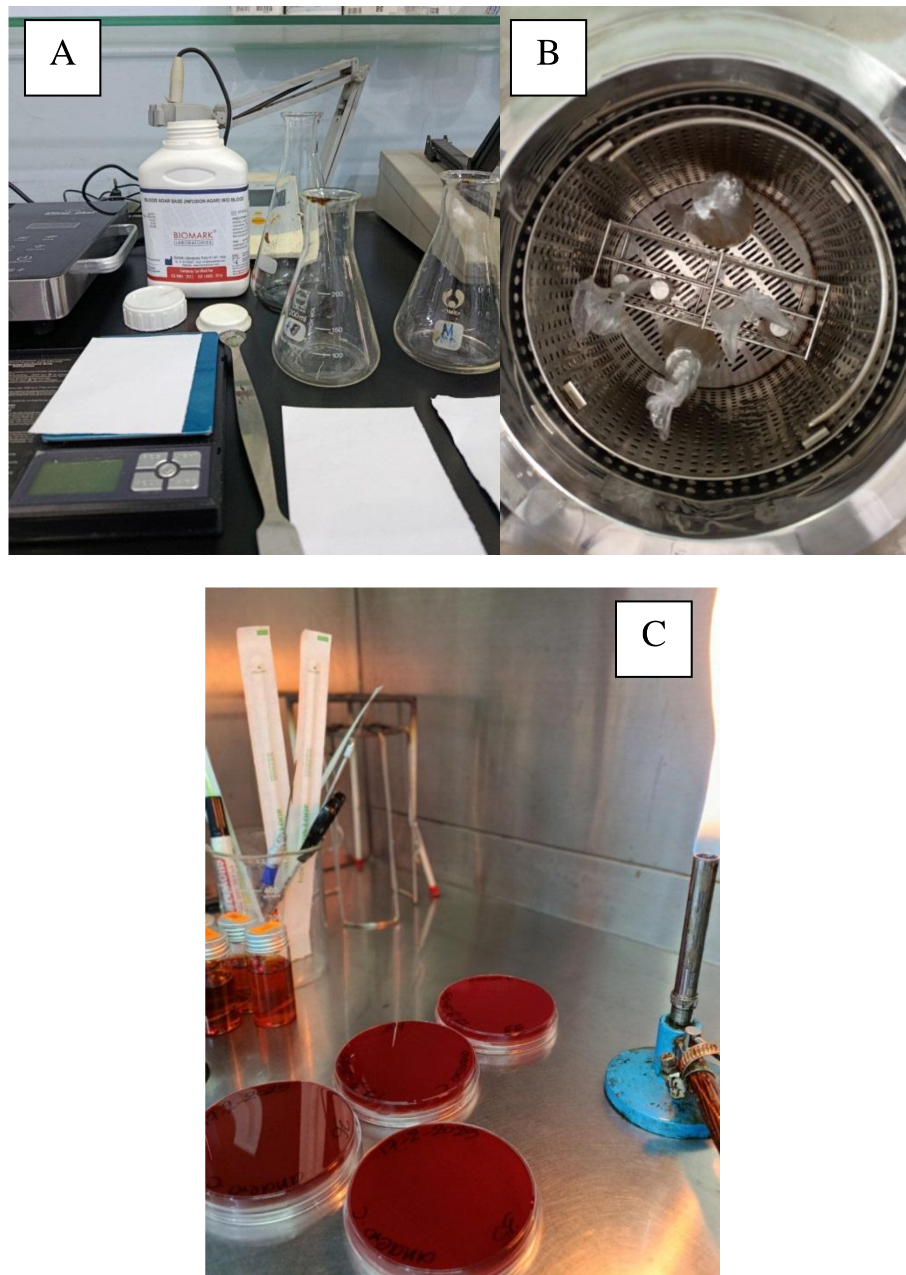

Figure (3): A, B, C, Preparing culture media for growing samples.

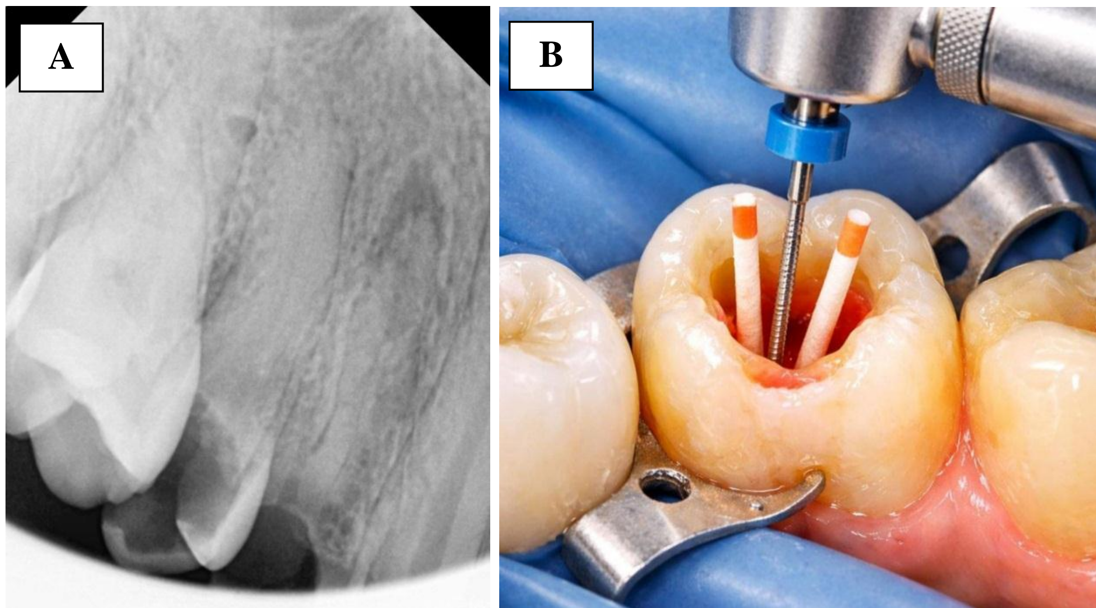

Figure (4): A, Radiograph showing tooth abscess resulting from chronic pulpitis. B, collecting the Sample from the root canal of the tooth using sterile paper points

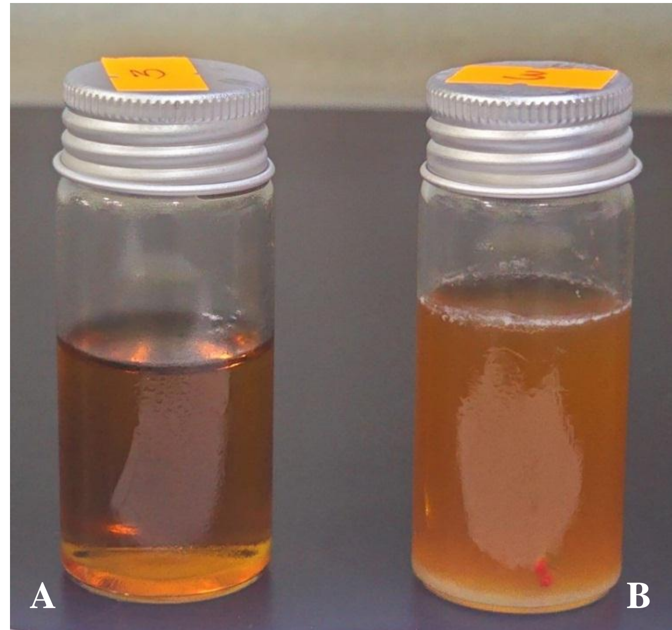

Figure (5): A, Control. B, Incoulation of paper points in thioglycollate broth after sample collection.

Figure (6): Anaero Gen 3.5 L gas pack.

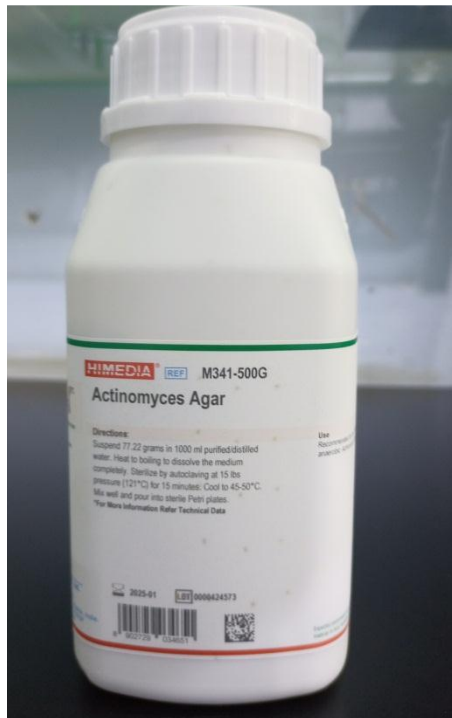

Figure (7): Actinomyces agar.

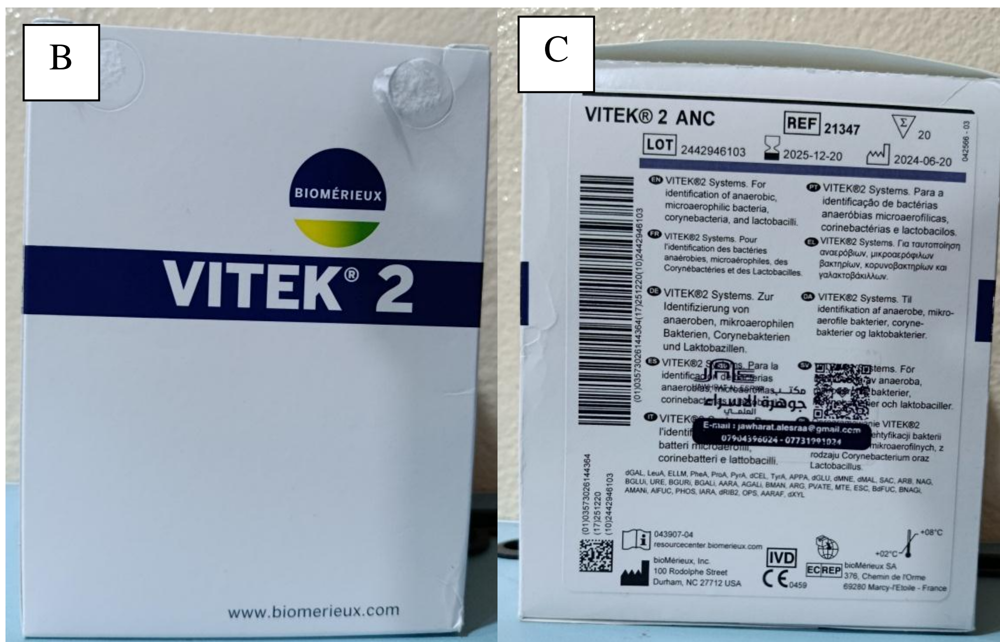

Figure (8): A, Vitek2 compact system B,C, ANC kit.

Figure (9): A, McFarland 0.5 B, Antibiotics disc.

Figure (10): A, Mini g DNA Bacteria kit B, Gel loading DNA.

Figure (11): A, Gel Electrophoresis System B, Thermocycler PCR Machine.

bioMérieux Customer:

Microbiology Chart Report

Printed January 27, 2025 6:38:40 AM CST

Patient Name: nahda5, .
Location:
Lab ID: 206

Patient ID: 206
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces odontolyticus

Source: Mouth

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.97 hours	**Status:**	Final
Selected Organism	90% Probability **Bionumber:**	**Actinomyces odontolyticus** 2360000400101		
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	-									

Page 1 of 1

bioMérieux Customer:

Microbiology Chart Report

Printed March 25, 2025 7:46:58 AM CDT

Patient Name: nahda3, .
Location:
Lab ID: 429

Patient ID: 429
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces odontolyticus

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.78 hours	**Status:**	Final
Selected Organism	93% Probability	**Actinomyces odontolyticus**		
	Bionumber:	2320010420001		
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	-									

Page 1 of 1

bioMérieux Customer: | Microbiology Chart Report | Printed March 25, 2025 7:45:27 AM CDT

Patient Name: nahda4, .
Location:
Lab ID: 430

Patient ID: 430
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces odontolyticus

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.80 hours	**Status:**	Final
Selected Organism	94% Probability **Bionumber:**	**Actinomyces odontolyticus** 2320000020001		
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	-									

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bioMérieux Customer: Microbiology Chart Report Printed March 25, 2025 7:51:08 AM CDT

Patient Name: nahda7, . Patient ID: 433
Location: Physician:
Lab ID: 433 Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces meyeri

Source: swab **Collected:**

Comments:	

Identification Information	**Analysis Time:** 5.82 hours	**Status:** Final
Selected Organism	50% **Actinomyces meyeri** **Bionumber:** 6360000400101	
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	-									

Page 1 of 1

bioMérieux Customer: Microbiology Chart Report Printed February 16, 2025 6:38:02 AM CST

Patient Name: Nahda1, .
Location:
Lab ID: 293

Patient ID: 293
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces meyeri

Source: mouth

Collected:

Comments:	

Identification Information	**Analysis Time:** 5.78 hours	**Status:** Final
Selected Organism	50% **Actinomyces meyeri** **Bionumber:** 6360000600001	
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed March 25, 2025 7:51:46 AM CDT

Patient Name: nahda8, .
Location:
Lab ID: 434

Patient ID: 434
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Actinomyces odontolyticus

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	93% Probability	**Actinomyces odontolyticus**		
	Bionumber:	2360000400101		
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed January 27, 2025 6:27:15 AM CST

Patient Name: Nahda 1, .
Location:
Lab ID: 202

Patient ID: 202
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: Mouth

Collected:

Comments:	

Identification Information	**Analysis Time:** 6.00 hours	**Status:** Final
Selected Organism	**Unidentified Organism** **Bionumber:** 0417300630401	
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed January 27, 2025 6:44:47 AM CST

Patient Name: nahda2, .
Location:
Lab ID: 203

Patient ID: 203
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: Mouth

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.23 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 6537723070741		
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed January 27, 2025 6:45:33 AM CST

Patient Name: nahda7, .
Location:
Lab ID: 208

Patient ID: 208
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: Mouth

Collected:

Comments:	

Identification Information	**Analysis Time:** 6.20 hours	**Status:** Final
Selected Organism	**Bionumber:** 6537700030441	**Unidentified Organism**
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed January 27, 2025 6:40:21 AM CST

Patient Name: nahda8, .
Location:
Lab ID: 209

Patient ID: 209
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: Mouth

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.93 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 0113302420421		
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	-									

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bioMérieux Customer: | Microbiology Chart Report | Printed March 25, 2025 7:52:21 AM CDT

Patient Name: nahda9, .
Location:
Lab ID: 435

Patient ID: 435
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 0017300020441		
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	-									

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bioMérieux Customer: | Microbiology Chart Report | Printed March 24, 2025 8:31:19 AM CDT

Patient Name: nahda 6, .
Location:
Lab ID: 420

Patient ID: 420
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: moth | **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.98 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 4417300430421		
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	?									

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bioMérieux Customer: Microbiology Chart Report Printed March 24, 2025 8:31:19 AM CDT

Patient Name: nahda 6, .
Location:
Lab ID: 420

Patient ID: 420
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: moth **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.98 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 4417300430421		
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	?									

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bioMérieux Customer:

Microbiology Chart Report

Printed April 16, 2025 7:43:09 AM CDT

Patient Name: nahda8, .
Location:
Lab ID: 489

Patient ID: 489
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.97 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 3737716776771		
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	?									

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bioMérieux Customer:

Microbiology Chart Report

Printed April 16, 2025 7:43:09 AM CDT

Patient Name: nahda8, .
Location:
Lab ID: 489

Patient ID: 489
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:** 5.97 hours	**Status:** Final
Selected Organism	**Bionumber:** 3737716776771	**Unidentified Organism**
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	?									

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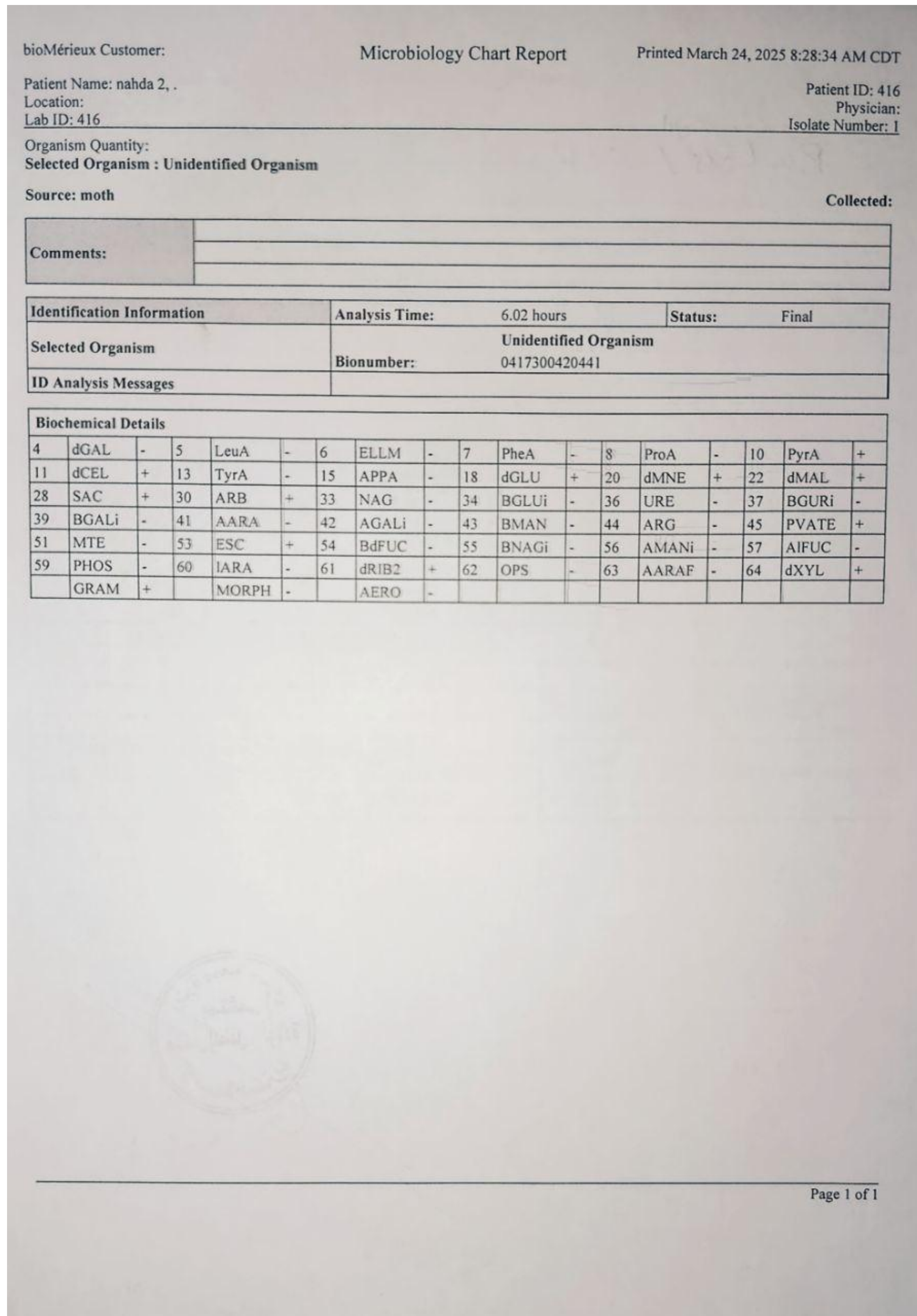

bioMérieux Customer:

Microbiology Chart Report

Printed March 24, 2025 8:28:34 AM CDT

Patient Name: nahda 2, .
Location:
Lab ID: 416

Patient ID: 416
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: moth

Collected:

Comments:	

Identification Information	**Analysis Time:** 6.02 hours	**Status:** Final
Selected Organism	**Unidentified Organism** **Bionumber:** 0417300420441	
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	-									

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bioMérieux Customer:

Microbiology Chart Report

Printed April 16, 2025 7:42:54 AM CDT

Patient Name: nahda7, .
Location:
Lab ID: 488

Patient ID: 488
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Unidentified Organism

Source: swab

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.00 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Unidentified Organism** 3007101713021		
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	-									

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Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1

Range 1: 3 to 483

Score	Expect	Identities	Gaps	Strand	Frame
706 bits(382)	0.0()	450/483(93%)	3/483(0%)	Plus/Minus	

```
Query  1    TCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACA  60
Sbjct  483  TCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCAAA  424
Query  61   TCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCC  120
Sbjct  423  TATCTACGCATTTCACCGCTACACTTGGAATTCCACTTTCCTCTTCTGCACTCAAGTTCC  364
Query  121  CCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC  180
Sbjct  363  CCAGTTTCCAATGACCTTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACC  304
Query  181  GCCTGCGCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGC  240
Sbjct  303  ACCTGCGCGCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGC  244
Query  241  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGCCCTA  300
Sbjct  243  GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTAC-GAGCAG  185
Query  301  TT-CGAACGGTACTTGTTCTTCCCTAACAACAGAGTTTACGATCCGAAAACCTTCATCA  359
Sbjct  184  TTACTCTC-ATACTTGTTCTTCCCTAACAACAGAACTTTACGACCCGAAGGCCTTCTTCG  126
Query  360  CTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT  419
Sbjct  125  TTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCT  66
Query  420  CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGC  479
Sbjct  65   CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGCCTGGC  6
Query  480  TAC  482
Sbjct  5    TAC  3
```

Actinomycetes bacterium strain KAC7 16S ribosomal RNA gene, partial sequence
Sequence ID: **PP550672.1** Length: 639 Number of Matches: 1
Range 1: 50 to 630

Score	Expect	Identities	Gaps	Strand	Frame
1009 bits(1118)	0.0()	578/584(99%)	5/584(0%)	Plus/Plus	

```
Query  1    CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCATTC  60
Sbjct  50   CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATC-TTC  108
Query  61   GCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG  120
Sbjct  109  -CGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCG  167
Query  121  TAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA  180
Sbjct  168  TAAAACTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA  227
Query  181  CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA  240
Sbjct  228  CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA  287
Query  241  GCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGA  300
Sbjct  288  GCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGA  347
Query  301  AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGG  360
Sbjct  348  AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGG  407
Query  361  AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCG  420
Sbjct  408  AGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCG  467
Query  421  AAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGA  480
Sbjct  468  AAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGA  527
Query  481  TTAGATACCCTGGTAGTCCACGCCGTAGACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC  540
Sbjct  528  TTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGC  587
Query  541  CCTTTAGTGCTGCAGCAA-CGCATTTAAGCACCCCGC-Tggggg  582
Sbjct  588  CCTTTAGTGCTGCAGCAAACGCATT-AAGCACCCCGCCTGGGGG  630
```

Actinomycetes bacterium strain PPB 16S ribosomal RNA gene, partial sequence
Sequence ID: **PP704658.1** Length: 1487 Number of Matches: 1
Range 1: 383 to 632

Score	Expect	Identities	Gaps	Strand	Frame
407 bits(450)	6e-109()	240/250(96%)	0/250(0%)	Plus/Plus	

```
Query  1    CGTGAGTGATGAAGGTTTTCGGATCTGTAAACTCTGTTGTTAGGGAAGAAAAAGTACCGT  60
Sbjct  383  CGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACCGT  442

Query  61   TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG  120
Sbjct  443  TCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAG  502

Query  121  CAGCCGCGGTAATACGTAAGTTGCAGGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG  180
Sbjct  503  CAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCG  562

Query  181  CAGGCGATTTCTTGGGTCTGATGTGAAAGCCCCGGCTCAACCGGGGAGGGTCATTGGAA  240
Sbjct  563  CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGGGAGGGTCATTGGAA  622

Query  241  ACTGGGGAAC  250
Sbjct  623  ACTGGGGAAC  632
```

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1
Range 1: 90 to 464

Score	Expect	Identities	Gaps	Strand	Frame
427 bits(231)	5e-120()	328/376(87%)	1/376(0%)	Plus/Minus	

```
Query  25   ACAGACAAGAAAGTCCCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGC  84
Sbjct  464  ACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCAAATATCTACGCATTTCACCGC  405

Query  85   TACAGGAGAAATTCCACTCTCCTCTTCTGCCCTCAAGTGTCGGAGTTTCCAATGAAcccc  144
Sbjct  404  TACACTTGGAATTCCACTTTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCTTC  345

Query  145  cccGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGC  204
Sbjct  344  CACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGC  285

Query  205  CCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCGCGGCACGTAGTT  264
Sbjct  284  CCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT-GGCACGTAGTT  226

Query  265  CCCCGGGGGTTCCTTGTTAAGTACCGTCCAGGAGTGAGCAGTTACTCTCGCACTTGCCCT  324
Sbjct  225  AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACGAGCAGTTACTCTCATACTTGTTCT  166

Query  325  TCCCTAACAACAAATCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGT  384
Sbjct  165  TCCCTAACAACAGAACTTTACGACCCGAAGGCCTTCTTCGTTCACGCGGCGTTGCTCCGT  106

Query  385  CAGACTTTCGTCCATT  400
Sbjct  105  CAGACTTTCGTCCATT  90
```

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1

Range 1: 30 to 433

Score	Expect	Identities	Gaps	Strand	Frame
372 bits(201)	2e-103()	340/407(84%)	10/407(2%)	Plus/Plus	

```
Query  1    ACTCTGGGA-TGAGACACGGGCCAAACTCCTACTGGAGG-AG-GGTGGGGAA-CTTCCGC  56
Sbjct  30   ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC  89
Query  57   AATGGACGAAAGTGTGAAGGAGCAACGCCTCGTGAGTGATGAAGGTTTTCGGATCCTAAA  116
Sbjct  90   AATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAACGAAGAAGGCCTTCGGGTCGTAAA  149
Query  117  GCTCTGTTGTTAGGGAAGAAAAAGTACCGTTCGAAT-AGGGC-GGTACCTTGACGGTACC  174
Sbjct  150  GTTCTGTTGTTAGGGAAGAACAAGTA---TGAGAGTAACTGCTCGTACCTTGACGGTACC  206
Query  175  TAAACGGAAAGCCACGGGTTGCTACGTGCCTTTC-CCGCGGTAATTCGTAGGTGGCAAGC  233
Sbjct  207  TAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC  266
Query  234  GTTGTCCGGAATTATTGGGTTTAAAGGGCTCGCAGCCACCTTCTTATGTCTGATGTGAAA  293
Sbjct  267  GTTGTCCGGATTTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAA  326
Query  294  GCCCCCGGCTCAACCGGGGGCTTTCTTTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAG  353
Sbjct  327  GCCCACGGCTCAACCGTGGAAGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAA  386
Query  354  AGTGGAATTCCCCGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAA  400
Sbjct  387  AGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAA  433
```

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1
Range 1: 239 to 551

Score	Expect	Identities	Gaps	Strand	Frame
302 bits(334)	4e-83()	259/313(83%)	8/313(2%)	Plus/Minus	

```
Query  1    AACACTTAGGACTCATCGTTTTGGGCCTGAACCACCAGGGAAACTCATCCTGTTCGCTCC  60
Sbjct  551  AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  492
Query  61   CCTCGCTTTCGTTCCTCAGCGTCAGAAACAGACCAGAGAGTCGGCTTCGGCACTGGTGTT  120

Sbjct  491  CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  432
Query  121  CCTCCACATCTCTACGCATTTCACCGCTACACAAGAAAATCCACTCTCCTCTTCTGCACT  180
Sbjct  431  CCTCCAAATATCTACGCATTTCACCGCTACACTTGGAATTCCACTTTCCTCTTCTGCACT  372
Query  181  CCAGTTCCCCGGTTTCCAATGGCCAGCCCCGGTTGAGCCGT-GCCATTCACATCAGACTT  239
Sbjct  371  CAAGTTCCCCAGTTTCCAATGACCTTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  312
Query  240  -CGAAACCGCCTGCG-ATGCTTTAACCC---TGAATTCCGGGAACGCTTG-CACCT-CTT  292
Sbjct  311  AAGAAACCACCTGCGCGCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGT  252
Query  293  ATTACCGCGGCTG  305
Sbjct  251  ATTACCGCGGCTG  239
```

Uncultured Actinomyces sp. clone EMLACT1IV 16S ribosomal RNA gene, partial sequence
Sequence ID: **JQ285871.1** Length: 801 Number of Matches: 1
Range 1: 33 to 174

Score	Expect	Identities	Gaps	Strand	Frame
76.1 bits(83)	1e-14()	104/143(73%)	2/143(1%)	Plus/Minus	

```
Query  399  ACTTCTATTTCCTTAACAACAGAA-TTTACGATAGGGTAACCTTCATCATTCAAGCGAAT  457
Sbjct  174  ACTTGTTCTTCCCTAACAACAGAACTTTACGACCCGAAGGCCTTCTTCGTTCACGCGGCG  115
Query  458  TGGCTCCGTCTGACTTTCATCCGTTTGGGAGGCCTTCTTGACTGCGTCTTCCCATAGGAG  517
Sbjct  114  TTGCTCCGTCAGACTTTCGTCCATTGCGGAAG-ATTCCCTACTGCTGCCTCCCGTAGGAG  56
Query  518  TCGGGGCCGTGACTAGGTCACAG  540
Sbjct  55   TCTGGGCCGTGTCTCAGTCCCAG  33
```

Uncultured Actinomycetales bacterium clone Otu128 16S ribosomal RNA gene, partial sequence
Sequence ID: **MH728125.1** Length: 407 Number of Matches: 1
Range 1: 127 to 355

Score	Expect	Identities	Gaps	Strand	Frame
125 bits(138)	3e-24()	184/229(80%)	30/229(13%)	Plus/Minus	

```
Query  52   GCCTGCTCCATCG-TGT--CTCCTG-TATCT--GCCTTCCACCG-TA-ACCAGG-AT--C  100
Sbjct  355  GCCTTCGCCATCGGTGTTCCTCCTGATATCTGCGCATTCCACCGCTACACCAGGAATTCC  296
Query  101  AG-CT-CCCTA-CTGACTCTAG-CTG-CCG-ACCCACTG--AGGCCGG-GTTAAGCCC--  149
Sbjct  295  AGTCTCCCCTACCATACTCTAGCCTGCCCGTACCCACTGCAAGCCCGGAGTTAAGCCCCG  236
Query  150  GGATTTCA-AGCAGAC-CGA-AAACCGCCTACAAGCTCTTTACGCC-AATA-TTCCGGA-  203
Sbjct  235  GGATTTCACAGCAGACGCGACAAACCGCCTACAAGCTCTTTACGCCCAATAATTCCGGAC  176
Query  204  AACGTCTGC-CCCTTC-TAATACCGGGGCTGCTGGAAGGAAGTTAGCCG  250
Sbjct  175  AACGCTCGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG  127
```

Uncultured actinomycete isolate DGGE gel band F12 16S ribosomal RNA gene, partial sequence
Sequence ID: **JN572604.1** Length: 209 Number of Matches: 1
Range 1: 8 to 113

Score	Expect	Identities	Gaps	Strand	Frame
169 bits(91)	3e-37()	102/107(95%)	1/107(0%)	Plus/Plus	

```
Query  1    GCAGTGGGGAATATTGCACAATGGGCGGAGCCTGATGCAGCGACGCCTCGTGAGGGATGA  60
Sbjct  8    GCAGTGGGGAATATTGCACAATGGGCGGAGCCTGATGCAGCGACGCCGCGTGAGGGATGA  67
Query  61   CGGCCTTCGGGATGTAAACCTCTTTCGCCAGGGACGAAGCTTTATTT  107
Sbjct  68   CGGCCTTCGGGTTGTAAACCTCTTTCACCAGGGACGAAGCGT-ATTT  113
```

Uncultured Actinomyces sp. clone 07_3_C11 16S ribosomal RNA gene, partial sequence
Sequence ID: **GU227168.1** Length: 1467 Number of Matches: 1
Range 1: 679 to 862

Score	Expect	Identities	Gaps	Strand	Frame
60.8 bits(66)	2e-10()	136/194(70%)	13/194(6%)	Plus/Plus	

```
Query  435   gggAA-AAC-CCTATGGCGAAgggggggCTCTTGGCCCCTAACTTGAGCCCTGGGAAGAG  492
Sbjct  679   GGGAAGAACACCTATGGCGAAGGCAGGTCTCTGGGCTGTTA--CTGA-CGCTGAGGAGCG  735
Query  493   AAAACCTgggggggggAAAAAGAATTAAAAACCCCGGGTAG-CCAAGCCGTAAAgggggg  551
Sbjct  736   AAAGCGTGGGGAGCG--AACAGGATTAGATA-CCCTGGTAGTCCACGCTGTAAACGTTGG  792
Query  552   ggcctaagggggggggggCATTTCCACGGGTTTCGGGGCGGGAGCTAAAGAATTAAGGGc  611
Sbjct  793   GCACT-AGGTGTGGGGGCCA---CCCGTGGTTTCTGCGCCGTAGCTAACGCTTTAAGTGC  848
Query  612   cccccccGGGGGGT  625
Sbjct  849   CCCGCCTGGGGAGT  862
```

Uncultured Actinomyces sp. partial 16S rRNA gene, isolate W153T_18573
Sequence ID: **LT693156.1** Length: 760 Number of Matches: 1
Range 1: 530 to 694

Score	Expect	Identities	Gaps	Strand	Frame
106 bits(57)	3e-23()	130/166(78%)	2/166(1%)	Plus/Plus	

```
Query  236   CGTAATTATTGGGCGTTAAGGGCTCGCTGGCGAATCCTTAAGTCTGATGTGAAAGCGCCG  295
Sbjct  530   CGGAATAACTGGGCGTAAAGGGCACGCAGGCGGTTATTTAAGTGAGGTGTGAAAGCCCCG  589
Query  296   GGGTCAACCGGGGAGTGTCTTTGTAAACTGGGGAACTTGAGTCCCGAAGAGGAGAGGG-G  354
Sbjct  590   GGCTTAACCTGGGAATTGCATTTCAGACTGGGTAACTAGAGTACTTTAG-GGAGGGGTAG  648
Query  355   AATTCCACGTGTTGCGGTGAAATGCGTAGAGATGTGGAGGAACACC  400
Sbjct  649   AATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACC  694
```

الخلاصة

هدفت هذه الدراسة إلى عزل و تشخيص أنواع البكتيريا الممرضة من جنس .Actinomyces spp من تجاويف الفم لدى مرضى التهابات عصب السن في مركز محافظة ميسان، وتحديدًا الفئة العمرية بين ١٣ و٧٥ عامًا ممن راجعوا مركز طب الأسنان التخصصي وعيادات الأسنان في مدينة العمارة، جنوب العراق. تم تحديد جميع هذه العينات باستخدام الطرق التقليدية (صبغة كرام، والاختبارات الكيميائية الحيوية، ونظام Vitek 2 compact)، والطرق الجزيئية، بما في ذلك تقنية تفاعل البوليميراز المتسلسل (PCR) باستخدام البادئات العامة و الخاصة، وتسلسل جينات الحمض النووي (DNA) بالاعتماد على بنك الجينات NCBI.

جُمعت العينات من ٢٥ نوفمبر ٢٠٢٤ إلى ٥ مارس ٢٠٢٥. جُمعت ٥٠ عينة من العزلات البكتيرية من ٥٠ مريضًا يعانون من أمراض ناتجة عن التهاب جذور الأسنان في مركز محافظة ميسان لسبع فئات عمرية تتراوح بين ١٣ و٧٥ عامًا (أقل من ٢٠ عامًا، ٢٠-٢٩ عامًا، ٣٠-٣٩ عامًا، ٤٠-٤٩ عامًا، ٥٠-٥٩ عامًا، ٦٠-٦٩ عامًا، ٧٠-٧٩ عامًا) بما في ذلك ٢٦ أنثى (٥٢٪) و٢٤ ذكرًا (٤٨٪). كانت أعلى نسبة عزل في الفئتين العمريتين (٣٠-٣٩ عامًا) و(٥٠-٥٩ عامًا).

وأخيرًا، تمت دراسة مقاومة هذه العزلة للمضادات الحيوية. تم اختبار مقاومة هذه العزلات للمضادات الحيوية التالية باستخدام طريقة كيربي-باور: Amoxyclav, ampicillin, cephalexin, azithromycin, erythromycin, clindamycin, doxycycline, tetracycline, vancomycin, cefoxitin, levofloxacin, and imipenen. أظهرت النتائج أن عزلتين ذات مقاومة متعددة للمضادات الحيوية (MDR)، حيث أظهرت مقاومة لكل من المضادات الحيوية التالية: Amoxyclav, Ampicillin, Erythromycin, Clindamycin, Cephalexin, Cefoxitin, Imipenem, Vancomycin, and Azithromycin.

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

دراسة جزيئية لبكتيريا *Actinomyces* spp. المقاومة للمضادات الحيوية المعزولة من قناة الجذر لبعض مرضى الأسنان في مركز محافظة ميسان

رسالة مقدمة إلى

مجلس كلية العلوم / جامعة ميسان

كجزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

ناهدة محمد هاشم

بكالوريوس علوم حياة / كلية العلوم / جامعة ميسان

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بإشراف

أ.د محمد عباس عبد علي

أ.د سامي خلف جبار

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