SURVEY AND ANALYSIS ON ORAL MICROBIAL PROFILE OF DHAKA CITY DWELLERS AND THEIR ASSOCIATION WITH DIFFERENT DISEASES IN THE COMMUNITY

A DISSERTATION TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

SUBMITTED BY

MAHMUDA KHATUN ANAMIKA

STUDENT ID: 19136048

SEPTEMBER 2023

FATIMA TUJ ZOHRA

STUDENT ID: 19236010

BIOTECHNOLOGY PROGRAM

DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES

BRAC UNIVERSITY

DHAKA, BANGLADESH

Declaration

It is hereby declared that

1. The thesis submitted titled **"Survey and analysis on oral microbial profile of Dhaka city dwellers and their association with different diseases in the community"** is our own original work while completing our undergraduate degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.

4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

_____

Mahmuda Khatun Anamika

19136048

Department of Mathematics and Natural Science,
Biotechnology Program

_____

Fatima Tuj Zohra

19236010

Department of Mathematics and Natural Science,
Biotechnology Program

Approval:

The thesis titled **"Survey and analysis on oral microbial profile of Dhaka city dwellers and their association with different diseases in the community"** submitted by 1. Mahmuda Khatun Anamika Student ID- 19136048. 2. Fatima Tuj zohra, Student ID- 19236010. has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Biotechnology in September 2023

Examining Committee:

Supervisor:

(Member)

_____

Iftekhar Bin Naser, PhD,
Associate professor,
Department of Mathematics and Natural Sciences
BRAC University

Co-Supervisor:

_____

Afia Nowshen
Lecturer, Department of Life Sciences.
School of Environment and Life Sciences.
Independent University Bangladesh

Program Director:

_____

Dr. Munima Haque, PhD,
Associate Professor and Director of Biotechnology program
Department of Mathematics and Natural Sciences,
BRAC University

Department Head:

Chair

_____

A.F.M Yusuf Haider PhD,
Professor and Chairperson,
Department of Mathematics and Natural Sciences
BRAC University

Ethics Statement

For the completion of this study, samples from selected venues were collected following all the necessary precautions. All the experiments were done at BRAC University Life Sciences Laboratory. It should also be noted that no animal or human models were used in this study.

Abstract

This research aims to determine the composition of the human oral microbiota, comprising various microorganisms inhabiting the oral cavity exist in the human mouth. The oral microbiota plays a vital role in human health, with growing evidence linking it to systemic conditions such as diabetes, obesity, cancer, and even diseases beyond the oral cavity, including rheumatoid arthritis, adverse pregnancy outcomes, and cardiovascular diseases. Furthermore, a substantial number of oral microorganisms can transit to the digestive tract, fostering close associations with digestive disorders. Notably, a large number of oral microorganisms enter the downstream digestive tract from the oral cavity through saliva, and they present a particularly close relationship with digestive diseases. (Iyer, P. 2023). The presented study was undertaken to find the mostly available bacteria in teeth, among the isolates, the most prevalent organism was *Klebsiella* and *Pseudomonas* species as the most abundant, followed by *Staphylococcus* species. Clinical oral samples were collected from two distinct dental clinics, encompassing 25 patient samples. However, probable microorganism *Klebsiella* was identified in 96% of the samples, *Pseudomonas* species microorganism (96%) and *Staphylococcus* species microorganism (88%). Subsequently, antibiotic susceptibility test was carried out for 15 antibiotics from different antibiotic classes in conjunction with biochemical assessments following gram staining. Additionally, patient surveys encompassing oral health, lifestyle, and dietary habits were conducted. All 25 clinical samples exhibited 100% resistance to four common antibiotics: Doxycycline, Penicillin, Ampicillin, and Cefixime. which is alarming. Furthermore, the study observed that many patients harbored diverse microorganisms while undergoing antibiotic treatment. In terms of microflora, *Klebsiella* and *Pseudomonas* was in maximum number of samples. Comparatively, non-clinical isolates displayed a lower prevalence of pathogenic microorganisms, with 16% Klebsiella, 24% Pseudomonas, and 8% Staphylococcus. After the study, and comparing both the non-clinical and clinical isolates, it could be said that Clinical isolates tend to exhibit higher pathogenicity. Along that study, statistical tests were also performed to find out significant associations from the data.

Keywords: *Klebsiella, pseudomonoas*, *Staphylococcus*, Antibiotic Resistant, Clinical, Oral bacteria.

Acknowledgment

We would like to start by thanking Almighty Allah for giving us the chance and the willpower to finish this research.

We are indebted to our supervisor, **Iftekhar Bin Naser PhD**, Associate professor, Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University for his ongoing oversight, frank feedback, knowledgeable direction, and never-ending inspiration during the whole duration of our study.

Additionally, we want to thank our co-supervisor **Afia Nowshen**, Lecturer, Department of Life science, Independent University Bangladesh, for her amazing generosity, consistent supervision, support and help. We would like to express our genuine appreciation for helping and consistently encouraging us in our endeavors.

We acknowledge our gratitude to Professor **A F M Yusuf Haider, PhD,** Chairperson of the Department of Mathematics and Natural Sciences, BRAC University, for allowing and encouraging us to complete our undergraduate thesis.

Also, our heartfelt gratitude and appreciation goes to **Dr. Munima Haque, PhD,** Program Director of Biotechnology program, Department of Mathematics and Natural Sciences, BRAC University, for all the help, love, and direction she always gave us when we needed it.

We would like to extend our appreciation to the respective Laboratory Officers, **Md. Mahmudul Hasan,** and **Shamima Akhter Chowdhury** for their suggestions, moral support, and cordial help throughout our working period.

Finally, we would like to extend a token of gratitude towards our family members and friends, without whose support and prayers we would not have come as far as we did.

List of Graphs:

List of Tables:

List OF Figures:

Chapter 1
Introduction and Literature review

1.1 Introduction

Oral cavity is a diverse ecosystem which harbors immense diversity of microorganisms. Some of these microorganisms are involved in causing multiple infections along with cavities. The human oral cavity provides a habitat for oral microbial communities. However, oral infectious diseases, such as caries, periodontal disease, and oral candidiasis, can be caused by the oral microbiota. Cavities are actually an infection of the tooth created from common bacteria that are present in everyone's mouth. In fact, there are over 500 different species of bacteria in the human mouth which can cause oral cavities. (Zheng., et al. 2019). Cavities are permanently damaged areas in the hard surface of the teeth that develop into tiny openings or holes. Cavities, also called tooth decay or caries, are caused by a combination of factors, including bacteria in the mouth, frequent snacking, sipping sugary drinks and not cleaning your teeth well. If cavities aren't treated, they get larger and affect deeper layers of the teeth. They can lead to a severe toothache, infection and tooth loss. The mucosa of the oral cavity provides a unique ecological site for microbiota. Saliva plays a considerable role in influencing the oral microbiome (Zhang. R. at al. 2021). The mouth is a rich environment for hundreds of species of bacteria since saliva is mostly water and plenty of nutrients pass through the mouth each day. Additionally, according to a report from the World Health Organization in 2022, poor oral health and the resulting ability of the (Iyer, P., 2023).

Oral health is an over looked aspect of well-being where men might also experience disease outcomes that differ from women. Just like men are less likely than women to take care of their physical health, studies suggest they similarly pay less attention to their oral health (McGrath & Bedi,2000). Identifying and understanding gender disparities related to oral health is important since poor oral health can adversely affect well-being, nutrition, speech, appearance, and impair self-confidence and socialization (General, Dental, & Research, 2000). Sometimes referred to as the gateway to health, research links good oral health to longevity and poor oral health to systemic disease including heart disease, stroke, diabetes, and respiratory disease (Li. X et al., 2019). Healthy People 2030 also highlights the importance of oral health and gender by including it as a key aim and that efforts to improve oral health need to address both sexes. Conventional microbiological approaches that rely on cultivation for the detection of microorganisms in the oral cavity are not sufficient for such comprehensive and intensive monitoring.

This particular study was performed to isolate and identify the common oral bacteria and their characterization for production in the oral cavity in Dhaka city.

Also, the comparison between non-clinical and clinical oral samples for identifying the microbial flora was observed. For this study, 25 non-clinical and 25 clinical samples were collected from different areas of Dhaka city Next, the samples were cultured in different agar media for the isolation of pathogenic organisms. Eventually, organisms that are isolated from the cultures go through gram staining and simultaneously biochemical identification. Different types of antibiotics are being consumed frequently by us, and for this reason, organisms are also becoming antibiotic resistant. To see the antibiotic sensitivity pattern of these organisms, an antibiotic sensitivity test was also done.

1.2 Oral cavities and infection

Oral infections, also known as mouth infections which includes dental infections, are a group of infections that occur around the oral cavity. Mouth infections typically originate from dental caries at the root that spread to adjacent structures. As such, mouth infections are more common in populations with poor access to dental care or populations with health-related behaviors that damage one's teeth and oral mucosa. This is a common problem, representing nearly 36% of all encounters within the emergency department related to dental conditions. (Sampaio et al.2016) Although mouth infections can present in many different ways. Cavities are actually an infection of the tooth created from common bacteria that are present in everyone's mouth. When you add factors in like having a sugary diet or slacking on brushing and flossing you are at risk even more. These bacteria build up and start to leak acid onto the teeth which will erode the teeth over time. This will in fact drain the tooth’s enamel of the minerals necessary to protect your teeth.

Usually bacterial in origin, that causes demineralization of the hard tissues and destruction of the organic matter of the tooth, usually by production of acid by hydrolysis of the food debris accumulated on the tooth surface. If demineralization exceeds saliva and other demineralization factors such as from calcium and fluoridated toothpastes, these tissues progressively break down, producing dental caries. Tooth decay disease is caused by specific types of bacteria that produce

acid in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. (Zheng, S. et al. 2015). The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. As the enamel and dentin are destroyed, the cavity becomes more noticeable. The affected areas of the tooth change color and become soft to the touch. (Keenleyside, 2019) Once the decay passes through enamel, the dentinal tubules, which have passages to the nerve of the tooth, become exposed, causing a toothache. The pain may worsen with exposure to heat, cold, or sweet foods and drinks. Dental caries can also cause bad breath and foul tastes. In highly progressed cases, infection can spread from the tooth to the surrounding soft tissues.

One of the main criteria required for caries formation is a caries-causing bacteria. Dental caries can occur on any surface of a tooth that is exposed to the oral cavity, but not the structures that are retained within the bone. Bacteria in a person's mouth convert glucose, fructose, and most commonly sucrose (table sugar) into acids such as lactic acid through a glycolytic process called fermentation. Bacteria can penetrate the enamel to reach dentin and cause caries. These caries, sometimes referred to as "hiddencaries" which can cause gum infections.

Gum infection is a harmful infection in the mouth that causes damage and inflammation in the gums and jaw also an accumulation of bacteria in the tissue around the teeth. Gum infections generally arise from poor dental hygiene over time, as this can allow bacteria to become lodged between the gums and teeth, where it will grow freely. Bacteria form a film around the teeth called plaque. The buildup of the bacteria under the gum line can easily lead an area to become infected. Cavities and oral infections can start on any tooth surface and it is one of the most common chronic diseases affecting people of all ages. People in their 20s often develop this form of tooth decay between their teeth. Adults who have receding gums are more prone to root decay. More than 80% of Americans have at least one cavity by the time they enter their mid-30s. (Vytla, S. Gebauer, D., 2017). cavities are more common in children. This is because many children don't brush properly or regularly enough and they tend to consume sugary foods and drinks.

1.3 Oral cavities and Bangladesh

The study was done from February 2017 to June 2017. From the study different dental problems, causes of problems were noticed as well as daily habits of patients have come to be noticed. Over the past 20 years, a marked decline in the prevalence of dental diseases has been observed in

several Western industrialized countries. Oral Health the general condition of the mouth, mouth cavity, teeth, and associated structures. it was apparent that the precise burden of dental diseases in Bangladesh was largely unknown. In Bangladesh more than 80 percent of the population have at least one or more oral and dental diseases. (Mostarin, et.al. 2019) Many suffer from periodontitis, gingivitis, dental caries, pulpitis, alveolar abscess etc. Dental cysts, carcinoma of the oral cavity are also common oral health problems in Bangladesh. The people of rural areas are comparatively much ignorant regarding their oral hygiene. They normally use neem sticks, ashes, etc. to clean their teeth in the morning. As there is no facility for dental treatment in villages, dental patients have to report to the nearby upazila health complexes for their emergency treatment. With the existing treatment facilities, it is really difficult to provide conservative and prosthetic dental treatment to the village people. So, after the loss of teeth, they mostly live without artificial denture. (Mostarin, et.al. 2019) In rural areas there is no facility available for regular dental treatment except voluntary dental camps at some places. The line of treatment in such temporary camps are usually minor extractions, scaling and temporary restoration.

In several areas there are indigenous dental practitioners. Quite a good number of people in Bangladesh are in the habit of chewing betel leaf and nut without knowing its ill-effects. Other ingredients, such as jarda, khaer, tamak pata (tobacco leaf), etc. are also mixed with betel leaf, nuts and lime to make the betel leaf eating tasty. (Mostarin, et.al. 2019). It irritates the mucous membrane of mouth and intensifies the respiration. The tannins in the betel nut slightly shrinks the cells. Tartars are formed at teeth roots of those who take betel leaf regularly, and suffer from gum troubles. The gum decays and in many cases the bone which holds tooth root in jaws gradually decays. Many superstitions among the people exist in Bangladesh regarding various sorts of diseases and their treatment. Many people still believe that worms grow in the decayed teeth causing pain in the teeth. Due to this wrong belief, many persons for relief of their dental pain do not even care. When the teeth are decayed small holes known as dental caries are formed in them. When this decay causes inflammation in the dental pulp, the victim experiences severe pain.

1.4 Oral Microbes:

Dental caries, gingivitis and gum infections are caused by overgrowth of oral bacteria, as a result of insufficient dental hygiene. Other infections of the mouth include oral thrush, an infection caused by overgrowth of *staphylococcus* species bacteria. Staph bacteria are also capable of

colonizing the mouth. Furthermore, *Pseudomonas aeruginosa* is bacterium commonly observed in gum infections, associated with high morbidity and mortality this pathogen has a great ability to develop antibiotics resistance leading to clinical complications. Moreover, it was frequently reported that *K. pneumoniae* was detected in human oral cavities as opportunistic pathogens. In healthy people, they live in the mouth peacefully, crowded by other microbes and cause trouble.

1.5 Risks associated with oral microbes:

Poor oral hygiene is considered a risk factor for esophageal squamous cell carcinoma and esophageal squamous epithelial dysplasia. Esophageal squamous cell carcinoma is closely related to oral conditions such as tooth loss and tooth brushing frequency. The micro-ecological imbalance caused by poor oral hygiene may be one of the reasons that cause the accumulation of carcinogens, and the body is in a state of continuous inflammation. Recently, increasing evidence has shown that the oral microbiota is closely related to the physical state of humans, such as diabetes, obesity, and cancer. oral microbiota is also closely related to systemic diseases, including rheumatoid arthritis, adverse pregnancy outcomes, and cardiovascular disease. (Alves et al. 2018). Unlike gut microbiota, these types of bacteria do not change significantly. Diet and the environment have a great impact on gut microbiota. *Klebsiella pneumoniae* is a bacterium that normally lives inside oral function it can lead to a range of illnesses, including pneumonia, bloodstream infections, meningitis, and urinary tract infections. *Staphylococcus aureus* is the most pathogenic; it typically causes skin infections and sometimes pneumonia, endocarditis, and osteomyelitis. It commonly leads to abscess formation. *Pseudomonas aeruginosa* has emerged as an important pathogen during the past two decades. (Keenleyside, et al 2019). The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia. The likelihood of recovery from pseudomonas infection is related to the severity of the patient's underlying disease process.

1.6 *Pseudomonas* species:

Pseudomonas species is distinguished in the world of microbiology due to its large genomic size and being a prolific, opportunistic human pathogen. These are Gram negative, rod shaped, motile and aerobic in character. Additionally, they are oxidase and catalase positive Being oxidase positive is a distinguishing feature of Pseudomonas. (Souza, et al., 2018). They also release

exopoly saccharide alginate, Psi and Pe which are used in the formation of biofilms. (Souza, L. C. D., Lopes, F. F., Bastos, E. G., & Alves, C. M. C., 2018). Especially those found in nosocomial infections are typically highly resistant forms. There have been reports of Pseudomonas being resistant to beta-lactam antibiotics and penicillin group. The antibiotic resistance may be as a result of their large genomes, poring channels that facilitates efflux pumps and once again, biofilm formation.

1.7 *Klebsiella* species:

Another opportunistic pathogen that is responsible for nosocomial infections is *Klebsiella*. Rod shaped gram negative *Klebsiella* species are facultative anaerobes and have a slimy capsule layer outside it. These bacteria are capable of causing many severe diseases that include septicemia, pneumonia, meningitis, urinary tract infection and more. Normally, they are a regular part of human nasal, oral and gastrointestinal floras. (M. T., 2023) However, when a patient is immunocompromised or has an infection where the bacteria are present, they can turn pathogenic Cancer patients with oral ulcer who have weakened immunity are highly vulnerable to *Klebsiella* infections. Additionally, there is no available vaccine against these bacteria and their resistance to regularly used antibiotics makes them far more dangerous. (Bush, et al.2023)

1.8 *Staphylococcus* species:

Staphylococcus is a genus of Gram-positive bacteria in the family *Staphylococcaceae* from the order Bacillales. *Staphylococcus aureus*, from the less virulent coagulase-negative *staphylococcal* species. Coagulase-positive *S. aureus* is among the most ubiquitous and dangerous human pathogens, for both its virulence and its ability to develop antibiotic resistance. A carrier state is common. Pathogenic *staphylococci* are ubiquitous. They are carried, usually transiently, in the anterior nares of about 30% of healthy adults and on the skin of about 20%; from these locations, *staphylococci* can cause infection in the host and others. (Bush, et al. 2023). Some have local effects; others trigger cytokine release from certain T cells, causing serious systemic effects.

1.9 *Antibiotic Resistance*:

Although a more recent term than cancer, antibiotic resistance has been appearing to be destructive in equal magnitude. Since the emergence of antibiotics as a means to treat bacterial infections, the global health scenario has improved multi-fold. However, with the rise of microbes which are no longer susceptible to commonly used antibiotics. Some bacteria could evade the deadly effect of antibiotics by acquiring mutations. (Stoopler, E. T. 2014). Then, by the process of natural selection, those bacteria may carry on and pass the resistant genes into the remaining gene pool. There are 3 major ways by which bacteria can show antibiotic resistance. These involve enzymatic degradation of the antibiotic agent, alteration of the site where the antibiotic would have initially worked and lastly by pumping out the agent out of the cell. Efflux pumping that Pseudomonas undertakes is an example of the latter. Initially, antibiotic resistance was seen in the Gram-positive *Staphylococcus aureues* (Kumarasamyet al., 2010); however, recently, it is the gram negative organisms which are showing more resistance to antibiotics. Due to horizontal gene transfer, antibiotic resistance is being spread out. The gene transfer is taking place mainly through plasmid (Zhang et al, 2011) and chromosomal DNA that can include mobile elements such as transposons, integrons and R-plasmid. As mentioned before, cancer patients often lose their natural immune responses due to the treatment regimen. In such situations, they are highly at risk of various microbial infections. Especially, those patients which already have infections in their oral cancer sites fall under an even greater risk. However, determination of the most suitable antibiotic is also important as all classes of antibiotics have a degree of side effects that can be lethal to cancer patients. Therefore, it becomes crucial to administrate the most suitable antibiotic. Moreover, antibiotic usage affects the normal flora of the body (Yassouret al, 2016) which can later aggravate the patients. By conducting antibiotic susceptibility testing, the effects of various antibiotics on a certain bacterial isolate can be tested.

1.10 Antibiotics used for the analysis:

For oral microbiota, not all antibiotics can be used. The groups of antibiotics that can be used are cephalosporin's, aminoglycosides, quinolones, carbapenems, penicillin and several other antibiotic classes. Sometimes, a mixture of antibiotics is given to cover both gram positive and gram negative bacterial pathogens.

Penicillin

Penicillin is a group of antibiotics that contain a beta-lactam ring which is capable of destroying the peptidoglycan layer, which is a major constituent of bacterial cell walls The antibiotics that fall into this vast group are penicillin -G, penicillin -V, ampicillin, amoxicillin, and many more. As time is proceeding, most bacterial species are showing resistance against the penicillin group. The enzyme beta-lactamase that certain gram negative bacteria can produce degrades the active beta-lactam ring. Hence, the antibiotics can no longer work. Even though, these are therefore not very effective, they are administered in combination with other antibiotics to the general and cancer patients.

Cephalosporin

Cephalosporin These are also beta-lactam antibiotics that halt the peptidoglycan layer formation of the bacterial cell wall (Kalmanet al, 1990). Contrary to beta-lactam antibiotics, these are less susceptible to beta-lactamase degradation. However, bacteria such as *E. coli, Pseudomonas aeuroginosa* are showing increased resistance to this group. Some of the members of this antibiotics class are cefepime, cefuroxime, ceftazidime.

Carbapenems

Carbapenems are the latest lines of defense against bacteria. Similar to other beta-lactam antibiotics, these disrupt cell wall formation, although, these offer a broader range of effectiveness. However, bacteria which are resistant to this group's antibiotics are also alarmingly becoming more frequent. *Pseudomonas* and *Klebsiella* are some of the common bacterial pathogens that are showing resistance against this group (Gazinet al, 2012). It is often prescribed in combination/conjugation/addition with aminoglycosides to attain control over *Pseudomonas* infections. Despite the combinations, some microbes are escaping its action, resulting in chronic infections. The antibiotics that fall under this group are imipenem and meropenem.

Aminoglycosides

This group of antibiotics is mostly effective against gram negative bacteria. They work by inhibiting protein synthesis. The antibiotics that fall into this group are kanamycin, gentamicin and

amikacin. There have been reports of genes such as aac, an and aph that can inhibit the effect of aminoglycosides by enzymatic modification. The bacteria harboring these genes can therefore become resistant to this group of antibiotics.

Quinolones

Among the quinolones, the antibiotics that fall under this class are ciprofloxacin, levofloxacin. These antibiotics have a 4-quinolone ring in their structure. These exhibit bactericidal activity by inhibiting DNA synthesis (Normack & Normack, 2002. Quinolones are effective against both gram positive and negative bacteria. Similar to other groups, antibiotic resistance is seen against these as well.

Others

Others Apart from the major groups mentioned above, other antibiotics that are used in oral treatment and for prophylaxis are linezolid, metronidazole, colistin and so on. Colistin is another protein synthesis inhibitory antibiotic that works well against many resistant bacteria. Colisitin is used when other antibiotics appear to be ineffective. However, colistin has many side effects; hence it is reserved as a last resort.

1.11 Objectives of the study

The whole paper very precisely focuses on some particular key points. The main objectives of this research are:

1. Analysis of probable oral microbiological profile of Dhaka city people.

2. To understand the susceptibility and resistance patterns of the pathogens that are found from the sample.

3. Finding the associates risk and evaluating survey from the patient's lifestyle.

Chapter 2
Materials and Methods

2.1. Materials:

2.2. Study Place:

This study was conducted in the BRAC University. All investigations and experiments related to this project were performed in the Biotechnology and Microbiology Laboratory of the Department of Mathematics and Natural Sciences in BRAC University, Mohakhali, Dhaka.

2.3. Study Duration:

The duration of the study was from November,2022 to September,2023.

2.4. General Procedure and Equipment:

By autoclaving at 121ºC for 60 minutes all media were sterilized in an autoclave such as pipettes, Petri dishes, test tubes, cotton swab, small vials etc. before use. All agar media, agar slant, and biochemical reagents were prepared freshly and kept in a refrigerator at 3-4ºC.Only LB Luria Broth media was stored at room temperature. All inoculations and sub-culture were done in a laminar airflow cabinet under aseptic conditions. The inoculated cultures were incubated in the incubator at 37ºC and 44ºC.

2.5 Sample site:

We collected a total of 75 cotton swab samples from 25 clinical patients, with each patient providing three swab samples. These samples were gathered from two branches of Anam Laser Dental & Implant Center.

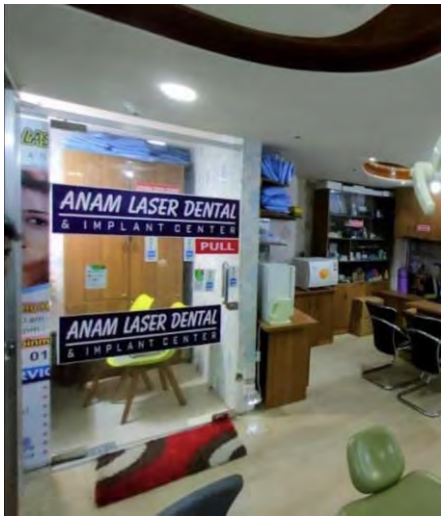


Figure 2.1: Oral sample taken from Anam laser dental & implant center.

2.6. Sample transportation:

An ice box was used for collecting the oral microbial sample to maintain the temperature and also prevents the growth and proliferation of the sample. For the collection of hospital samples, an autoclaved test tube was used and for the non-clinical sample collection also an autoclaved test tube was used. Before the sample collection, lab equipment such as test tube, cotton swab was autoclaved because all the equipment must be in sterile condition. Also, necessary precautions were maintained such as using gloves and masks because various types of unwanted microorganisms can be infected during collecting the samples.

Figure 2.2: Ice box

2.7. Culture media

For total viable count (TVC), *Staphylococcus, Klebsiella* and *Pseudomonas* different types of media were used. Media like Nutrient agar (NA), Mannitol Salt Agar (MSA), Cetrimide Agar, Hi chrome Agar, Mueller Hinton Agar (MHA) were used for bacterial isolation purposes. Details of these culture media are as below:

Nutrient Agar (NA):

Nutrient Agar is used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for bacterial growth. It was prepared by weighting 28g of its powder and dissolving in 250ml of distilled water in a conical flask. After stirring on heat it was boiled. Later, the flask was covered in aluminum foil and kept in autoclave for sterilization. After the liquid was sterile, it was plated into Petri dishes.

Figure 2.3: Survey question form for Clinical Patient:

A Survey on Oral Microbial Profile and Their Association with Different Diseases in Dhaka City Population

Sample Type	Clinical (C)	Non Clinical (N)	Date

Subdivision 01: Participant and Interviewer Information:

PAETICIPANT

ID Number	
Name (optional)	
Address/ Location	
Contact Number (optional)	
Venue of Interview	
Signature and Date	

INTERVIEWER

Name	
Institution	
Supervisor	
Signature and Date	

Subdivision 02: Socio Economic Information of Participant:

Sex	Male	
	Female	
	Other	
Age (In Number)		
Education	No Education/ Home Schooled	
	Primary	
	Secondary	
	Higher Secondary	
	Graduate	
	Masters	
	Other	

Figure 2.4 Consent form for Clinical Patient:

PARTICIPANT INFORMED CONCENT FORM

School of Data and Sciences:
Department of Mathematics and Natural Sciences:
(Biotechnology Program)

NAME OF THE STUDY	Oral Microbial Profile of Dhaka City Dwellers and Their Association with Different Diseases in the Community

INFORMATION ABOUT INVESTIGATOR:

Principle Investigator:	
Co-Investigator(s):	
Faculty Advisor(s):	
Institute/ Department Information:	Name: Address: E- mail: Contact Number(s):

PURPOSE OF STUDY:

To have a view of the overall dental health of people living in Dhaka city, identification of bacteria that gives early warnings of plaque development and other oral diseases. Furthermore, we can identify relationships between dental health and other systemic diseases in the community.

CAN YOU PARTICIPATE?

Everyone who is at least **18 years or older,** who are able to read and understand the terms and conditions of this study and free to give his/her own consent, are welcome to participate in this study.

STUDY PROCEDURES:

- Explaining the informed consent form to the patient
- Taking the informed consent
- Collect the survey information
- Collect the dental swab in sterile method
- Transportation of sample To Life Science Laboratories, BRAC University
- Analysis and characterization of dental bacteria in the lab
- Analyze survey data by statistical methods
- Identifying correlations in survey data and analyzed samples
- Deposition of data

Form ID Number:_____

Mannitol Salt Agar (MSA):

Mannitol salt agar or MSA is a commonly used selective and differential growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. It is also a differential medium for mannitol-fermenting staphylococci. It was prepared by weighting 500g of its powder and dissolving in 250ml of distilled water in a conical flask. After stirring on heat it was boiled. Later, the flask was covered in aluminum foil and kept in autoclave for sterilization. After the liquid was sterile, it was plated into Petri dishes.

Cetrimide Agar:

Cetrimide agar is a type of agar used for the selective isolation of the gram-negative bacterium, Pseudomonas aeruginosa. It contains selective agent against alternate microbial flora. Cetrimide also enhances the production of Pseudomonas pigments, which show a characteristic blue-green and yellow-green color, respectively. Cetrimide agar is widely used in the examination of pharmaceuticals and clinical specimens to test for the presence of Pseudomonas aeruginosa. It was prepared by mixing 46.7g of the laboratory grade powder with 250ml of distilled water. After dissolving the powder in the water through heating, it was sealed in aluminum foil and autoclaved. Later, the liquid media was poured into dry sterilized Petri dishes. The dishes were later used for bacterial culture.

Hi-Chrome KPC Agar:

Hi-Chrome agar is used to differentiate among many opportunistic pathogens that cause nosocomial and urinary tract diseases. For consisting several dyes and compounds that different bacteria break with their unique enzymes, this is called a chromogenic dye. The preparation of Hi-Chrome included mixing 56.8g powder into conical flask containing 250ml distilled water. Later boiling and autoclaving followed by pouring into Petri dishes.

Mueller-Hinton Agar:

Mueller-Hinton Media is used for the antibiotic susceptibility test of bacteria. By adding 38g of Mueller Hinton agar powder in 250ml distilled water and boiling while stirring, MHA was

prepared. The opening of the flask containing the mixture was wrapped in aluminum foil and autoclaved for sterilization. After sterilization, the liquid was poured into sterile Petri dishes.

2.8. Biochemical test media:

Simmon's citrate agar: Simmons citrate agar tests were used to observe the ability of organisms to utilize citrate as a carbon source. If the medium turns blue, the organism is citrate-positive. If there is no color change, the organism is citrate-negative.

TSI (Triple Sugar Iron) agar: Triple sugar iron agar is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate enteric based on the ability to reduce sulfur and ferment carbohydrates.

MR (Methyl Red) broth: Methyl Red test determines the microbe performs mixed acids fermentation when glucose is supplied. After the addition of methyl red reagent to the culture media, if it turns pink then it is a positive result and if it remains yellow then it is a negative result.

VP (Voges-Proskauer) broth: This test is used to detect acetoin in a bacterial broth culture. The test is performed by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth which has been inoculated with bacteria. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result.

2.9. Methods

2.10. Samples Collection:

Twenty-five different oral microbial clinical samples and twenty-five non- clinical samples were collected from different patients in different places in Dhaka city. We are involving people who visited a dentist for dental problems as well as those he did not yet. after getting respondents informed consent, we will collect dental swab from their mouth. Samples were collected in test tube. All samples should be brought to the laboratory for culture as soon as possible. Next, we will culture and identify the oral bacteria in different methods and relate it with the information from

the survey. Finally, the study will give us a view of the overall lifestyle dental health of people living in Dhaka city. We can also identify the bacteria that gives early warnings of plaque development and other oral diseases. Furthermore, we can identify relationships between dental health and other systemic diseases in the community.

2.11. Sample Processing and Inoculation:

Sample Collection and Inoculation: The collection and inoculation of samples were conducted with sterility and precision. A cotton swab containing 0.9% saline solution was employed as the collection tool in a test tube. The swab was gently inserted into the target area around the teeth where caries has formed and rotated to ensure effective sample collection. We prepared selective culture media appropriate for our research objectives. These media were selected to encourage the growth of target microorganisms while inhibiting the growth of others. The collected samples were evenly spread, or "lawned," onto the prepared selective culture media. This was done to encourage the growth and proliferation of any microorganisms present in the sample. The inoculated culture plates were then incubated at a controlled temperature of 37 degrees Celsius for a standardized duration of 24 hours. This incubation period allowed sufficient time for microbial colonies to develop. Following the incubation period, the culture plates were carefully examined. The growth and morphology of microbial colonies were observed and documented.

2.12 Sample spreading:

Equipment: Cotton swab, test tube, saline solution, spirit lamp, 70% alcohol.

Media: MSA agar, Cetrimide agar & Hi-Chrome KPC agar.

Sample: Oral sample collected from clinic and non-clinical sample collected from laboratory

Procedure:

I. At first, the hand was cleaned with 70% ethanol and all media plates were labeled by a marker.
II. One milliliter dental sample was taken in a test tube containing 9 ml
saline (0.9%NaCl solution).
III. Now through the cotton swab sample was taken and spread on a Cetrimide agar plate. Before spreading, the spreader was burnt into the burner to avoid contamination.
IV. For Non clinical samples, the same technique was followed.

V. Samples were spread by the same process on Hi-chrome KPC agar, MSA and MacConkey agar.

VI. Next, the plates were incubated for 24-48 hours at 37°C.

VII. After the incubation period, the results were observed and recorded.

2.13. Stock culture method:

LB Agar media was used to stock bacteria that were found in the samples. To prepare 200ml LB agar media, we used 1.2g agar,1g yeast, 2g tryptone and 2g NaCl. LB media was preserved at room temperature after the growth of bacteria. First, the bacterial colony will be taken by a needle from 24 hours of subculture plate. Subculture should be done from the nutrient agar plate. Then bacterial colonies were inoculated by stabbing and incubated for 24 hours. Now this stock culture can keep for several months at room temperature.

2.14. Biochemical test methods:

Different types of unknown bacteria were found after spreading the sample on different types of agar plates. For the identification of those unknown bacteria, different types of biochemical tests were performed. They are as below:

Gram-staining: Gram staining allows bacteria to be differentiated in terms of their cell wall composition. From overnight culture of the organism, at first, clean glass slides were obtained. Using a sterile technique smears of the organisms were prepared. The smear was allowed to air dry and then heat fixed. The smear was flooded with crystal violet for 1 minute. Then the smear was gently washed with tap water. Then the smear was gently flooded with Gram's iodine mordant for 1 minute. After that, the smear was gently washed with tap water again. In the next step, the smear was decolorized with 95% ethyl alcohol. The reagent was added 10 drops for 10 seconds until crystal violet failed to wash from the smear. Later the smear was gently washed with tap water. After that smear was counterstained with safranin for 45 seconds. Again the smear was gently washed with tap water. Finally, the slide was air-dried and examined under a microscope with oil immersion. Under the microscope if the bacterial cells appear pink then it is gram-negative and if they appear purple then gram-positive. Shapes of the cells can be different types like rods, cocci, spiral, bacilli, etc.

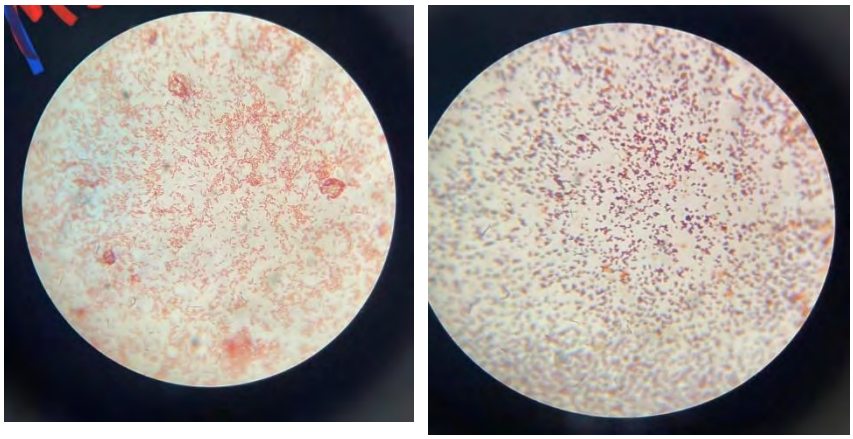


Figure 2.5 :(a) gram negative red pink rod shape (b) gram positive purple color cocci shape (c) gram staining procedure

Oxidase test: The oxidase test is used to determine if an organism possesses the cytochrome-c oxidase enzyme. Oxidase positive bacteria contain cytochrome c oxidase and produce a change in color of the reagent from colorless to bluish or purplish in less than 30 seconds. oxidase negative bacteria do not contain cytochrome c oxidase and do not change the color of oxidase reagent in less than 30 seconds. The oxidase test is used to identify bacteria that produce cytochrome oxidase, an enzyme of the bacterial electron transport chain. At first, enough quantity of bacteria from the nutrient agar plate was picked and placed on a piece of filter paper. One drop of the oxidase reagent (Tetramethyl-p-phenylenediaminedi-hydrochloride) was added at the place of the culture on the filter paper and mixed with a sterile toothpick. A positive reaction turned the bacteria from violet to purple within 20 seconds.

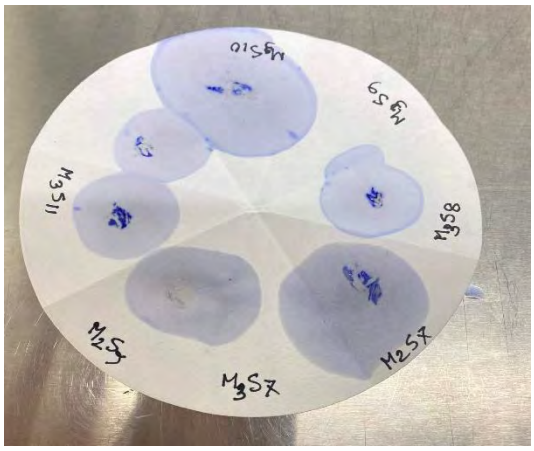


Figure2.6: Oxidase test

Catalase test: The catalase test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. At first, the inoculating loop was flamed and cooled. Then a small amount of a bacterial colony (18 to 24 hours old) was placed on a clean glass slide. The inoculating loop was flamed and cooled again. One or two drops of 3% hydrogen peroxide (H2O2) were added. A positive result gave a rapid evolution of oxygen within 5-10 seconds and was evidenced by a bubbling reaction. A negative result showed no bubble.

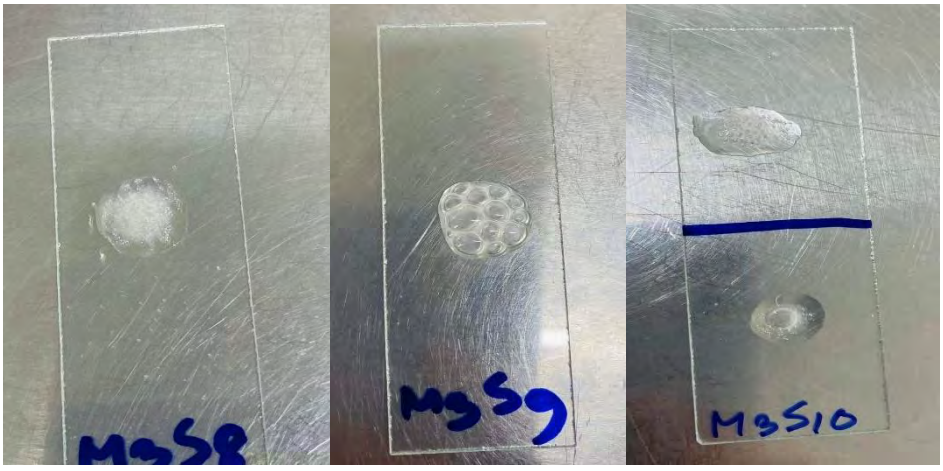


Figure 2.7: catalase test positive and negative

TSI (Triple Sugar Iron) test: TSI media was used for determining whether bacteria can ferment glucose, and lactose or they can produce Hydrogen Sulfide or other gas. This will help to differentiate between various enterobacteriaceae including 24 intestinal pathogens *Salmonella* and

Shigella. TSI media have two parts one is a butt with a poorly oxygenated area at the bottom and the other is a slant with a well-oxygenated area at the top.

Interpretation of Triple Sugar Iron Agar Test:

i. If lactose or sucrose is utilized, then a huge amount of acid production is indicated by changing the color from red to yellow.

ii. If gas is produced, then there will be a crack in the media.

iii. If H2S is produced, then there will be black precipitation.

iv. If lactose is not fermented but a small amount of glucose is fermented, then the butt will be Yellow and the slant will be Red.

v. If neither glucose nor lactose nor sucrose is fermented then both butt and slant will remain red.

vi. If Ammonia is produced, then the slant can become a deeper red-purple.

Equipment: Bunsen burner, inoculating loop.

Procedure:

1. Using a marker, the TSI tubes were labeled.
2. Using a sterile technique, each experimental organism was inoculated into its appropriately labeled tube using stab and streak inoculation (inoculated by stabbing into the agar butt with an inoculating wire and streaking the slant in a wavy pattern).
3. The tubes were incubated for 24 to 48 hours at 37°C.
4. After the incubation period results were observed and recorded.

The expected results of the TSI agar test are:

- Yellow slant/yellow butt: lactose fermentation. No H2S formation
- Red slant/red butt/no black color: No fermentation and no H2S formation
- Yellow slant/Yellow butt: lactose fermentation, No H2S formation.

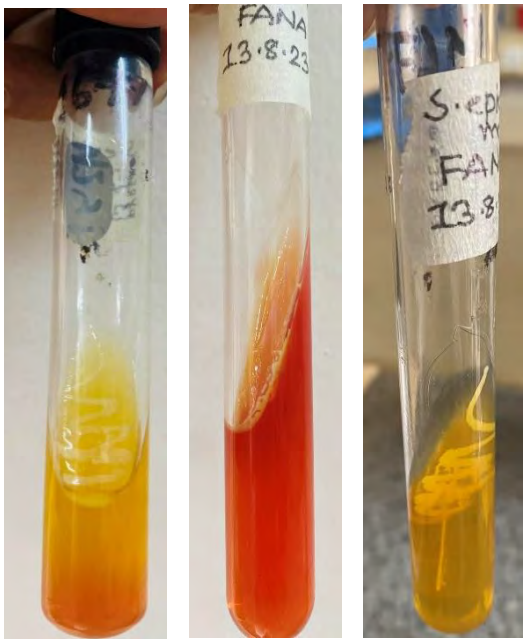


Figure2.8 :(a) no fermentation, no H2S formation, (b) no lactose fermentation (c) no lactose but glucose fermentation

Simmon's citrate test:

This test was done to differentiate among enteric organisms based on their ability to ferment citrate as the sole source of carbon and energy. Citrate is used by microorganisms as a carbon source for their energy when there is no easily fermentable glucose or lactose. This can be possible if that organism contains citrate permease. For this test bacterial culture was obtained from a 24-hour subculture plate. Sterile citrate agar media was taken and using a marker it was labeled. Then using an inoculating needle and aseptic technique, a bacterial colony was taken. The needle was inserted into the butt of the citrate and as the needle was pulled out of the butt, the slant was streaked in a zigzag manner. Next, it was incubated for 24 to 48 hours at 37°C. After the incubation period, the simmon's citrate slants were taken out of the incubator and observed for color changes. The Prussian blue color of the agar indicates positive results and the green color indicates a negative result.

figure 2.9: Citrate test results, Green is negative result, and Blue ones are the positive Results.

Methyl Red (MR) test

Methyl red test detects the ability of an organism to produce and maintain stable acid end products from glucose fermentation. In this test methyl red is the pH indicator which detects the presence of a large concentration of acidic end products. Methyl Red test determines the microbe performs mixed acids fermentation when glucose is supplied. In sterile peptone water loop-full of bacterial colony was inoculated. For performing this test fresh 24-hour bacterial for MR test.

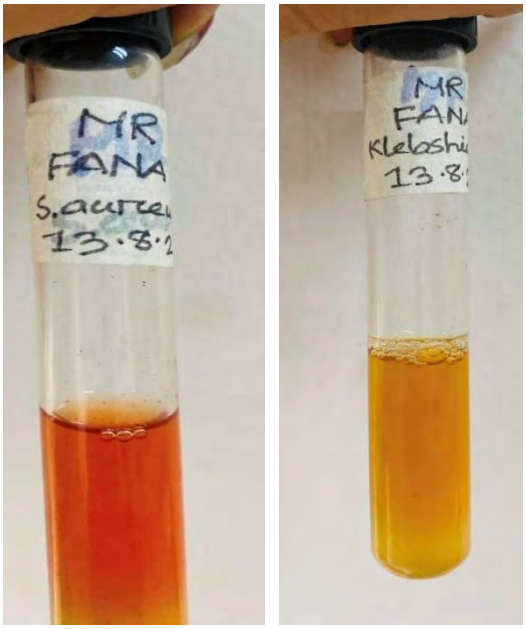


Figure 2.10: (a) positive result for MR test (b) negative result for MR test

subculture was used. One sterile tube of MR broth was taken. Then, using a marker it was labeled. With an inoculating loop and ascetic technique, a loop full of bacterial culture was transferred into the MR broth. It was assured that the loop was shaken and touched to the side of the tube to remove excess broth. Next, this tube was placed in the incubator for 24 to 48 hours at 37°C. After incubation, inoculated MR broth was taken out of the incubator. Five drops of methyl red indicator were added to the tube and rolled between the hand palms to mix The red color rings indicated positive results for the MR test and the yellow color ring indicated negative results.

Voges-Proskauer (VP) test: VP test detects some organisms that produce neutral or non-acidic end products like acetyl methyl carbinol, from organic acids that result from glucose metabolism. When 40% KOH and 5% alpha-naphthol are added to the solution which is known as Barritt's reagent, it reacts with the acetoin produced by the bacteria in the solution. This reaction will produce diacetyl and a deep rose/pink color in the medium within 30 minutes after the addition of the reagent which is a positive VP test. On the other hand, yellow or the absence of rose/pink color represents a negative result. For performing this test fresh 24-hour bacterial subculture was used. One sterile tube of VP broth was taken. Then, using a marker it was labeled. With an inoculating loop and ascetic technique, a loop full of bacterial culture was transferred into the VP broth. It was assured that the loop was shaken and touched to the side of the tube to remove excess broth. Next, this tube was placed in the incubator for 24 to 48 hours at 37°C. After incubation, inoculated VP broth was taken out of the incubator, and 10-14 drops of Barritt's reagent were added to the tube. The tube was shaken gently for several minutes and waited for 15-20 minutes for color change. Rose/pink color formation is a positive result and yellow or no rose/pink color formation is a negative result.

2.15. Disk diffusion method for antibiotic susceptibility test

Antibiotic Susceptibility test: Antibiotic susceptibility testing was done on the isolated bacterial samples. It was done to check the in vitro effect of selected antibiotics on the isolated organisms. The Kirby Bauer disc diffusion method was done for the antibiotic susceptibility testing. Bacterial suspension was lawn over Mueller Hinton agar plates and antibiotic discs were placed on the law culture.

Mueller Hinton Agar (MHA): Mueller Hinton agar is a type of growth medium used in microbiology to culture bacterial isolates and test their susceptibility to antibiotics. By adding 38g of Mueller Hinton agar powder in 250ml distilled water and boiling while stirring, MHA was prepared. The opening of the flask containing the mixture was wrapped in aluminum foil and autoclaved for sterilization. After sterilization, the liquid was poured into sterile Petri dishes. Mueller Hinton Media is used for the antibiotic susceptibility test of bacteria. For the antibiotic susceptibility test first off all MacFarlane solutions were made.

Equipment: Antibiotic disks, forceps, burner, OD (Optical density) machine, vortex machine, loop, cuvette, saline solution, sterile cotton stick.

Media: Mueller Hinton agar.

Sample: Bacteria collected from tap water.

Procedure:

One to two specific bacterial colonies were taken by a sterile loop from 24 hours of fresh subculture plate. Then it was inoculated into 0.9%NaCl solution (physiological saline) and mixed by vortexing.

Next, the turbidity of the saline solution was compared with the MacFarlane solution. Turbidity was observed by the OD machine at 360 nm. If the turbidity of the saline solution and MacFarlane solution becomes the same then this saline solution containing bacteria can be used for the test.

After taking turbidity, a cotton swab was dipped into the turbid saline solution, and the bacterial lawn was made on Muller Hinton agar media.

Through sterile forceps, specific antibiotics were placed on the inoculated agar media, and disks were slightly pressed on the agar to place them well.

Then inoculated plates were incubated at 37ºC for 24 hours.

After the incubation period plates were observed and results were recorded.

Results were taken by observing and measuring the diameter of the clear zone around the antibiotics disks. According to the diameter of the clear zone, it was determined whether the organisms were susceptible, intermediate, or resistant to antibiotics. No clear zone also indicates resistance to the antibiotic

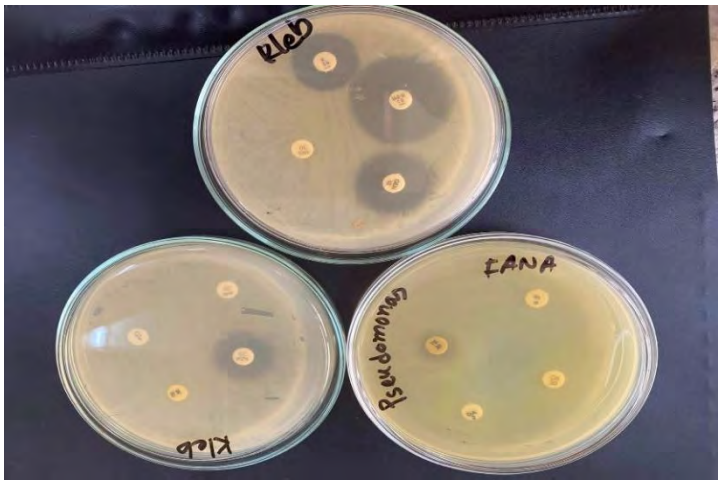


Figure 2.11: Antibiotic susceptibility test: Clear zone indicates susceptibility to antibiotics and no clear zone indicates resistance to the antibiotic

2.16 List of Antibiotic Used

Table 2.1: Name of antibiotics and their sensitivity level

Antibiotics Name	Sensitivity level (mm)		
	Resistant	Intermediate	Susceptible
Doxycycline	≤10 mm	11-13	≥14
Ampicillin	≤ 13	14-16	≥ 17
Gentamicin	≤ 12	13-14	≥ 15
Streptomycin	≤ 11	12-14	≥ 15
Ciprofloxacin	≤ 15	16-20	≥ 21
Penicillin	≤14	15-16	≥ 17
Tetracycline	≤ 14	15-18	≥ 19
Meropenem	≤ 13	14-15	≥ 16
Amikacin	≤ 14	15-16	≥ 17
Imipenem	≤ 19	20-22	≥ 23
Colistin	≤ 12	13-14	≥ 15
Cefixime	≤ 15	16-18	≥ 19
Kanamycin	≤ 12	13-14	≥ 15
Levofloxacin	≤13	14-16	≥ 17
Amoxicillin	≤ 13	14-17	≥ 18

Chapter 3
Results

3.1 Results from growth on selective media

Test tubes containing saline that were inoculated with oral swabs both non-clinical; and clinical were lawn on 3 selective media namely, Hi-chrome KPC agar, Cetrimide agar and Mannitol salt agar and then streaked on the Nutrient agar media. Total clinical and non-clinical 50 samples (25 each) were collected, some of the specimen showed growth and some not. In terms of clinical samples, 24 patients showed growth on the both Hi-chrome KPC agar media and Cetrimide agar media where 22 sample's showed positive growth result on MSA agar media.

Table 3.1: Growth of the isolates in selective media.

Clinical Sample no	Media name						
	Hi-chrome KPC		Cetrimide		MSA		
	Colony Appearance		Colony Appearance		Colony Appearance		
	Greenish Blue	No growth	Light green	No growth	yellow	Pink	No growth
25	24	1	24	1	22	5	3

3.2 Biochemical tests result of isolated bacteria

The individual distinct colonies that were found from the selective media were streaked on nutrient agar ton observe visual similarities in terms of colony morphology. A total 25 samples of clinical patients under treatment were taken and later biochemical tests were done for further identification of organism's. Bacteria were isolated and sub cultured for biochemical testing after spreading and streaking. In biochemical testing, a 24-hour fresh culture was used. The required biochemical tests were performed, and the results were recorded. The results were listed in a chart according to the findings obtained from the tests.

Table 3.2: Biochemical test results of the isolates from clinical patient and their probable identity.

Isolates no	Media used for selection	MR Methyl Red	(VP) Voges proskeur	Catalase	Oxidase	Citrate	TSI				Gram staining Color and shape	Probable Organism	
							Slant/butt color	Glucose	H_2S Production	Gas Production			
1	Hi-chrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
2	Hi-chrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink, Rod	*Pseudomonas* species	
3	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
4													
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	

Y=Yellow; R= Red; "+" = Positive; "-"= Negative

Isolates no	Media used for selection	MR Methyl Red	(VP) Voges proskeur	Catalase	Oxidase	Citrate	TSI				Gram staining Color and shape	Probable Organism	
							Slant/butt color	Glucose	H_2S Production	Gas Production			
5	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
6	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
7	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
8	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
9	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
10	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	

Y=Yellow; R= Red; "+" = Positive; "-" = Negative

Isolates no	Media used for selection	MR Methyl Red	(VP) Voges proskeur	Catalase	Oxidase	Citrate	TSI				Gram staining Color and shape	Probable Organism	
							Slant/butt color	Glucose	H_2S Production	Gas Production			
11	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
12	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
13	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
14	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
15	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
16	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	

Y=Yellow; R= Red; "+" = Positive; "-" = Negative

Isolates no	Media used for selection	MR Methyl Red	(VP) Voges proskeur	Catalase	Oxidase	Citrate	TSI				Gram staining Color and shape	Probable Organism	
							Slant/butt color	Glucose	H_2S Production	Gas Production			
17	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
18	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
19	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
20	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
21	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
22	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	

Y=Yellow; R= Red; "+" = Positive; "-" = Negative

Isolates no	Media used for selection	MR Methyl Red	(VP) Voges proskeur	Catalase	Oxidase	Citrate	TSI				Gram staining Color and shape	Probable Organism	
							Slant/butt color	Glucose	H_2S Production	Gas Production			
23	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
24	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	
25	Hichrome KPC	–	+	+	–	+	Y/Y	+	–	–	Pink, rod	*Klebsiella* species	
	Cetrimide	–	–	+	+	+	R/R	–	–	+	Pink,Rod	*Pseudomonas* species	
	MSA	+	–	+	–	+	Y/Y	+	–	+	Purple, Cocci	*Staphylococcus* species	

Y=Yellow; R= Red; "+" = Positive; "-" = Negative

3.3 Identification of the isolates (Clinical):

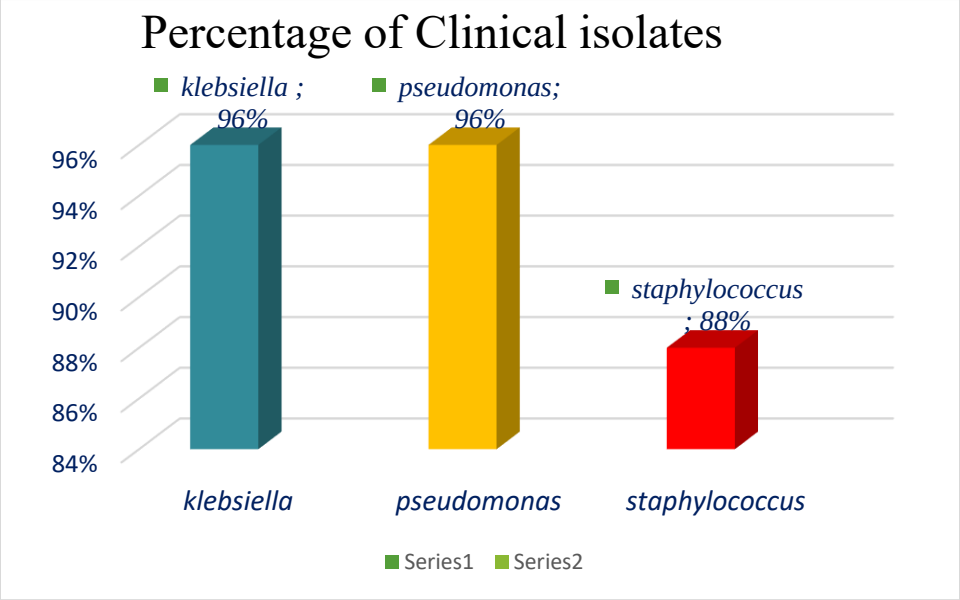
After the selection from selective media and biochemical tests the probable organisms were *pseudomonas* species, *Klebsiella* species and *Staphylococcus* species.

The number and ratio of isolated organisms are given below

Table 3.3: percentage of Clinical Isolates

Organism	Total sample number	Probable organisms in isolates (100%)
Klebsiella	25	24 (96%)
Pseudomonas	25	24(96%)
Staphylococcus	25	22(88%)

This table shows the highest number of isolates were *klebsiella* and *pseudomonas* followed by Staphylococcus species.



Graph 3.1: Percentage of Clinical isolates

3.4 Identification of the isolates (Non-Clinical):

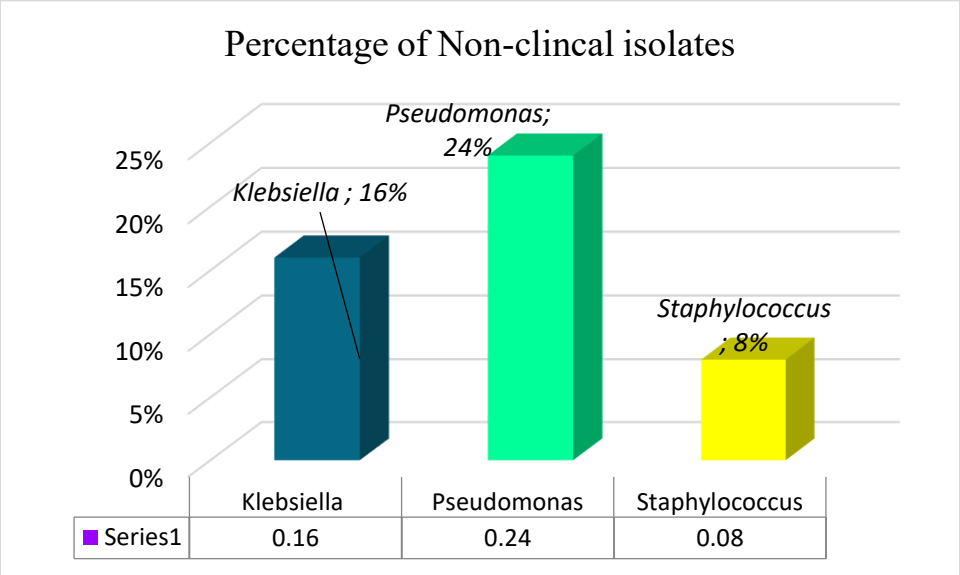
After the selection from selective media and biochemical tests the probable organisms were *pseudomonas* species, *Klebsiella* species and *Staphylococcus* species.

The number and ratio of isolated organisms are given below

Table 3.4: percentage of Non-clinical isolates

Organism	Total sample number	Probable organisms in isolates (100%)
Klebsiella	25	4(16%)
Pseudomonas	25	6(24%)
Staphylococcus	25	2(8%)

This table shows the highest number of isolates were *klebsiella* and *pseudomonas* followed by Staphylococcus species.



Graph 3.2: percentage of Non-clinical isolates

Table 3.5: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. For (***Klebsiella)***

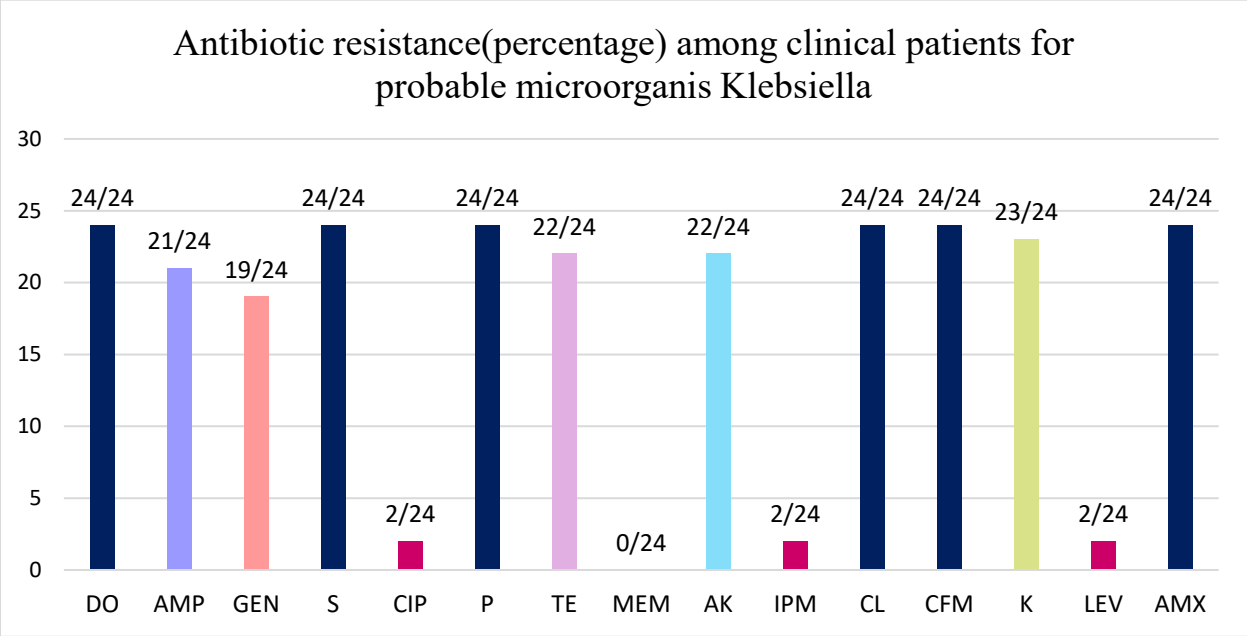
SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M1S1	**-R**	**- R**	**10R**	**8R**	**22S**	**-R**	**18S**	**8R**	**10R**	**23S**	**-R**	**12R**	**4R**	**24S**	**-R**
M1S2	**-R**	**-R**	**9R**	**-R**	**28S**	**-R**	**28S**	**10R**	**12R**	**28S**	**-R**	**12R**	**-R**	**25S**	**-R**
M1S3	**-R**	**- R**	**12R**	**6R**	**26S**	**-R**	**24S**	**6R**	**12R**	**22S**	**-R**	**10R**	**-R**	**26S**	**-R**
M1S5	**-R**	**- R**	**8R**	**-R**	**24S**	**-R**	**18S**	**6R**	**8R**	**24S**	**-R**	**4R**	**-R**	**24S**	**-R**
M1S6	**-R**	**-R**	**10R**	**-R**	**14R**	**-R**	**20S**	**8R**	**6R**	**26S**	**-R**	**8R**	**-R**	**25S**	**-R**
M1S7	**-R**	**- R**	**12R**	**-R**	**28S**	**-R**	**24S**	**8R**	**10R**	**28S**	**-R**	**12R**	**-R**	**25S**	**-R**
M1S8	**-R**	**-R**	**4R**	**-R**	**30S**	**-R**	**28S**	**8R**	**10R**	**30S**	**-R**	**10R**	**-R**	**25S**	**-R**
M1S9	**-R**	**- R**	**6R**	**4R**	**12R**	**-R**	**30S**	**18S**	**8R**	**19R**	**-R**	**8R**	**-R**	**23S**	**-R**
M1S10	**-R**	**-R**	**10R**	**10R**	**22S**	**-R**	**32S**	**12R**	**18S**	**26S**	**-R**	**4R**	**-R**	**22S**	**-R**
M1S11	**-R**	**18S**	**15S**	**7R**	**24S**	**-R**	**18S**	**8R**	**14R**	**32S**	**-R**	**12R**	**4R**	**25S**	**-R**
M1S12	**-R**	**-R**	**4R**	**-R**	**22S**	**-R**	**22S**	**22S**	**14R**	**28S**	**-R**	**10R**	**4R**	**28S**	**-R**
M1S13	**-R**	**- R**	**8R**	**-R**	**24S**	**-R**	**24S**	**12R**	**13R**	**24S**	**-R**	**4R**	**2R**	**22S**	**-R**
M1S14	**-R**	**-R**	**4R**	**10R**	**26S**	**-R**	**22S**	**10R**	**10R**	**24S**	**-R**	**6R**	**2R**	**26S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible

Table 3.6: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. For (***Klebsiella)***

SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M1S15	**-R**	**- R**	**10R**	**-R**	**20S**	**-R**	**22S**	**8R**	**8R**	**24S**	**-R**	**8R**	**18S**	**23S**	**-R**
M1S16	**-R**	**-R**	**15S**	**-R**	**22S**	**-R**	**21S**	**8R**	**10R**	**24S**	**-R**	**10R**	**-R**	**24S**	**-R**
M1S17	**-R**	**- R**	**6R**	**-R**	**28S**	**-R**	**18S**	**12R**	**6R**	**14R**	**-R**	**12R**	**-R**	**24S**	**-R**
M1S18	**-R**	**-R**	**8R**	**-R**	**30S**	**-R**	**32S**	**12R**	**10R**	**26S**	**19S**	**12R**	**-R**	**23S**	**-R**
M1S19	**-R**	**- R**	**18S**	**4R**	**32S**	**-R**	**26S**	**10R**	**12R**	**22S**	**-R**	**10R**	**-R**	**12R**	**-R**
M1S20	**-R**	**17S**	**4R**	**-R**	**24S**	**-R**	**30S**	**10R**	**6R**	**24S**	**-R**	**8R**	**17S**	**24S**	**-R**
M1S21	**-R**	**- R**	**12R**	**6R**	**22S**	**-R**	**32S**	**12R**	**10R**	**24S**	**-R**	**9R**	**4R**	**26S**	**-R**
M1S22	**-R**	**-R**	**16S**	**-R**	**24S**	**-R**	**25S**	**11R**	**12R**	**24S**	**1**	**8R**	**-R**	**22S**	**-R**
M1S23	**-R**	**- R**	**9R**	**-R**	**21S**	**-R**	**28S**	**14R**	**14R**	**28S**	**-R**	**12R**	**-R**	**22S**	**-R**
M1S24	**-R**	**19S**	**16S**	**4R**	**25S**	**-R**	**36S**	**12R**	**10R**	**28S**	**-R**	**10R**	**4R**	**10R**	**-R**
M1S25	**-R**	**20S**	**18S**	**9R**	**24S**	**-R**	**23S**	**6R**	**12R**	**23S**	**-R**	**12R**	**4R**	**22S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible



Graph: 3.3: antibiotic resistance among clinical patients for probable microorganism *klebsiella*

All the isolates from clinical samples were fully resistant to Doxycycline, Streptomycin, penicillin, Colistin, and Amoxicillin. Also followed by partially resistant to Ampicillin, Gentamicin, Tetracycline and Amikacin and Cefixime. The least resistance was seen against Imipenem, Ciprofloxacin, Meropenem and Levofloxacin.

Table 3.7: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. For (***Pseudomonas)***

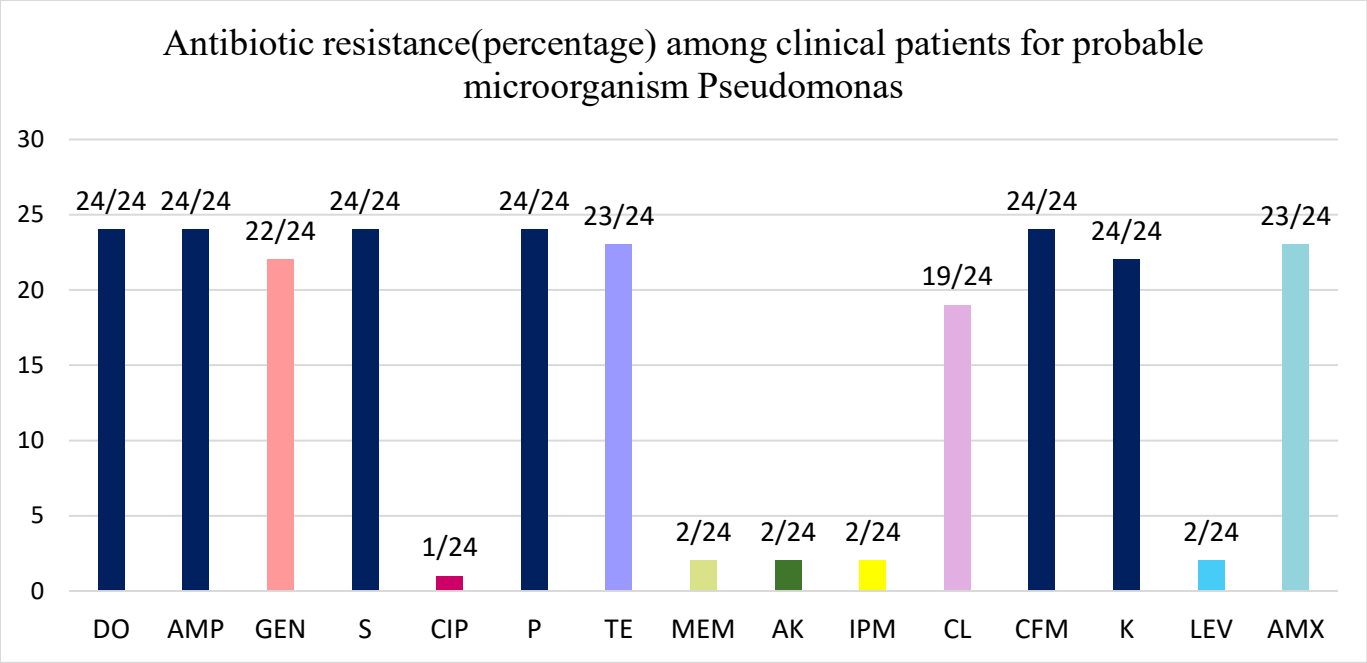
SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M2S1	**-R**	**- R**	**10R**	**8R**	**22S**	**-R**	**18S**	**8R**	**18S**	**23S**	**-R**	**12R**	**4R**	**24S**	**-R**
M2S2	**-R**	**-R**	**9R**	**-R**	**28S**	**-R**	**28S**	**10R**	**22S**	**28S**	**-R**	**12R**	**-R**	**25S**	**-R**
M2S3	**-R**	**- R**	**12R**	**6R**	**26S**	**-R**	**24S**	**6R**	**25S**	**22S**	**-R**	**10R**	**-R**	**26S**	**-R**
M2S4	**-R**	**-R**	**4R**	**-R**	**24S**	**-R**	**28S**	**4R**	**28S**	**24S**	**-R**	**8R**	**6R**	**22S**	**-R**
M2S5	**-R**	**- R**	**8R**	**-R**	**24S**	**-R**	**18S**	**6R**	**24S**	**24S**	**-R**	**16S**	**-R**	**24S**	**-R**
M2S6	**-R**	**-R**	**10R**	**-R**	**14R**	**-R**	**20S**	**8R**	**6R**	**26S**	**-R**	**8R**	**-R**	**25S**	**-R**
M2S7	**-R**	**- R**	**12R**	**-R**	**28S**	**-R**	**24S**	**8R**	**22S**	**28S**	**-R**	**12R**	**-R**	**25S**	**-R**
M2S8	**-R**	**-R**	**4R**	**-R**	**30S**	**-R**	**28S**	**8R**	**26S**	**30S**	**-R**	**10R**	**-R**	**25S**	**-R**
M2S10	**-R**	**-R**	**16S**	**10R**	**22S**	**-R**	**32S**	**12R**	**32S**	**26S**	**-R**	**4R**	**-R**	**22S**	**-R**
M2S11	**-R**	**-R**	**15S**	**7R**	**24S**	**-R**	**18S**	**8R**	**18S**	**32S**	**-R**	**12R**	**4R**	**25S**	**-R**
M2S12	**-R**	**-R**	**4R**	**-R**	**22S**	**-R**	**22S**	**22S**	**20S**	**28S**	**-R**	**10R**	**4R**	**28S**	**-R**
M2S13	**-R**	**- R**	**8R**	**-R**	**24S**	**-R**	**24S**	**12R**	**22S**	**24S**	**-R**	**4R**	**2R**	**22S**	**-R**
M2S14	**-R**	**-R**	**4R**	**10R**	**26S**	**-R**	**22S**	**10R**	**25S**	**24S**	**-R**	**6R**	**2R**	**26S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible

Table 3.8: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. For (***Pseudomonas)***

SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M2S15	**-R**	**- R**	**10R**	**-R**	**20S**	**-R**	**22S**	**8R**	**8R**	**24S**	**-R**	**8R**	**18S**	**23S**	**-R**
M2S16	**-R**	**-R**	**8R**	**-R**	**22S**	**-R**	**21S**	**8R**	**26S**	**11R**	**-R**	**18S**	**-R**	**24S**	**-R**
M2S17	**-R**	**- R**	**6R**	**-R**	**28S**	**-R**	**18S**	**12R**	**22S**	**14R**	**-R**	**12R**	**-R**	**24S**	**-R**
M2S18	**-R**	**-R**	**8R**	**-R**	**30S**	**-R**	**12R**	**12R**	**23S**	**26S**	**-R**	**12R**	**22S**	**23S**	**-R**
M2S19	**-R**	**- R**	**8R**	**4R**	**32S**	**-R**	**26S**	**10R**	**22S**	**22S**	**-R**	**22S**	**-R**	**12R**	**-R**
M2S20	**-R**	**-R**	**4R**	**-R**	**24S**	**-R**	**30S**	**10R**	**20S**	**24S**	**-R**	**8R**	**17S**	**24S**	**18S**
M2S21	**-R**	**- R**	**12R**	**6R**	**22S**	**-R**	**32S**	**12R**	**19S**	**24S**	**-R**	**24S**	**4R**	**26S**	**-R**
M2S22	**-R**	**-R**	**4R**	**-R**	**24S**	**-R**	**25S**	**11R**	**26S**	**24S**	**-R**	**8R**	**-R**	**22S**	**-R**
M2S23	**-R**	**- R**	**9R**	**-R**	**21S**	**-R**	**11R**	**14R**	**28S**	**28S**	**-R**	**20S**	**-R**	**22S**	**-R**
M2S24	**-R**	**-R**	**10R**	**4R**	**25S**	**-R**	**36S**	**12R**	**25S**	**28S**	**-R**	**17R**	**4R**	**10R**	**-R**
M2S25	**-R**	**-R**	**6R**	**9R**	**24S**	**-R**	**23S**	**6R**	**25S**	**23S**	**-R**	**19S**	**4R**	**22S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible



Graph: 3.4: antibiotic resistance among clinical patients for probable microorganism *pseudomonas*

All the isolates from clinical samples were fully resistant to Doxycycline, Ampicillin, penicillin, Streptomycin, Ciprofloxacin. Where samples were partially resistance to Amoxicillin The least resistance were seen against Imipenem, Tetracycline, Kanamycin and Amikacin, all having a same percentage of (2%).

Table 3.9: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. For (***Staphylococcus)***

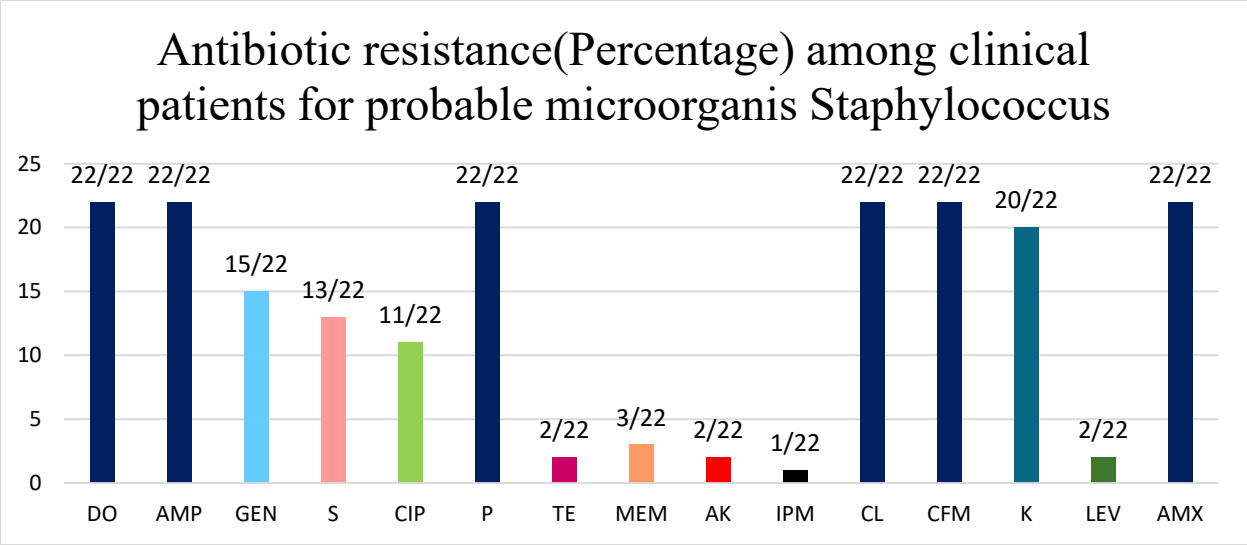
SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M3S1	**-R**	**- R**	**10R**	**8R**	**22S**	**-R**	**18S**	**23S**	**18S**	**23S**	**-R**	**12R**	**4R**	**24S**	**-R**
M3S3	**-R**	**- R**	**12R**	**6R**	**26S**	**-R**	**24S**	**20S**	**25S**	**22S**	**-R**	**10R**	**-R**	**26S**	**-R**
M3S4	**-R**	**-R**	**4R**	**20S**	**14R**	**-R**	**28S**	**22S**	**28S**	**24S**	**-R**	**8R**	**6R**	**22S**	**-R**
M3S5	**-R**	**- R**	**22S**	**-R**	**24S**	**-R**	**13R**	**24S**	**24S**	**24S**	**-R**	**4R**	**-R**	**24S**	**-R**
M3S7	**-R**	**- R**	**28S**	**18S**	**28S**	**-R**	**24S**	**30S**	**22S**	**28S**	**-R**	**12R**	**-R**	**25S**	**-R**
M3S8	**-R**	**-R**	**20S**	**-R**	**12R**	**-R**	**28S**	**19S**	**26S**	**30S**	**-R**	**10R**	**-R**	**25S**	**-R**
M3S9	**-R**	**- R**	**6R**	**22S**	**10R**	**-R**	**30S**	**18S**	**28S**	**19R**	**-R**	**11R**	**-R**	**23S**	**-R**
M3S10	**-R**	**-R**	**18S**	**19S**	**22S**	**-R**	**32S**	**20S**	**32S**	**26S**	**-R**	**4R**	**-R**	**22S**	**-R**
M3S12	**-R**	**-R**	**4R**	**26S**	**12R**	**-R**	**22S**	**22S**	**20S**	**28S**	**-R**	**10R**	**4R**	**28S**	**-R**
M3S13	**-R**	**- R**	**8R**	**-R**	**24S**	**-R**	**24S**	**26S**	**22S**	**24S**	**-R**	**4R**	**2R**	**22S**	**-R**
M3S14	**-R**	**-R**	**4R**	**10R**	**26S**	**-R**	**22S**	**31S**	**25S**	**24S**	**-R**	**6R**	**2R**	**26S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible

Table 3.10: Table of antibiotic sensitivity testing on the probable isolated microorganisms from clinical oral sample. (***Staphylococcus)***

SAMPLE NO.	DO	AMP	GEN	S	CIP	P	MEM	TE	AK	IPM	CFM	CL	K	LEV	AMX
M3S15	**-R**	**- R**	**10R**	**-R**	**12R**	**-R**	**22S**	**11R**	**8R**	**24S**	**-R**	**8R**	**18S**	**23S**	**-R**
M3S16	**-R**	**-R**	**15S**	**21S**	**14R**	**-R**	**21S**	**23S**	**26S**	**23S**	**-R**	**4R**	**-R**	**24S**	**-R**
M3S17	**-R**	**- R**	**6R**	**23S**	**28S**	**-R**	**10R**	**21S**	**22S**	**28S**	**-R**	**12R**	**-R**	**24S**	**-R**
M3S18	**-R**	**-R**	**27S**	**-R**	**15R**	**-R**	**32S**	**12R**	**23S**	**26S**	**-R**	**12R**	**22S**	**23S**	**-R**
M3S19	**-R**	**- R**	**8R**	**4R**	**32S**	**-R**	**26S**	**28S**	**11R**	**22S**	**-R**	**10R**	**-R**	**12R**	**-R**
M3S20	**-R**	**-R**	**4R**	**-R**	**12R**	**-R**	**30S**	**29S**	**20S**	**24S**	**-R**	**8R**	**17S**	**24S**	**-R**
M3S21	**-R**	**- R**	**12R**	**26S**	**9R**	**-R**	**11R**	**30S**	**19S**	**24S**	**-R**	**4R**	**4R**	**26S**	**-R**
M3S22	**-R**	**-R**	**20S**	**19S**	**24S**	**-R**	**25S**	**26S**	**26S**	**24S**	**-R**	**8R**	**-R**	**22S**	**-R**
M3S23	**-R**	**- R**	**9R**	**-R**	**11R**	**-R**	**28S**	**24S**	**28S**	**28S**	**-R**	**7R**	**-R**	**22S**	**-R**
M3S24	**-R**	**-R**	**9R**	**26S**	**25S**	**-R**	**36S**	**22S**	**25S**	**28S**	**-R**	**12R**	**4R**	**10R**	**-R**
M3S25	**-R**	**-R**	**6R**	**9R**	**13R**	**-R**	**23S**	**19S**	**25S**	**23S**	**-R**	**8R**	**4R**	**22S**	**-R**

*R= Resistant *I= Intermediate *S= Susceptible



Graph: 3.5: antibiotic resistance among clinical patients for probable microorganism *Staphylococcus*

All the isolates from clinical samples were fully resistant to Doxycycline, Ampicillin, penicillin, Colistin, Cefixime and Amoxicillin. Also followed by partially resistant to Streptomycin (6%) and Gentamicin (7%). The least resistance was seen against Imipenem, Tetracycline, Kanamycin, Ciprofloxacin and Amikacin, all having a same percentage of (1%).

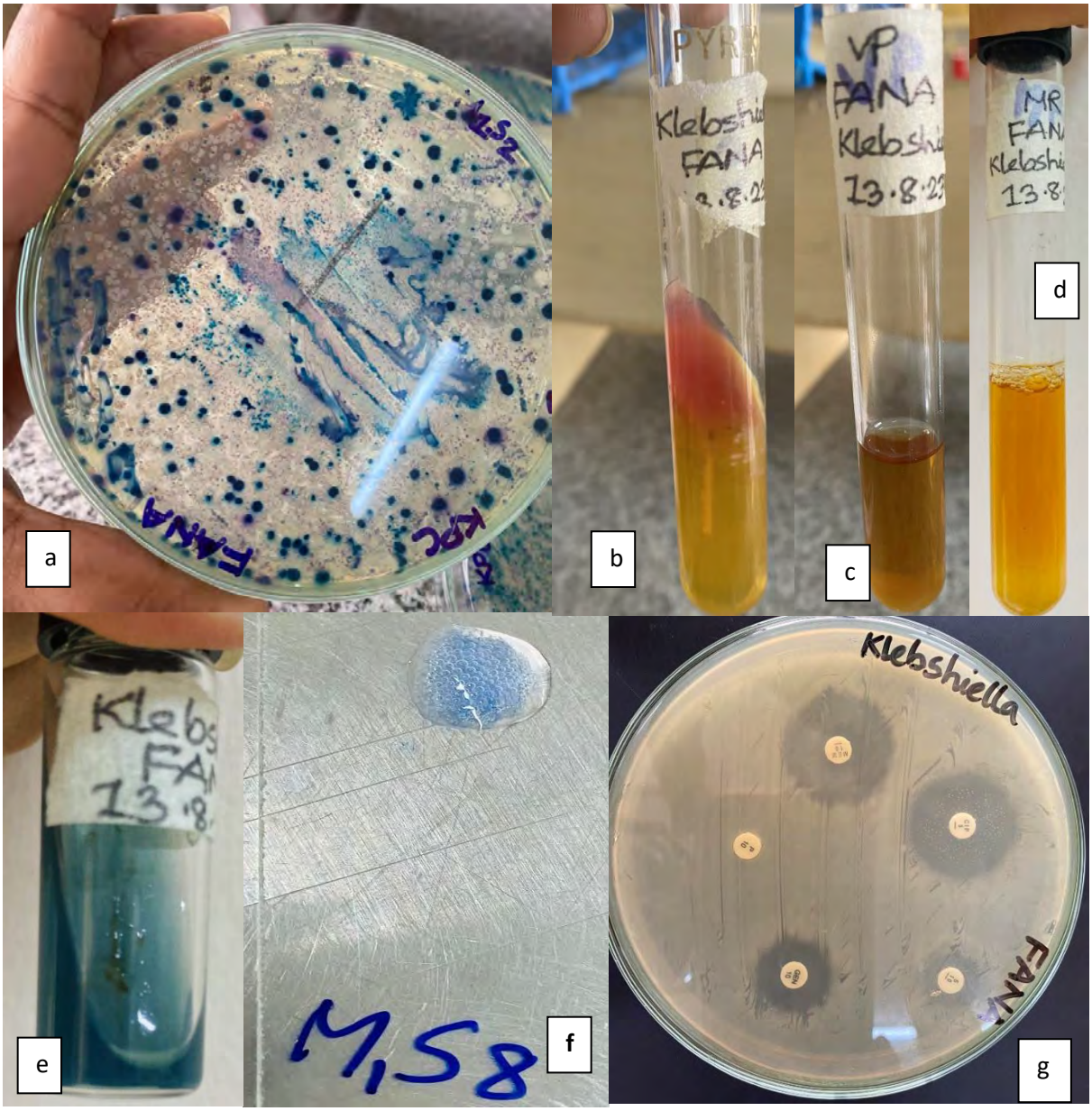


Figure 3.1: a. *Klebsiella* species grown on Hi-chrome KPC agar. b. TSI slant. c. Voges-Proskauer test positive. d. Methyl red negative. e. Citrate positive. f. Catalase positive result. g.Antibiotic susceptibility test with antibiotic discs

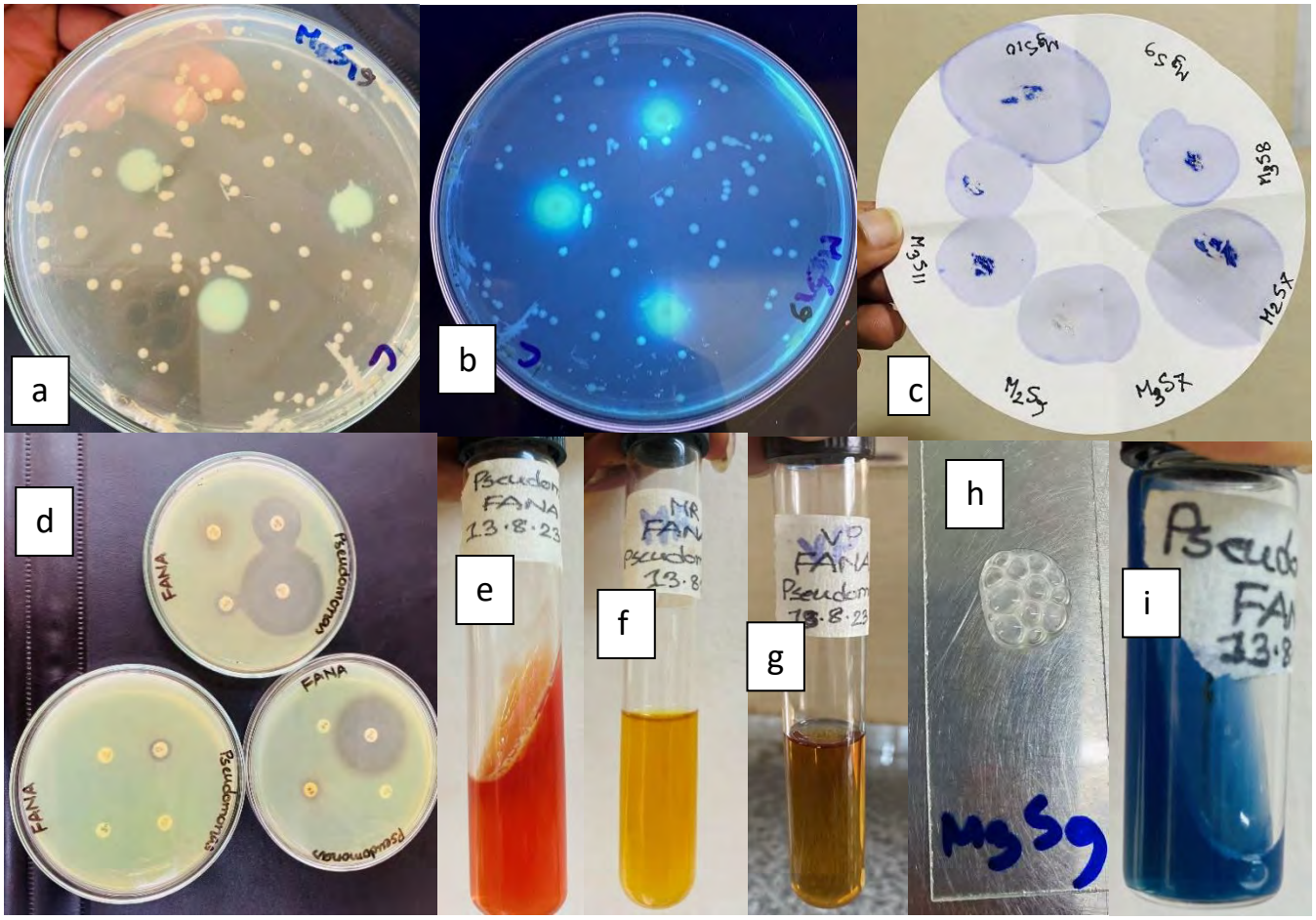


Figure3.2: a *Pseudomonas* species grown on Cetrimide agar. b. Cetrimide agar under UV light containing pseudomonas species.c. Oxidase test positive. d. Antibiotic susceptibility test with antibiotic discs. e. TSI slant red. f. MR test negative. g. VP test negative. h. Catalase test positive. i. Citrate test positive

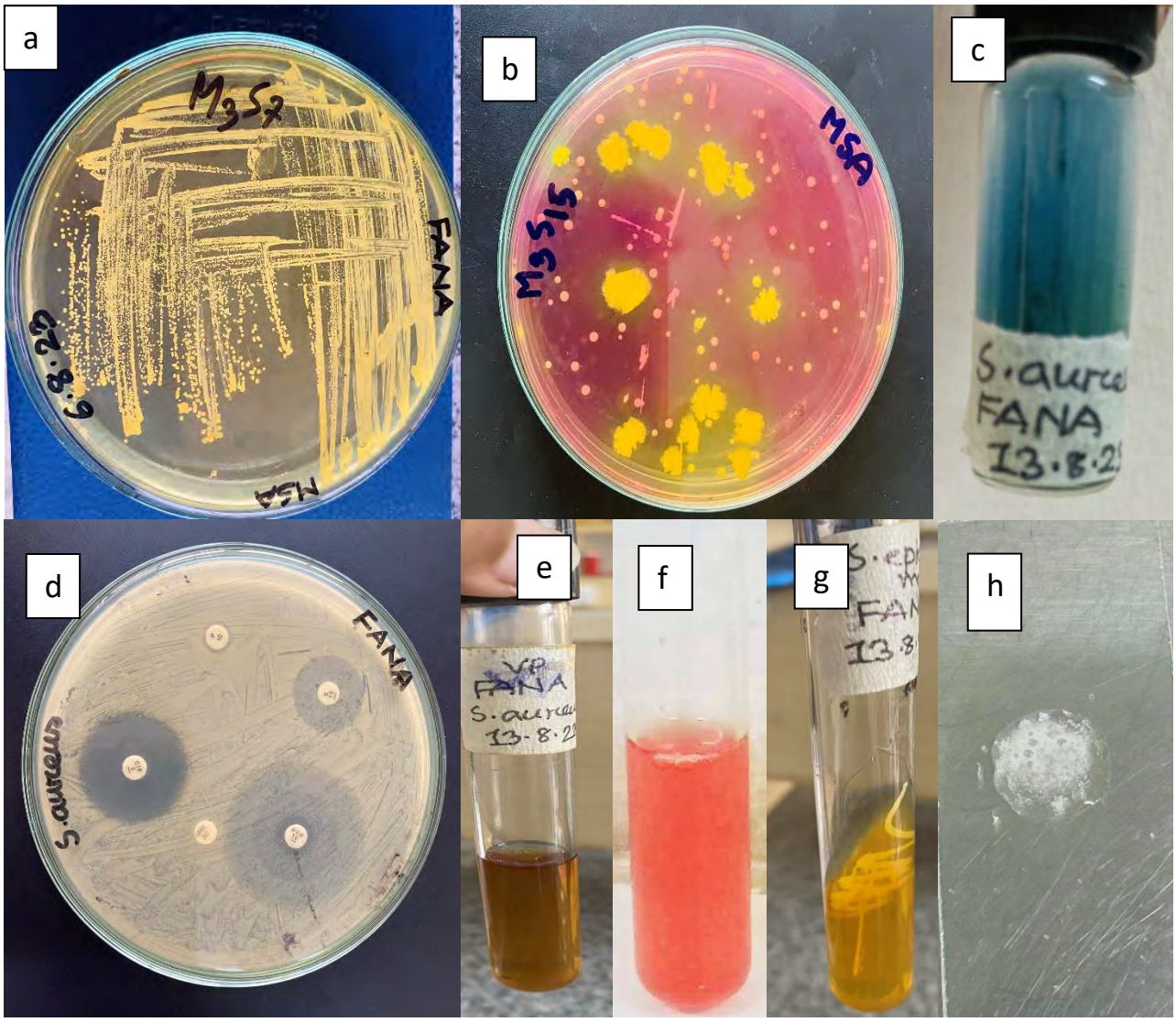
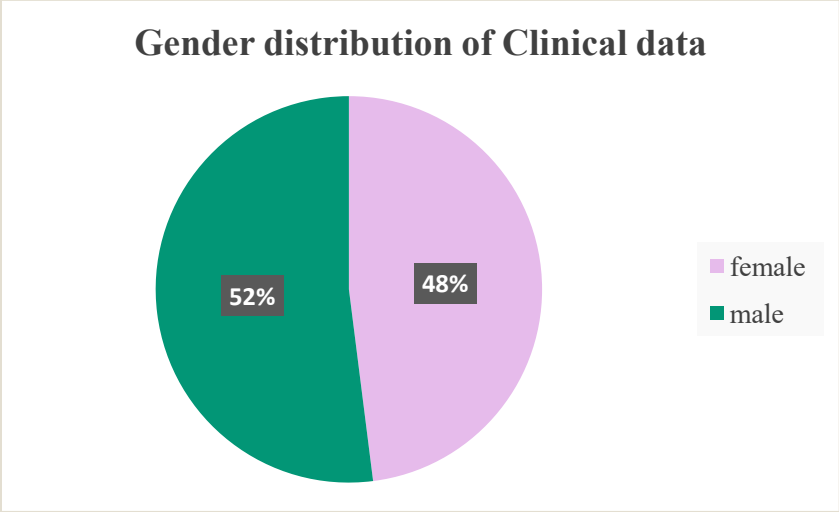


Figure 3.3: a and b. *Staphylococcus* species grown on MSA agar media. c. Citrate test positive d. Antibiotic susceptibility test with antibiotic discs. e. VP test negative. f. MR test positive. g. TSI slant yellow. h. Catalase test positive

3.5 Analysis of survey according to the Questionnaire

The statistical analysis was done with the data collected from the survey between clinical patients who were taking dental treatment and non- clinical patient who are not taking any treatment. It was done to check the gender, age, oral health, oral treatment under processing and demographic distribution of the patient that went to the treatment facility in the time being. Along with that lifestyle, food habit, brushing habit was also analyzed. Apart from that, connection between treatment, progression and medication were also checked.

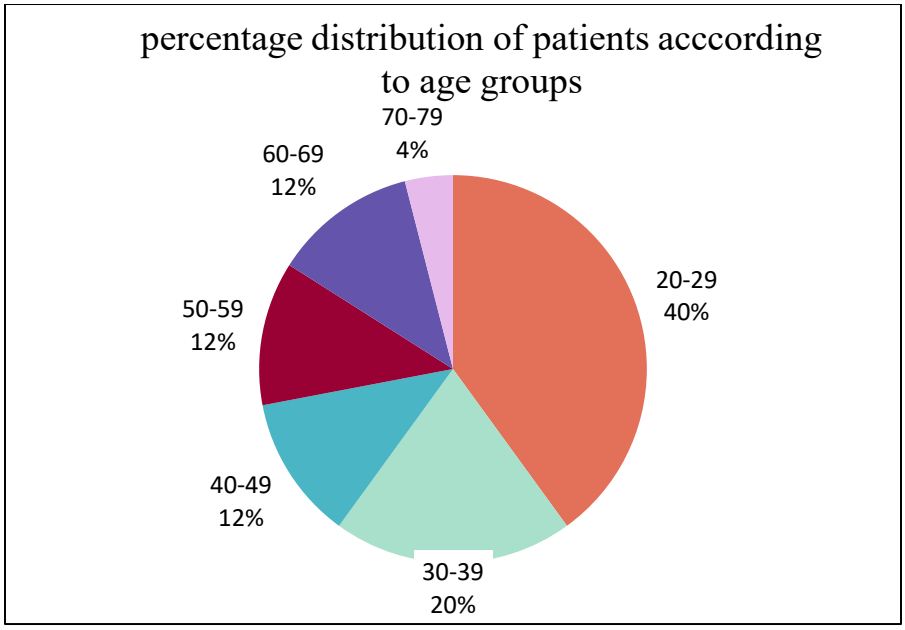
3.6 Distribution of the gender in the study population in terms of Clinical patient



Graph3.6: pie chart for male and female patients

This graph shows how gender is distributed among the cancer patient in the study. Majority of the patients were male with a percentage of 52% and the percentage of female patient is 48%. The difference between two gender is 4%.

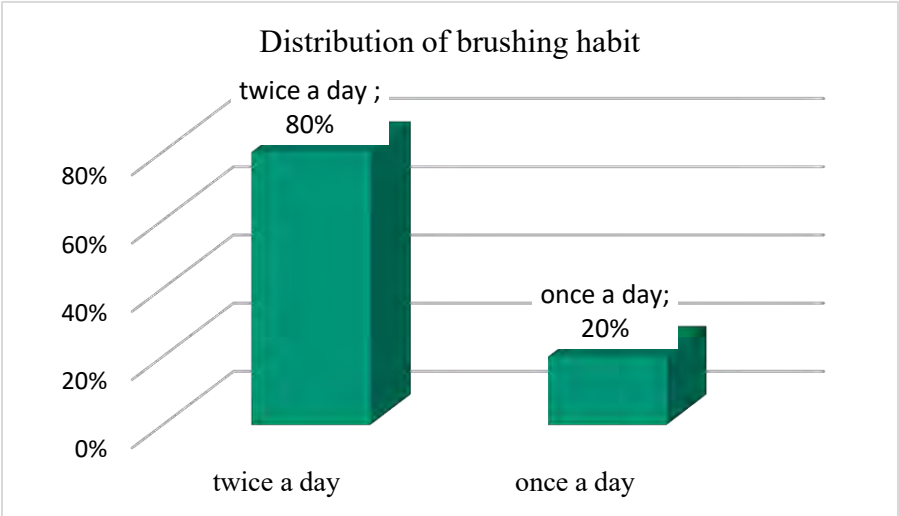
3.7 Distribution of age among the patients



Graph 3.7: percentage distribution of age groups of patients

The distribution of patients in terms of age is given above. While the pie chart shows the illustration and values of age distribution. The group with the highest value is 20-29 years' old which consists of 40%. On the other hand, the lower group with the lowest percentage is 70-79-year-old consist of 4%. It can be seen that majority of age between 20-29-year-old patient are taking oral treatment.

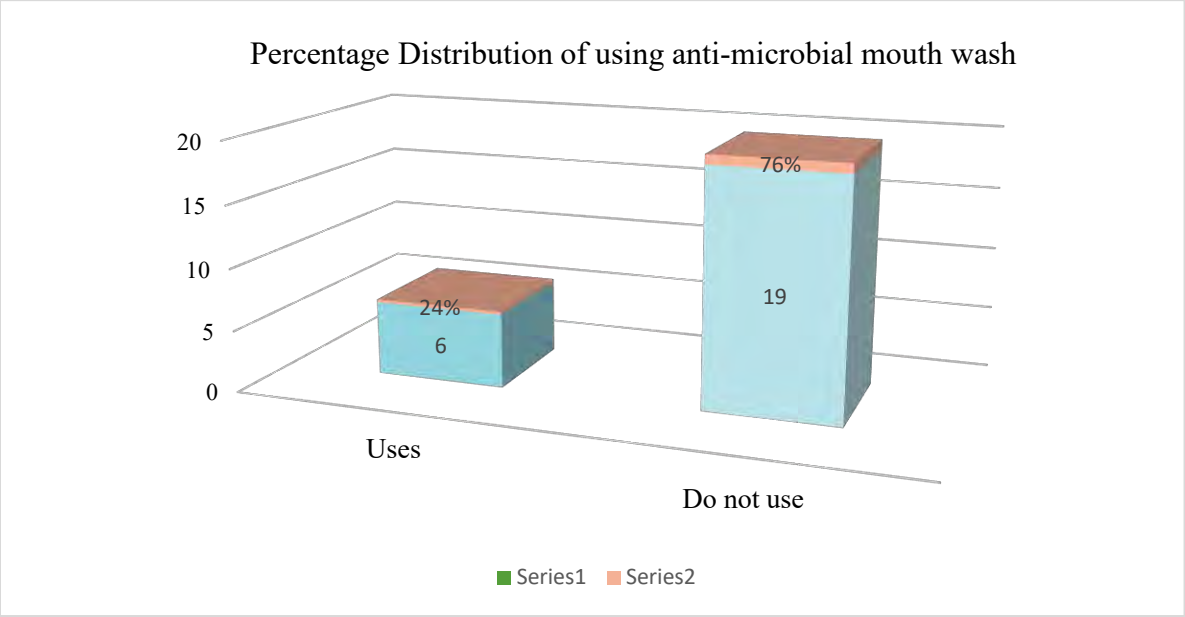
3.8 Distribution of brushing habit



Graph 3.8: percentage distribution of age groups of patients

The distribution of patients in terms of brushing habit is given above. While the bar chart shows the illustration and values of the distribution. The group with the highest value is brushing teeth twice in a day which consists of 80%. On the other hand, brushing teeth once in a day has percentage of 20%. It can be seen that majority of people follows brushing teeth twice a day.

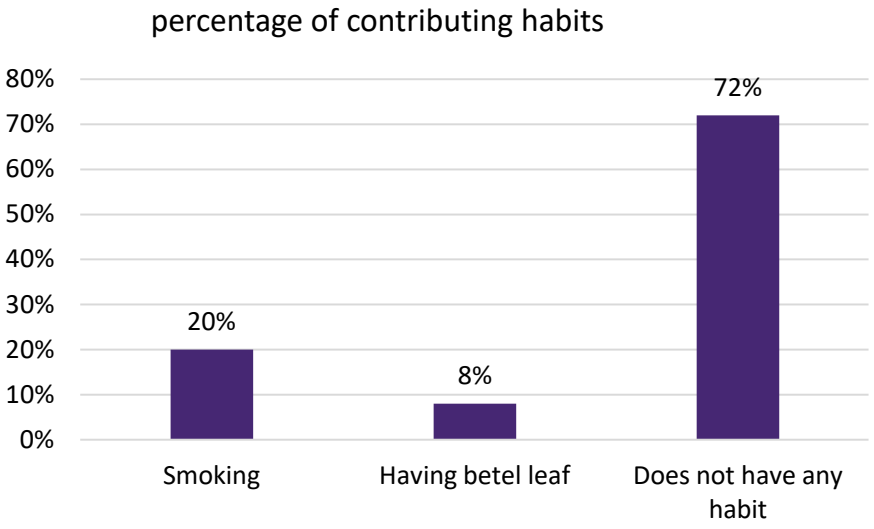
3.9 Percentage Distribution of using anti-microbial mouth wash



Graph 3.9: Percentage Distribution of using anti-microbial mouth wash

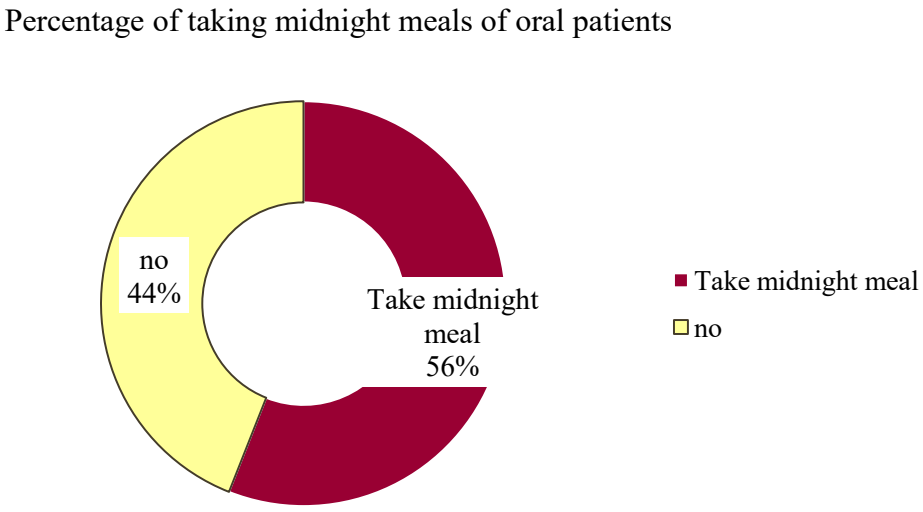
3.10 Distribution of predisposing factors of oral disease

Consuming nicotine, betel leaf and other intoxicant leaves are considered predisposing factors of oral disease. Here, the distribution bar is given below which shows 20% of patient's uptake nicotine and 8% of the people have the habit of taking betel leaf. Surprisingly majority percentage is not related to any habit but having oral discomfort and various diseases.



Graph 3.10: percentage of user contributing habits.

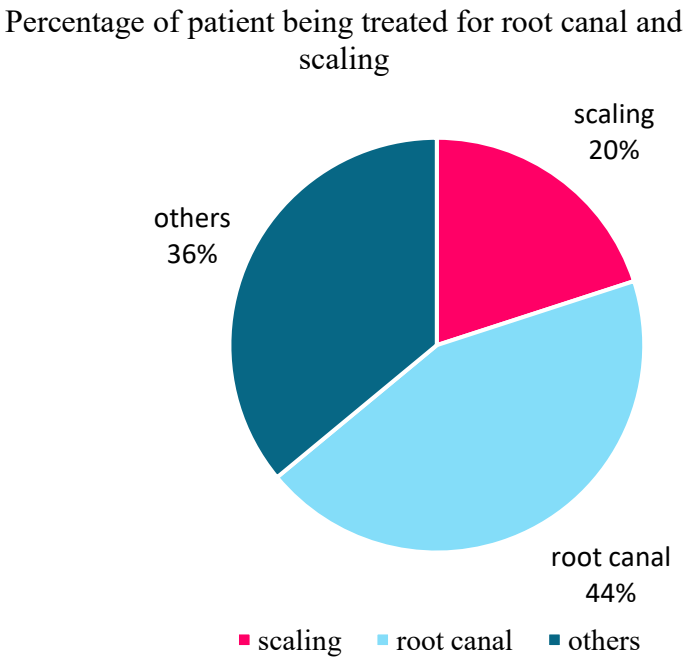
3.11 Percentage of taking midnight meals of oral patients



Graph 3.11: Percentage of taking midnight meals of oral patients

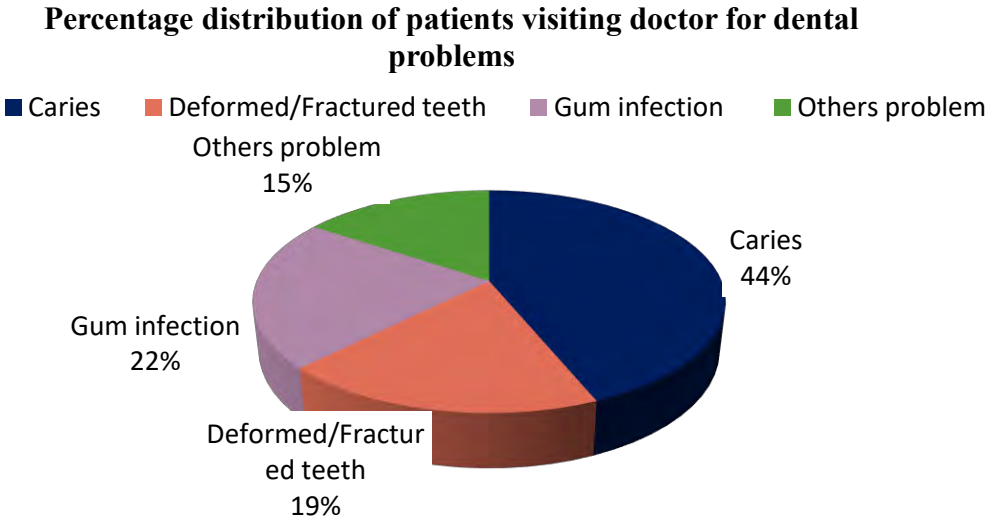
The distribution of patients in terms of having meals at midnight is given above. It shows majority of patient takes meals at midnight consisting 56% which can lead to increase of oral microbiota. And 44% patient said they do not take any kind of meal at midnight.

3.12 Percentage of patient being treated for root canal and scaling



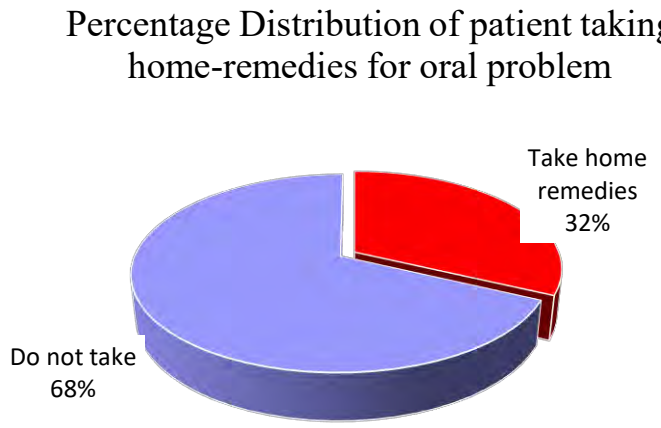
Graph 3.12: Percentage of patient being treated for root canal and scaling

3.13 Percentage distribution of patients visiting doctor for dental problems



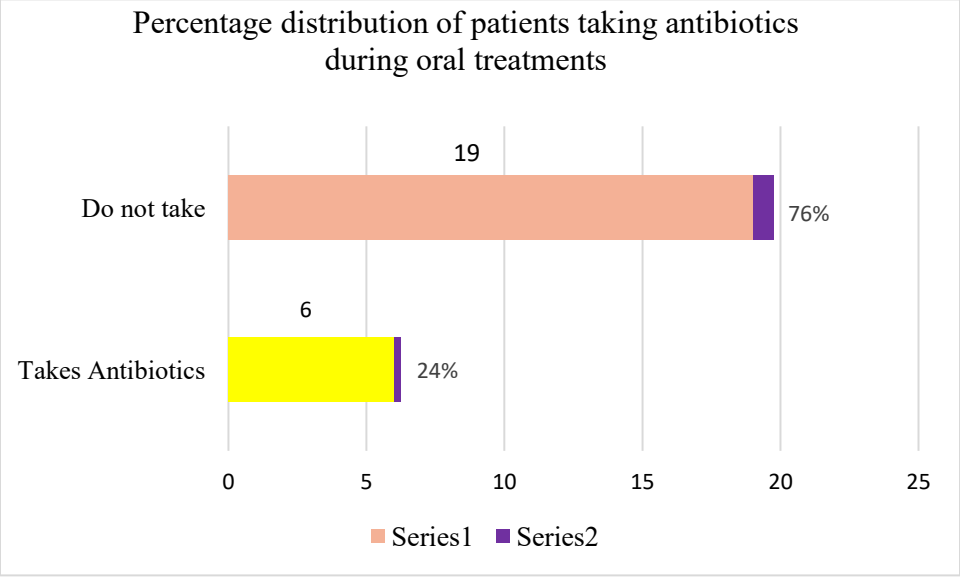
Graph 3.13: Percentage distribution of patients visiting doctor for dental problems

3.14 Percentage Distribution of patient taking home-remedies for oral problem.



Graph 3.14: Percentage Distribution of patient taking home-remedies for oral problem

3.15 Percentage distribution of patients taking antibiotics during oral treatments



Graph 3.15: Percentage distribution of patients taking antibiotics during oral treatments

Chapter 4
Discussion & Conclusion

4.1 Discussion

The results obtained from our study offer a glimpse into the microbial diversity within the oral samples collected. A critical aspect of our investigation was the incubation period, which played a pivotal role in the cultivation of various microbes. After 24 hours of incubation, we observed the development of bacterial colonies exhibiting diverse morphologies and colors, providing valuable insights into the composition of the oral microbiome.

In particular, Hi-chrome KPC and MSA agar media revealed an array of colony colors, including Light/Dark Pink, Yellow, Purple, Off-white, Transparent, Black, and Brown. This rich variety of colors signifies the presence of different microbial species with distinct biochemical characteristics.

Among the 25 clinical samples analyzed, an intriguing pattern emerged in the specific types of microorganisms identified. The majority of the samples, 22 out of 25, displayed the growth of *Staphylococcus* species on MSA agar plates. This finding suggests the prevalence of *Staphylococcus* in the oral microbiome of our study population.

Furthermore, both Hi-chrome KPC and Cetrimide Agar media plates revealed the presence of *Klebsiella* and *Pseudomonas* species, with 24 out of 25 samples exhibiting growth of these microorganisms on each respective plate. This high prevalence underscores the significance of *Klebsiella* and *Pseudomonas* in the oral microbiome.

The differential growth of these microbial species in our samples attributed to various factors, including individual differences in oral hygiene, dietary habits, and exposure to external pathogens.

All the isolated organisms were taken for gram staining to determine the shape, color and morphology of the organism and then different biochemical tests to figure out their characteristics by differentiating them based on biochemical activities. The presence of different types of pathogens was suspected by analyzing the results of the biochemical tests. Among them, some organisms can create different types of waterborne and enteric diseases.

All results were taken and recorded with the help of books and online articles. From the biochemical tests, *Staphylococcus, Pseudomonas, Klebsiella* species, were suspected. By completing the biochemical tests, three different organisms were suspected and taken for the antibiotic susceptibility test. A total of 15 antibiotics were used to treat each of the organisms:

Susceptibility of oral microorganisms isolated from patients receiving oral care treatments to a range of antibiotics, including Doxycycline, Ampicillin, Gentamicin, Ciprofloxacin, Penicillin, Tetracycline, Meropenem, Amikacin, Imipenem, Colistin, Cefixime, Kanamycin, Levofloxacin, and Amoxicillin. The diameters of the zones of inhibition were measured meticulously from both sides, and the average diameter was recorded. These measurements were then compared with reference values to determine the susceptibility or resistance of the microorganisms to the tested antibiotics. among all 25 clinical samples in our study,100% resistance observed in clinical samples to antibiotics Doxycycline, Penicillin, Ampicillin, and Cefixime is a cause for concern within the context of oral healthcare is indeed an alarming issue. This finding raises significant concerns about antibiotic resistance within the oral microbiome of patients undergoing oral care treatments

A significant proportion of patients undergoing oral care treatment harbored diverse microorganisms in their oral cavities. Among the identified microflora, *Klebsiella* and *Pseudomonas* species were found to be the most prevalent in the collected samples.

These findings highlight the importance of antibiotic susceptibility testing in the context of oral health care, as they demonstrate the presence of potentially resistant microorganisms within the oral microbiome. This information underscores the need for prudent antibiotic prescribing practices and the development of tailored treatment regimens to effectively combat infections in oral healthcare settings.

Among the isolates, 96% of *Klebsiella* species and *Pseudomonas* species were the most prevalent. *Pseudomonas* have been responsible for many infections and major cause of pneumonia. The *Staphylococcus* species organism were screened 88% among all the patient. This indicates that heavy amount of people are infected with *Staphylococcus* genus bacteria. However, in terms of isolates most of this bacteria found from those patients who have diabetes and other complications.

Among 25 samples, it was observed that one patient exhibited no presence of *Klebsiella* microorganisms, another patient showed no presence of *Pseudomonas*, and three patients displayed no presence of *Staphylococcus* species. These variations in microbial profiles among the patients highlight the individualized nature of oral microbiomes.

One of the key observations of this study is the disparity in the prevalence of these microorganisms between non-clinical and clinical samples. In our non-clinical patient group, we identified

Klebsiella in approximately 16% of samples, Pseudomonas in approximately 24% of samples, and Staphylococcus in approximately 8% of samples. These percentages were notably lower than what was observed in clinical samples, suggesting a significant difference in microbial composition between these two cohorts.

The higher prevalence of *Klebsiella, Pseudomonas*, and *Staphylococcus* in clinical samples could be attributed to the fact that these patients were actively seeking treatment for oral health issues. It is well-established that oral health problems often lead to shifts in the oral microbiota, favoring the growth of certain pathogens. Therefore, it is not surprising that these pathogenic bacteria were more prevalent in the clinical group, where oral health issues were actively addressed.

On the other hand, the lower prevalence of these microorganisms in non-clinical samples may indicate a healthier oral microbiome in this population. However, it is essential to note that even in non-clinical samples, the presence of these pathogens was not negligible. This finding suggests that these pathogenic bacteria can still persist in the oral cavity even in the absence of apparent clinical symptoms.

4.2 Conclusion

This study was carried out to determine the oral microbial profile of Dhaka city people. From the study, our research has provided valuable insights into the oral microbial profiles and pathogen presence among the residents of Dhaka City. Through extensive data collection, laboratory analysis, and collaboration with patients in this urban setting, we have gained a comprehensive understanding of the oral microbiota within this specific population. Our findings have shed light on the unique microbial landscape of Dhaka City's residents, revealing the coexistence of various commensal microorganisms alongside the presence of potentially harmful pathogens. These results underscore the importance of approaches to oral health management, taking into account the specific microbial dynamics of this urban environment.

our research has uncovered a significant and concerning aspect of oral health within our study population, particularly in Dhaka City. Through the analysis of oral samples, we have identified the presence of pathogens that exhibit resistance to commonly prescribed antibiotics, highlighting a growing issue in the oral microbiology and healthcare. The emergence of antibiotic-resistant pathogens in the oral cavity presents a formidable challenge for both clinicians and researcher. It underscores the need for a more comprehensive understanding of antibiotic resistance patterns specific to the oral microbiome. Our findings serve as a wake-up call, emphasizing the urgency of addressing this issue to prevent further complications and the spread of antibiotic resistance

Furthermore, our research has yielded crucial insights into the oral health of young individuals and their encounters with pathogenic bacteria, specifically *Klebsiella, Pseudomonas*, and Staphylococcus species. Through the analysis, we have unveiled the presence of these potentially harmful microbes within the oral cavities of our study population. The identification of *Klebsiella, Pseudomonas*, and *Staphylococcus* bacteria in the oral microbiome of young individuals highlights the dynamic nature of oral health, even among this age group. This discovery underscores the importance of monitoring and maintaining oral health from a young age to prevent potential complications and long-term health issues

Moreover, as we delve deeper into the oral microbiome of Dhaka City and other urban areas, it becomes increasingly evident that localized research is crucial for addressing regional oral health challenges. Our work serves as a testament to the importance of community engagement, interdisciplinary collaboration, and data-driven healthcare initiatives. We hope that the insights

gained from this study will guide future research endeavors and public health policies aimed at improving oral healthcare outcomes for the people of Dhaka City.

We hope that our work inspires further investigation and innovation in the field of oral microbiology, ultimately leading to improved healthcare outcomes for individuals worldwide. We must strive for innovative approaches to combat oral diseases effectively while mitigating the risk of further resistance development. We hope that this knowledge will pave the way for improved oral health outcomes among young individuals, emphasizing the importance of proactive dental care and the development of targeted treatment options.

Reference:

1. Bush, L. M., & Vazquez-Pertejo, M. T. (2023). (n.d.). Infections caused gram positive cocci 252(2), 91-106.
2. Gazin. M, Passch, F, Gosen, H, & Maltotakumar, S. C2012) Curent rend in culture- based and molecular detection of extended-specturm-B-lactamase-harboring and carbapenem-resistant Enterobacteriaceae. Journal of clinical microbiology, 50(4), 1140-1146.
3. Iyer, P. (2023). Oral Cavity is the Gateway to the Body: Role of Oral Health Professionals: A Narrative Review. Journal of the California Dental Association, 51(1).
4. Kalman, D., & Barriere, S. L. (1990). Review of the pharmacology, pharmacokinetics, and clinical use of cephalosporins. Texas Heart Institute Journal, 17(3), 203.
5. Keenleyside, W. (2019, July 23). 25.2 Microbial Diseases of the Mouth and Oral Cavity. Pressbooks.
6. L, B.G.R.V. (Accessed: 05 September 2023). Infections caused by pseudomonas aeruginosa, Reviews of infectious diseases.
7. L, G., Xu, T., Huang, G., Jiang, S., Gu, Y., & Chen, F. (2018). Oral microbiomes: more and more importance in oral cavity and whole body. Protein & Cell, 9(5), 488–500.
8. Li X, Liu Y, Yang X, Li C and Song Z (2022) The Oral Microbiota: Community Composition, Influencing Factors, Pathogenesis, and Interventions.
9. Ma, C., Chen, F., Zhang, Y., Sun, X., Tong, P., Si, Y., & Zheng, S. (2015). Comparison of Oral Microbial Profiles between Children with Severe Early Childhood Caries and Caries-Free Children Using the Human Oral Microbe Identification Microarray. PLOS ONE, 10(3), e0122075.
10. Madani, M., Berardi, T. R., & Stoopler, E. T. (2014). Anatomic and examination considerations of the oral cavity. Medical Clinics of North America, 98(6), 1225–1238.
11. Maoyang Lu a, a, b, & Oral microbiota is an important part of the human microbiota. Oral microbiota is associated with a variety of oral diseases. Recently. (2019).
12. Massour, M., Vatanen, T., Siljander, H., Hämälainen, A. M., Härkönen, I., Ryhänen, S. J.. & Lander, E. S. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Science translational
13. Mayo Foundation for Medical Education and Research. (2022, March 19). Cavities/Tooth Decay. Mayo Clinic

14. Mostarin, S. Z., Khyang, P., Roy, S. R., & Sultana, A. (2019). Dental health care in Bangladesh: Prospects and Challenges.
15. Normark, B. H., & Normark, S. (2002). Evolution and spread of antibiotic resistance. Journal of internal medicine, 252(2), 91-106.
16. Oral Health Bangladesh 2022 country profile. (n.d.). https://www.who.int/publications/m/item/oral-health-bgd-2022-country-profile/
17. Salazar-Flores, J., Lomeli-Martinez, S. M., Ceja-Gálvez, H. R., Torres-Jasso, J. H., Torres-Reyes, L. A., & Torres-Sánchez, E. D. (2022). Impacts of pesticides on oral cavity health and ecosystems: A review. International Journal of Environmental Research and Public Health, 19(18), 11257.
18. Sampaio-Maia, B., Caldas, I. M., Pereira, M. D. C., Pérez-Mongiovi, D., & Araujo, R. (2016). The oral microbiome in Health and its implication in oral and systemic diseases. In Advances in Applied Microbiology (pp. 171–210).
19. Singer, A. J., Jackson, M. S., Ohara-Nemoto, Y., Tawara, Y., MacFarlane, T. W., Goldberg, M. H., Bagg, J., Kronström, M., & Rokadiya, S. (2015, January 3). Staphylococcus aureus and the oral cavity: An overlooked source of carriage and infection. American Journal of Infection Control.
20. Souza, L. C. D., Lopes, F. F., Bastos, E. G., & Alves, C. M. C. (2018). Oral infection by Pseudomonas aeruginosa in patient with chronic kidney disease - a case report. Brazilian Journal of Nephrology, 40(1), 82–85.
21. Welch, A., Rauf, D., Upham, B., Cassata, C., Garrard, C., & Rapaport, L. (n.d.). Klebsiella-pneumoniae
22. Zhang, Y., Qi, Y., Lo, E. C. M., McGrath, C., Mei, M. L., and Dai, R. (2021a). Using next-generation sequencing to detect oral microbiome change following periodontal interventions: a systematic review.
23. Zhang, Y., Wang, X., Li, H., Ni, C., Du, Z., & Yan, F. (2018). Human oral microbiota and its modulation for oral health. Biomedicine & Pharmacotherapy, 99, 883–893.
24. Zheng, H., Xu, L., Wang, Z., Li, L., Zhang, J., Zhang, Q., et al. (2015). Subgingival microbiome in patients with healthy and ailing dental implants. Sci.
25. Zheng, J., Wu, Z., Niu, K., Xie, Y., Hu, X., Fu, J., et al. (2019). Microbiome of deep dentinal caries from reversible pulpitis to irreversible pulpitis.

26. Zijnge, V., van Leeuwen, M. B., Degener, J. E., Abbas, F., Thurnheer, T., Gmur, R., et al. (2010). Oral biofilm architecture on natural teth.