Screening of opportunistic microbes from the infected sites of oral cancer patients and a study of their antibiotic resistance pattern

By

Zareen Nawar

Student ID: 16136013

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology

Department of Mathematics and Natural Sciences

BRAC University

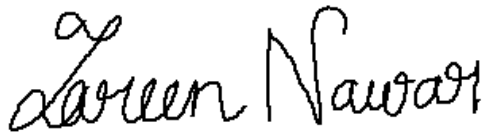
3 September, 2020

Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing 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 which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all the main sources of help.

Student's Full Name & Signature:



Zareen Nawar
Student ID:16136013

Approval

The thesis/project titled **"Screening of opportunistic microbes from the infected sites of oral cancer patients and a study of their antibiotic resistance pattern"** submitted by

1. Zareen Nawar (ID 16136013)

of Spring, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology on "date to be given of August 2020.

Examining Committee:

Supervisor:
(Member)

_____

M. Mahboob Hossain, PhD
Professor, Department of Mathematics and Natural Sciences
BRAC University

Program
Coordinator:
(Member)

_____

Iftekhar Bin Naser, PhD
Assistant Professor, Department of Mathematics and Natural Sciences
BRAC University

Departmental Head:
(Chair)

_____

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

Dedicated to

Almighty Allah, my beloved family, and some loving friends…

Acknowledgment

I would like to commence after expressing my earnest gratitude to the Almighty for endowing me with the opportunity of this research course and then supporting me throughout this journey to fulfill it successfully.

I acknowledge my gratitude to Professor **A F M Yusuf Haider**, Chairperson of the MNS Department of BRAC University for allowing me to complete my undergraduate thesis.

I express my sincere gratitude and respect to my supervisor Dr. M Mahboob Hossain, Associate Professor, Department of Mathematics and Natural Sciences, BRAC University for being constant support throughout my student life and guiding me during the research work which has inspired and enhanced my capability enormously.

My regards and gratitude go to **Aparna Islam,** Professor, Department of Mathematics and Natural Sciences, BRAC University, for all her support and encouragement throughout my undergraduate degree.

A special token of appreciation should be given to the director of the National Institute of Cancer Research and Hospital (NICRH), **Prof (Dr.) Md. Moarraf Hossen** for granting my application to work in the cancer hospital.

I would also like to thank **Dr. Nadimul Hasan** for being the co-supervisor of my thesis work and giving valuable knowledge. The study could not have been complete without his support. Also, I should duly thank **Dr. Waheed Akhtar** for arranging the facilities to work with the NICRH.

I would also like to thank all the Lab officers and teaching assistants for supporting us in the times of lab inquiries.

My regards and gratitude to my friend **Samiha Ashreen** for being with me throughout the study and for her endless help and also **Sujoy Sarker**, **Rifaquat Ahmed** and **Nusrat Jahan Annesha** for always encouraging me in all means.

Lastly and most importantly, I would like to declare my gratitude to my family for their substantial support and encouragement in every aspect of my life.

Zareen Nawar

ID: 16136013

Abstract

Cancer is one of the most lethal diseases in the world. It can be defined as the uncontrolled growth of cells that can spread throughout the body. Oral cancer is one of the most common cancers in South-Asian countries. Worldwide it is the 6th most prevalent cancer. The immune-comprised patients after the treatment of oral cancer may have a chance of infection by drug-resistant opportunistic microbes. In this study, opportunistic microbes were identified and isolated from the oral cancer patient with infection and their resistance profile with the common antibiotics used was determined. Oral swab samples from 55 oral cancer patients were taken to check the presence of the opportunistic organisms. Of them 24 had a post-operative infection, 31 had a pre-operative infection. So, a higher number of infections were found in pre-operative patients. On the other hand, swab samples were also taken from 50 healthy people (control). After screening from the patient group, 83(65.4%) of the organism were gram-negative bacteria and 44(34.6%) were gram-positive bacteria. Again, among the isolates from the patient group, the most prevalent organism was *Pseudomonas spp* 30(54.54%) followed by *Klebsiella spp* 27(49.09%), *Staphylococcus spp* 24(43.63%), *E. coli* 14(25.45%), *Streptococcus spp* 14(25.45%), *Proteus spp* 12(21.8%). The least prevalent was *Enterococcus spp* 6(10.9%). The isolates were all taken for antibiotic sensitivity testing (AST) against 13 antibiotics from 11 different groups used in hospitals. It was observed gram-positive isolates of the patient group exhibited 100% resistance to antibiotic metronidazole, erythromycin, oxacillin, cloxacillin, and amoxicillin. The resistance of these gram-negative organisms was followed in nalidixic acid with 95.5% resistance and ceftazidime with 90.9% resistance. Gram-positive organisms isolated from oral cancer patients didn't show any resistance towards the antibiotic imipenem. The gram-negative isolates exhibited 100% resistance to metronidazole, vancomycin, amoxicillin, penicillin. The resistance of these organisms was followed in azithromycin with 92.9% resistance, nalidixic acid with 89.3% resistance, tetracycline with 88.1% resistance, and amoxyclav with 81% resistance. The gram-negative isolates showed 16.7% resistance to imipenem, 7.1% to amikacin, and minimum resistance of 2.4% to gentamicin. Both gram-positive and gram-negative isolates of the patient group exhibited high resistance to metronidazole, nalidixic acid, amoxicillin. The least resistance was seen against amikacin, gentamicin, imipenem, and ciprofloxacin. In contrast, the microbes of the control group showed less resistance to these antibiotics and rather showed sensitivity to them. Thus, the isolates of the control group were less harmful than those from cancer patients.

Table of Contents:

List of tables:

List of figures:

Chapter 1

Introduction and Literature Review

1.1: Oral Cancer:

Oral Cancer also is known as 'mouth cancer' is cancer in the lining of lips, mouth, and upper throat. Cancer develops in the anterior tongue, gingival, buccal cavity, retromolar trigone, hard palate, salivary glands, and even tonsil glands. (Ahmed and Islam, 1990). This cancer is a major global public health problem and is the cause of death from an oral disease worldwide. This cancer includes malignancy of vermillion of lips and all surfaces of the oral cavity including two-thirds of the tongue. Globally Oral cancer is the sixth most common cancer in the world (Shin-Ichi *et al.*, 2002). It is prevalent in the area where the consumption of betel quid, smoking, and alcohol is more. 40% of cancer in Southeast Asia is Oral cancer (Rodrigues *et al., 1998*)

Oral cancer occurs initially as squamous of cell carcinomas, that is why it is defined as squamous cell carcinoma (OSCC). This is so named because 90% of all dental diseases originate from the squamous cell. Primarily, Oral Cancer arises as a lesion that is hyperplastic in growth. Due to the presence of external carcinogenic stimuli and the absence of internal cell regulations mechanism because or tumor repressor genes, the hyperplasia turns into metaplasia and anaplasia that leads to malignant invasion. Other factors that causes oral cancer are tobacco, betel leaf, catechu, alcohol, etc. Oral infection caused by Herpes virus, Human Papillomavirus, Candida albicans, *Treponema pallidum*, and even poor oral hygiene is a biological factor that increases the risk of oral cancer (Cawson, 1969). In some studies, a white lining that grows inside the oral cavity known as leukoplakia may become malignant and might increase the risk of oral cancer. (Brad *et al.*, 2009)

Oral cancer should be prevented based upon appropriate hygiene, control of the major risk factors and use of available HPV vaccines. The risk of oral cavity cancer increases at the age of 45 and affects men more than women.

1.2: Oral Cancer in Bangladesh:

According to the World Health Organization (WHO), oral cancer is the 11th most dominant cancer in the world and is affecting many people each year. In Bangladesh the rate of oral cancer is high. Cancer occurring in the whole body is 20,0000 per year and among them, oral cancer represents 20%, and it is the third leading cancer occurring in this country (Shaheed & Molla 1996, p. 8). More than 7000 people in Bangladesh are diagnosed with oral cavity cancer every year and many of them remain undiagnosed. Among the diagnosed 6.6% face mortality. In Bangladesh, the mortality of Oral Cancer has reached 15,010 or 1.90% of total deaths according to the latest WHO data published in 2017.

The majority of oral cancer patients are from rural areas of Bangladesh (Hussain, 2013). The cause of oral cancer in Bangladesh is mainly due to the regular consumption of tobacco, betel leaf, catechu, alcohol, smoking, etc. Arecoline is a compound that is found in catechu is known for carcinogenicity (Boucher and Mannan, 2002). Adding up to it, arsenic-contaminated groundwater, availability of chemical carcinogens mainly formalin treated fruits, and poor hygiene conditions increase the risks of oral cancer in Bangladesh.

Though the rate of oral cancer in Bangladesh is high, the treatment facilities compared to it has not developed. Bangladesh is now in a severe shortage of radiation therapy machines, hospital beds, trained oncologists, medical radiation physicists and technologists in comparison with the number of oral cancer patients. Again, the cost of the available treatment is also very high. Therefore, some of the diagnosed patients cannot avail of the treatment due to their financial instability (Singh & Singh, 2017).

1.3: Types of Oral Cancer:

Oral cancers are commonly referred to as head and neck cancers, and of all head and neck cancers, it comprises about 85% of that category.

Squamous cell carcinoma: This is the most common type of oral cancers that occur in the oral cavity and oropharynx. Some squamous cells are abnormal. About 90% or more patients are included in this category.

Verrucous carcinoma: Among the oral cavity tumors, verrucous carcinoma is about 5%. This is a very slow-growing type of cancer made up of squamous cells and rarely spreads to other parts of the body. It can also invade the tissue surrounding the site of origin.

Minor salivary gland carcinomas: This category develop on the minor salivary gland that is found throughout the lining of the mouth and throat.

Lymphomas: this type of oral cancer develops in the lymph tissue which is a part of the immune system. Lymphoid tissue is located both in the tonsil and on the base of the tongue.

Benign oral cavity and oropharyngeal tumors: In the oral cavity and oropharynx several types of non-cancerous tumors or tumor-like conditions can develop. Sometimes, these conditions may develop into cancer. The types of benign lesions include: Eosinophilic granuloma, Fibroma, Granular cell tumor, Karatoacanthoma, Lipoma, Neurofibroma, Papilloma, Odontogenic tumors (lesions that begin in tooth-forming tissues)

Leukoplakia and erythroplakia: These non-cancerous certain types of abnormal cells in the mouth or throat (Placeholder1). A white area can be seen in leukoplakia and in erythroplakia a red area, flat or slightly raised, and it often bleeds when scraped to determine whether the cells are cancerous biopsy or other tests are done (Whitmore & Lamont, 2014).

1.4: Infection and risk associated with oral cancer:

Despite significant development in oral cancer treatment, the cancer patients remain at risk of developing serious infections. The immune-compromised patients may have the chance of infections by drug-resistant opportunistic microbes like *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella, E. coli, etc.* This infection may take place during the cancer progressions or after surgery (Cloke *et. al.*, 2004). The infection decreases the recovery rate of patients and it also increases the mortality rate. Because of this infection cancer also spreads to the other parts of the body. After chemotherapy and radiotherapy treatment the cancer patients become more immune- suppressed and thus reduce the number of white blood cells (Gabrilove *et al.*, 1998). The lower level of neutropenia due to this radiotherapy increases the possibility of infectious disease. Significant agents involved in the etiology of oral cancer such as age, gender, food habit, race, tobacco uses, consumption of alcohol. Age is frequently named as a risk factor for oral cancer, as historically it occurs in those over the age of 40. This may indicate a time component in the biochemical or biophysical processes of aging cells that allows malignant transformation, or perhaps, immune system competence diminishes with age. Among the oral cancer patients, two-third-are men. However, tobacco use is the real culprit. Most people with oral cancer use tobacco in some form like pipe-smoking, using tobacco with betel nut. Historically at least 75% of those diagnosed at 50 and older have been tobacco users for years. (Yamashita *et al.,* 2013) Recently, strong evidence for an etiological relationship between the human papillomavirus and a subset of head and neck cancers has been noted (Khode, Dwivedi, Rhys-Evans, & Kazi, 2014). Other factors include poor nutrition, especially a diet low in fruits and vegetables, prolonged sun exposure, Long-term irritation caused by ill-fitting dentures.

1.5: Treatment:

Oral cancer is usually treated with surgery first. After surgery radiotherapy or sometimes radiotherapy and chemotherapy both are given. Reconstruction may be needed to repair structures in the mouth and jaw or to help with speech and swallowing. Reconstruction is planned at the same time as treatment.

1.6: Opportunistic microbes in oral cancer:

In oral cavity infection, the oral microflora may be subsequently replaced by potentially pathogenic microorganisms, such as pathogenic gram-positive cocci and gram-negative bacilli. Histological changes in oral mucosa and salivary glands such as oral mucositis, and for the facilitation of their growth reduced phagocytic activity of salivary granulocytes and reduced amount of salivary glands play a vital role. Opportunistic infections are common in cancer patients with poor health (an immunocompromised host) caused by several different microorganisms, among them representative microorganisms include *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans. Klebsiella spp, Proteus.* But there are some difficulties in curing the related infectious diseases as most of this organism has become drug-resistant. Colonization of pathogenic bacteria in the oral cavity is thought to increase the risk of infections such as pneumonia and bacteremia (Costerton *et al.*, 1999; Gosney *et al.*, 1999).

1.6.1: *Staphylococcus* spp:

S. aureus causes a high number of both human and animal infections, it is gram-positive, salt-tolerant, and commonly found on the skin. However, some of its strains are pathogenic as they can produce harmful toxins. For a wide range of pyogenic infections e.g. skin and soft tissue infections, endocarditis, osteomyelitis, pneumonia and sepsis, and toxin-related syndromes, *S. aureus* is one of the major causative agents. A recent European survey reported *S. aureus* in up to 27.5% of bloodstream infections and up to 19.3% of pneumonia acquired in intensive-care units. As part of the complex microbial community of the anterior nares, this species colonizes about 30% of the healthy human population. Although the majority of colonized individuals will suffer no adverse effects caused by the colonizing strain, colonization has been described as a major source and risk factor for *S. aureus* bacteremia and other invasive infections

1.6.2: *Streptococcus* spp:

Many types of bacteria are caused by Streptococcus which is gram-positive bacteria. The severity of mild throat infections to pneumonia varies from different types of Streptococci bacteria. Based on their hemolytic activity Streptococci are divided into many groups. The main two groups are alpha (α)-hemolytic Streptococci and beta (β)-hemolytic *Streptococci*.

1.6.3: *Enterococcus* spp

Enterococci are Gram-positive cocci that are difficult to distinguish from streptococci on physical characteristics alone. They are often in pairs or short chains. Earlier enterococcus was classified as group D *Streptococcus* until 1984 when genomic DNA analysis indicated a separate genus classification would be appropriate. They are commonly found in the intestine of humans: *E. faecalis* (90–95%) and *E. faecium* (5–10%). Enterococci may occasionally reside in the vagina and oral cavity. In oral cancer, a significant amount of enterococcus was found.

1.6.4: *Pseudomonas spp*

Pseudomonas species are gram-negative, rod-shaped, and polar-flagellated bacteria with some sporulating species found widely in the environment such as in soil, water, and plants. In the history of microbiology, pseudomonads were observed. Because of their widespread occurrence in water and plant seeds such as dicots. Under iron-limiting conditions, *Pseudomonas* species secrete pyoverdine which is a yellow-green fluorescent siderophore. (Lau *et al*., 2004) *Pseudomonas* species gives a positive result in the oxidase and catalase test (Tortora, 1982) and negative result in indole, methyl red, and Voges–Proskauer test. For biofilms production a significant number of this species secrete exopolysaccharides. The threat of *Pseudomonas* infection is severe among people with a weak immune system and it is also resistant to maximum antibiotics. The antibiotic resistance is due to their large genomes, porin channels that facilitates efflux pumps and once again, biofilm formation (Cornelis, 2008)

1.6.5 *Klebsiella spp*

Klebsiella species are gram-negative, facultative, rod-shaped with pointed ends, and with a prominent polysaccharide capsule (Tortora, 1982). In nature, it is found everywhere like in water, soil, and plants. They are also found in the human nose, mouth, and gastrointestinal tract as normal flora. They are responsible for nosocomial infections. Patients with a weak immune system are most likely to be infected with this species (Bagley, 1985). *Klebsiella* is notable for causing pneumonia, urinary tract infections, sepsis, meningitis, diarrhea, peritonitis, and soft tissue infections. Till now there is no vaccine and the resistance to regular antibiotics has made this species more dangerous

1.6.6 *Escherichia coli*

E.coli is a gram-negative, facultative anaerobic, non-sporing bacteria. It is rod-shaped and found in the lower intestine of a warm-blooded animal. *E.coli* strains those live in the gut are harmless, but some are serotypes and cause severe food poisoning in the host's body. Strains that cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease are said to be virulent. When too much virulent this bacterium is also resistant to many antibiotics.

1.6.7 *Proteus spp*

Proteus are gram-negative bacteria found in decomposing animal matter, sewage, manure soil, the mammalian intestine, and human and animal feces. It is rod-shaped with peritrichous flagella that give it swarming motility. They are opportunistic pathogens, responsible for septic and urinary infections.

1.7: Antibiotics for treating Oral cancer infected patients

All kinds of antibiotics can be used for treating infected oral cancer patients. A lot of antibiotics are seen resistant to both gram-positive and gram-negative bacteria. Sometimes the mixture of antibiotics is used for better treatment. The groups of antibiotics that are generally used are cephalosporins, aminoglycosides, quinolones, carbapenems, penicillin, and several other antibiotic classes (Ubeda & Pamer, 2012).

The commonly used antibiotics for both gram-positive and gram-negative bacterial infection of oral cancer patients are-

Aminoglycosides:

Aminoglycoside is a group of antibiotics traditionally for gram-negative bacteria and inhibits the synthesis of protein and contains as a portion of the molecule an amino-modified glycoside. The antibiotics in this group are tobramycin, kanamycin, gentamicin, and amikacin (Mingeot-Leclercq *et al.,* 1999). Aminoglycosides are drugs with poor gastrointestinal absorption for this intravenous or intramuscular administration is needed

Quinolone:

This antibiotic is effective against both gram-positive and gram-negative bacteria and contains a fluorine atom in its chemical structure. The most commonly used antibiotics of this group are Ciprofloxacin. Other antibiotics are nalidixic acid, levofloxacin, and moxifloxacin These antibiotic drugs inhibit the bacterial DNA gyrase enzyme which is necessary for DNA replication (Normack & Normack, 2002). Since a copy of DNA must be made each time a cell divides, interfering with replication makes it difficult for bacteria to multiply.

Cephalosporins:

Maintaining the integrity of cell wall peptidoglycan is an integral part. Cephalosporins disrupt the synthesis of this peptidoglycan layer. This disruption in synthesis leads to cell lysis or death of the cell. Cefepime, Ceftriaxone, Ceftazidime, Cefuroxime used from this group

Carbapenems:

Carbapenems are used for severe or high-risk bacterial infections. Like other cell wall disrupting antibiotics, Carbapenems also bind to the penicillin-binding protein and thus inhibit the synthesis of the cell wall. An allergic reaction is one of the adverse effects of this group of antibiotics. Imipenem and meropenem are the antibiotics of this group.

Penicillin:

Penicillin is an antibiotic derived from penicillium fungi. Penicillin hinders the bacterial activity of causing infection by preventing the cross-linking of amino acid chains in the bacterial cell wall (Green, 2002). The pre-existing bacteria is not hampered, but the new bacteria is formed with a weak cell wall that ruptures easily. The antibiotics that fall into this vast group are penicillin-G, penicillin-V, ampicillin, amoxicillin, cloxacillin and many more

Macrolide:

Macrolides are protein synthesis inhibitors. The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis, and they are thought to do this by preventing peptidyl transferase from adding the growing peptide attached to tRNA to the next amino acid as well as inhibiting ribosomal translation. Erythromycin falls in this group.

Others:

Apart from the above major groups, the antibiotics used in treating cancer patients are Linezolid, Metronidazole, Chloramphenicol, and many more. Linezolid is used for the treatment of infection caused by gram-positive bacteria by inhibiting the synthesis of bacterial protein. Metronidazole is used in combination with other antibiotics for treatment. Chloramphenicol also prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome.

1.8: Antibiotic Resistance:

Antibiotics that have saved millions of lives in the past are now becoming resistant to many bacterial infections. This antibiotic resistance crisis is increasing day by day and has been attributed to the overuse and misuse of these medications. Again, now the microbes are no longer susceptible to the commonly used antibiotics (Nikaido, 2010). By the process of mutation, the bacteria can evade the effect of the antibiotics. Then, by the process of natural selection, those bacteria may carry on and pass the resistant genes into the remaining gene pool (Davison, 1999).

For being antibiotic resistance there are 3 major ways. They are:

- Enzymatic degradation of the antibiotic agent
- Alteration of the site where the antibiotic would have initially worked
- By pumping out the agent out of the cell.

Gram-positive pathogens like S. aureus and Enterococcus species pose the biggest threat of being resistant (Kumarasamy *et al.,* 2010). But recently Gram-negative bacteria are becoming resistant to nearly all kinds of antibiotics also. This is mainly happening because of gene transfer which is being taken place through plasmid (Zhang *et al.,* 2011) and chromosomal DNA that can include mobile elements such as transposons, integrons, and R-plasmid (Hooper, 2000).

This antibiotic resistance is an alarming issue worldwide. Antibiotic-resistant microbes are prevalent in oral cancer infection also. This is because the cancer patients lose their natural immune response due to the cancer treatments. As a result, they become prone to various kinds of infections which might show resistance to numerous antibiotics. After hospital discharge, attending physicians check not only for cancer recurrence but also for wound healing, local infection, and oral mucositis. Clinicians are also aware that their patients may become carriers of drug-resistant microorganisms and may spread them to others.

1.9 Objectives:

The objective of this thesis work was to identify and screening of micro-organisms from oral cancer infection patients. Those results are compared with an isolates number of healthy people. Due to the emerging incidence of multi-drug resistant organisms, this study also aimed at determining the antibiotic resistance profile and detecting the multi-drug resistant bacteria. Then identification of most effective antibiotics so that this can contribute minimize the suffering of the patient. Also an estimation of the epidemiological, etiological and socio-economic status of oral cancer patients in Bangladesh

Chapter 2

Materials and Methods

2.1: Study Place:

The laboratory work for research was done in the Biotechnology and Microbiology laboratory of the Mathematics and Natural Sciences Department at **BRAC University**. The research was done in collaboration with the **National Institute of Cancer Research and Hospital**, Bangladesh. Data and clinical swab samples were collected from 55 oral cancer patients taking treatment from this hospital.

2.2: Study Duration:

The duration of this research work was from March 2019 to March 2020.

2.3: Study Population:

Swab samples and data were collected from the oral cavity of oral cancer patients taking treatment from the National Institute of Cancer Research and Hospital, Bangladesh. 55 swab samples were thus taken to find infection in their cancer site. Those of them who didn't have an infection in their cancer site were included in the control group. Again, 50 swab samples were taken randomly from healthy students and staff of BRAC University who didn't develop cancer.

2.4: Sample Collection:

2.4.1: Bacterial Collection:

The pus from the infection site in the oral cavity of oral cancer patients was collected by autoclaved cotton swabs and then streaked on autoclaved nutrient agar slant. The slant was taken in the laboratory and kept in incubation at 37^{o}C for the growth of bacteria. After the growth of bacteria in nutrient agar slant, further experiments were carried out. A similar process was followed for the control group who didn't develop any infection in their cancer site. Again, for another set of the control group, the autoclaved cotton swabs were rubbed on the gum area, under the tongue and cheeks of the healthy people who didn't develop any cancer.

2.4.2: Data collection:

A survey was also done to check the etiological, demographic, and socio-economic conditions of oral cancer patients in Bangladesh. The questionnaire for the survey is given in table 2.1 below:

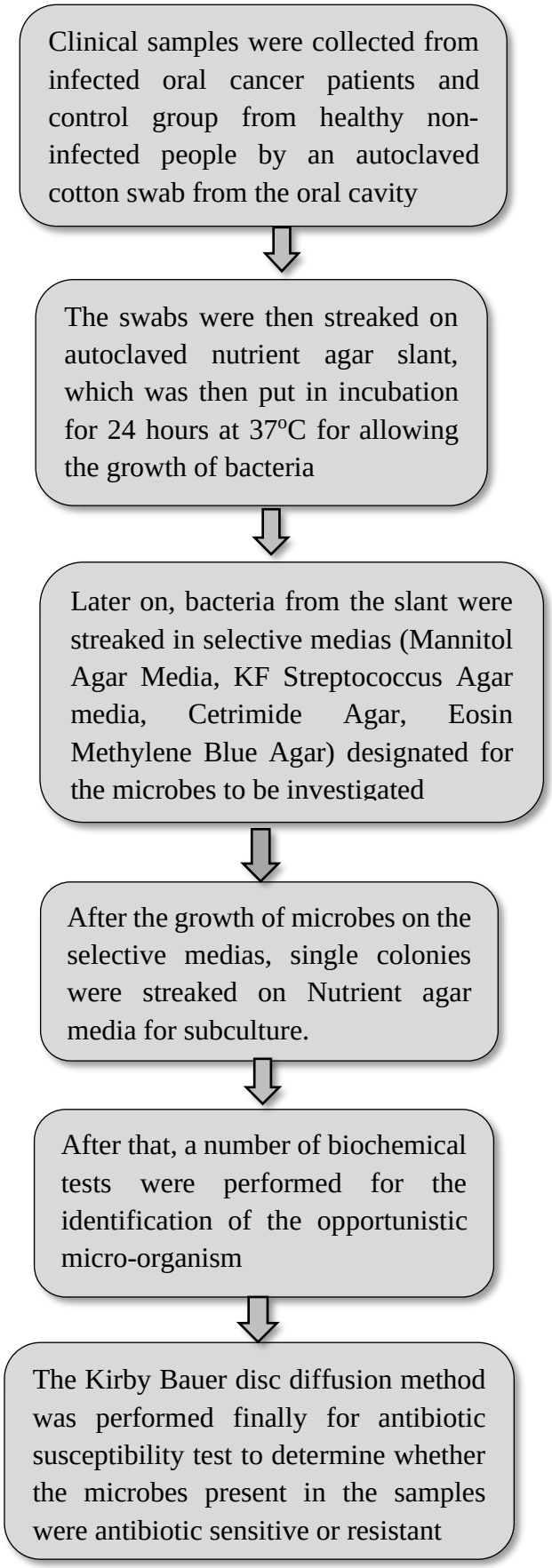
Table 2.1: Survey questionnaire for oral cancer patients

Name	Question
1) Particular(s) of the patients	• Name • Sex • Age • Address
2) Medical History	• History of cancer in any family members • History of oral cancer in any family members • History of non-infectious disease • History of infectious disease • History of mental illness
3) Lifestyle	• Hygiene practice • Consumption of betel leaf/ tobacco/betel nut/ alcohol
4) Clinical examination of cancer	• Duration of cancer • Location of infection • Treatment status
5) Socioeconomic	• Education • Occupation

2.5: Types of equipment:

- Laminar airflow cabinet (Model-SLF-V, vertical, SAARC group Bangladesh)
- Incubator (Model-0SI-500D, Digi system Laboratory Instruments Inc. Taiwan)
- Vortex machine (Digi system Taiwan, VM-2000)
- Autoclave machine (Model: WIS 20R Daihan Scientific Co. Ltd, Korea)
- Glasswares, laboratory distillation apparatus- fractional distillatory set up, microscope, pH meter Petri-dishes, slants, micro-pipettes, Bunsen burner, hot plate, clamp stands, electric balance, etc.

2.6: Experimental workflow:



2.7 Culture media for bacterial Isolation:

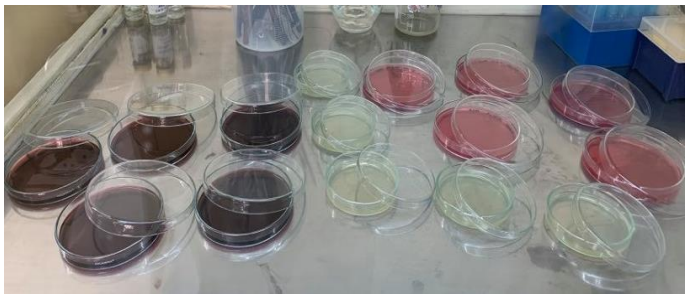


Figure 2.1: Different types of media were prepared

2.7.1: Nutrient Agar:

Nutrient agar is a nutrient media used for the growth of a wide range of non-fastidious microbes. It is popular because a wide range of bacteria and fungi can grow in this medium. It is prepared by weighing 28g of nutrient agar powder dissolved in 1 liter of distilled water in a conical flask. Then it was boiled. After boiling the conical flask was covered with aluminum foil and kept in the autoclave for sterilization. Completing sterilization, the lukewarm liquid media was plated in a petri dish

2.7.2: Mannitol Salt Agar:

Mannitol Salt Agar is a selective media used for the identification and isolation of gram-positive bacteria *Staphylococcus* and inhibits the group of others. It contains sugar mannitol and pH indicator phenol red. The organism ferments mannitol and produces an acidic byproduct that turns phenol red in agar to yellow. *Staphylococcus aureus* ferments mannitol.

In the research work, 111.02g of MSA powder was dissolved in 1 liter of distilled water in a conical flask. Then the mixture was boiled. After boiling the conical flask was covered with aluminum foil and was kept in the autoclave for sterilization. After finishing sterilization, the warm liquid media was plated in a petri dish.

2.7.3: KF Streptococcus agar media:

KF (Kenner Fecal) Streptococcus Agar media is a selective media for the isolation and identification of fecal streptococci. The nitrogen and carbon source in this media is from the enzymatic digestion of animal tissue. Yeast extract provides vitamins and trace elements in the medium. Maltose and lactose are metabolized by most fecal streptococci. Sodium Azide suppresses the growth of gram-negative bacteria. The acid formation is detected by Bromocresol blue, indicated by a color change from purple to yellow. The supplement, 1% Triphenyltetrazolium Chloride (TTC), results in the development of pink to red colonies.

In the research work, for making KF Streptococcus Agar Media, at first 0.2N NaOH was made by dissolving 0.08g of NaOH in 10ml dH_2O in a test tube and was kept in autoclave for sterilization. After sterilization 0.03g of Bromocresol purple was dissolved in the autoclaved 10ml of NaOH. Later on, 76.4g of powder KF Streptococcus Agar Media was dissolved in 1liter of distilled water. 5ml of Bromocresol purple dissolved in NaOH was added with the powder media and was boiled. After boiling 10ml of TTC was added in the boiled media and stirred well. Then the lukewarm liquid was plated in a petri dish.

2.7.4 Eosin Methylene Blue

Eosin methylene blue agar is a selective media for isolation and identification of gram-negative bacteria and inhibits the growth of gram-positive bacteria due to the presence of eosin and methylene dyes. In the media, bacteria that ferment lactose form colored colonies, and those that do not ferment lactose from colorless colonies. *Escherichia coli* forms green sheen in EMB media as it produces lactose and lowers the pH of the media. Other non-lactose fermenting gram-negative bacteria appear pink in the media and the *Aerobacter aerogenes* colonies have a brown center.

The preparation of eosin methylene blue agar involves dissolving 35.96g powder into 1 liter distilled water and boiling. After boiling, the flask containing it was sealed with aluminum foil and autoclaved. Later, it was poured into Petri dishes and used after hardening.

2.7.5: Cetrimide Agar media:

Cetrimide Agar is used for the isolation of gram-negative *Pseudomonas* species. It can produce pyocyanin, which gives the distinctive greenish hue (Leoboffe and Pierce, 2011).

It was prepared by mixing 46.7g of powder with 1 liter of distilled water. After dissolving the powder in the water through boiling, it was sealed with aluminum foil and autoclaved. Later, the liquid media was poured into dry sterilized Petri dishes.

2.7.6: HiChrome Agar:

Hi-Chrome is a differential medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections. This agar medium is selective for urine infection-causing microorganisms such as *Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus, Proteus spp, E. coli, Pseudomonas aeruginosa* and they produce distinctive different colors on media. *E. coli* gives pink-purple colonies, *Staphylococcus aureus* gives golden-yellow colonies, *Proteus spp.* give brown colonies, *Enterococcus faecalis* produce blue colonies, *Klebsiella pneumonia* produces blue mucoid colonies and *Pseudomonas spp.* give colorless colonies on Hi-Chrome agar.

2.8: Biochemical Test:

A set of biochemical tests were performed to confirm the identification of the bacteria formed in the media. The methods were done according to the microbiology laboratory manual (Cappuccino & Sherman, 2005).

- Gram staining
- Methyl Red (MR) test
- Voges– Proskauer (VP) test
- Citrate Utilization test
- Catalase test
- Oxidase test
- Triple Sugar Iron (TSI) test
- Motility Indole Urease (MIU) test
- Indole test

`1232.8.1: Gram staining:

Gram Staining is a technique used to distinguish between gram-positive and gram-negative bacteria. From an overnight culture of the organism, loopful of bacteria was smeared onto a sterile glass slide and gram staining was done

2.8.2: Methyl Red (MR) test:

Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of the high concentration of acid end products. MR-VP broth of 7 ml in each test tubes was prepared by dissolving 7g peptone, 5g dextrose, and 5g di-potassium hydrogen phosphate in 1 liter of distilled water and was autoclaved at 15 psi 121◦C. Using a sterile technique, a small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tube with an inoculating loop and the tubes were incubated for 24 hours at 37◦C. After 24 hours 3.5 ml from the culture tubes were transferred to clean test tubes for Voges- Proskauer test and the remaining broth were re-incubated for additional 24 hours. After 48-hour incubation 5 drops of methyl red indicator were added directly into the remaining aliquot of the culture tubes to observe the immediate development of a red color that indicates a positive result. (Cappuccino & Sherman, 2005)

2.8.3: Voges Proskauer:

Voges Proskauer test was done to differentiate further among enteric organisms for determining the capability of the organisms to produce non-acidic or neutral end products such as acetyl-methyl-carbinol. To the aliquot of MR-VP broth after 24hour incubation, 0.6 ml (12 drops) of 5% alpha naphthol (Baritte A) was added followed by 0.2 ml (4 drops) of 40% KOH (Baritte B). The tube was gently shaken to expose the medium to atmospheric oxygen (30seconds-1 minute) and the medium was allowed to remain undisturbed for 10-15 minutes. The test was read, but not
beyond, one hour following the addition of the reagents (McDevitt, 2009). A pink color indicated a positive result; no color change meant a negative result.

2.8.4: Citrate utilization test:

A citrate utilization test was done to differentiate among enteric organisms based on their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vial were prepared by autoclaving at 15 psi 121◦C. Small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials through a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37◦C (Cappuccino & Sherman, 2005). The blue color showed a positive result and the green color indicated a negative result.

2.8.5: Catalase test:

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase. A microscopic slide was placed inside a petri dish. Using a sterile inoculating loop, a small number of bacteria from 24-hour pure culture were placed onto the microscopic slide. 1 drop of 3% H_2O_2 was placed onto the organism on the microscopic slide using a dropper and observed for immediate bubble formation which indicated positive results (Reiner, 2010).

2.8.6: Oxidase test:

An Oxidase test was done to determine the presence of the enzyme cytochrome oxidase in the bacteria. A small piece of filter paper was soaked in Gaby and Hadley oxidase test reagent and let dry. Using an inoculating loop, a well-isolated colony from pure 24-hour culture was picked and rubbed onto filter paper and observed for color change (Shields & Cathcart, 2010).

2.8.7: Triple sugar iron test (TSI):

A triple sugar iron test was done to differentiate among the different groups or genera of the *Enterobacteriaceae* based on the ability to reduce sulfur, ferment carbohydrates, and produce gas. The base powder of the Triple sugar iron was added to distilled water and boiled, and poured into test tubes. Then it was autoclaved at 15 psi 121◦C. While it remained warm, it was put on an angled position and hardened into slants. A small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes employing a stab and streak inoculation method with an inoculating needle. The screw caps were not fully tightened and the tubes were incubated for 24 hours at 37◦C (Cappuccino & Sherman, 2005).

2.8.8 Motility Indole Urease test (MIU):

MIU test was done to simultaneously determine the ability of the bacteria to produce indole, check motility, and degrade urea using the enzyme urease. MIU media was prepared by autoclaving at 15 psi 121◦C. The media was cooled to about 50-55◦C and 100ml of urea glucose solution was added aseptically to 900 ml base medium. After that, a 6ml solution was transferred to each sterile test tube and allowed to form a semi-solid medium. Using a sterile technique, a small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes using a stab inoculation method with an inoculating needle, and the tubes were then incubated for 24 hours at 37◦C (Acharya, 2015). The appearance and color of the media were observed after incubation (Cappuccino and Sherman, 2005).

2.8.9: Indole test:

An indole production test was done to determine the ability of the bacteria to degrade the amino acid tryptophan by the enzyme tryptophanase. Tryptophan broth of 5 ml in each test tube was prepared by autoclaving at 15 psi 121◦C. Using a sterile technique, a small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes utilizing a loop inoculation method with an inoculating loop and the tubes were incubated for 48 hours at 37◦C. To test for indole production, 5 drops of Kovac's reagent was added directly into the tubes (MacWilliams, 2009). Red color meant a positive result and yellow color meant a negative result.

2.9: Antibiotic resistance and susceptibility analysis

It is an important task to check the performance of antimicrobial susceptibility testing of significant bacterial isolates. This test aims to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Muller-Hinton agar following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial susceptibility pattern was determined using thirteen selected antibiotics from commercial antimicrobial disks with a wide range of mechanisms of action, including drugs that target cell wall, nucleic acid, and protein. The bacterial suspension was inoculated in Mueller Hinton agar plates and antibiotic discs were placed on the culture After incubation, the antimicrobial efficacy was determined by measuring the diameter of the zones of inhibition and bacterial strains were classified as susceptible (S) or resistant (R) depending on the diameter of the inhibition zone

2.9.1: Preparation of Muller Hinton Agar (MHA)

Muller Hinton agar is a suitable medium for antibiotic susceptibility testing. All micro-organisms plated in this medium will grow as its a non-selective and non-differential medium.

38g of Mueller Hinton agar powder was dissolved in 1-liter distilled water by boiling and stirring. The opening of the conical flask was wrapped in aluminum foil and autoclaved for sterilization. After sterilization, the liquid was poured into sterile Petri dishes.

2.9.2: Bacterial Suspension preparation:

With a sterile loop, the bacterial colony from 24 hours old culture was taken and mixed with sterile 0.9% saline. The concentration was kept at 1 McFarland Standard solutions.

2.9.3: List of antibiotics:

The antibiotics used in the susceptibility test were selected based on their usage. The list of antibiotics with their zone of inhibition used in this research work are given in the table below-

Table 2.2: List of antibiotics with their zone size for interpretation of susceptibility pattern:

	Group of Antibiotic(s)	Name of Antibiotic(s)	Disc Code	Disc Potency (µg)	Range		
					Resistance (mm)	Intermediate (mm)	Susceptible (mm)
1	Aminoglycosides	Gentamicin Amikacin	GEN AK	10 30	12 14	13-14 15-16	15 17
2	Carbapenems	Imipenem	IMI	10	13	14-15	16
3	Cephalosporins	Ceftazidime Ceftriaxone	CAZ CTR	30 30	14 13	15-17 14-20	18 21
4	Glycopeptides	Vancomycin	VA	30	14	15-16	17
5	Macrolides	Erythromycin Azithromycin	E AZM	15 15	13 13	14-22 14-17	23 18
6	Penicillin	Amoxicillin Penicillin-G Oxacillin Cloxacillin	AMX P OX COX	10 10 1 5	13 14/28 10 15	14-17 12/21-21/28 11-12 16-19	20 15/19 13 20
7	Penicillin combination	Amoxyclav	AMC	10	13	14-17	20
8	Quinolones	Ciprofloxacin Nalidixic Acid	CIP NA	5 30	15 13	16-20 14-18	21 19
9	Tetracycline	Tetracycline	TE	30	14	15-18	19
10	Others	Linezolid Metronidazole Chloramphenicol	LZ MT C	30 30	20 < 12	21-22 13-17	23 > 18

2.9.4: Inoculation and disc diffusion:

Muller Hinton agar plate was inoculated by an autoclaved cotton swab. The autoclaved cotton swab was dipped into the bacterial suspension mixture. The swab was then spread into the entire MHA plate to make a lawn culture. After the streaking was complete, the plate was allowed to dry for 5 minutes. Later on, with sterile forceps, the antibiotic discs were placed on the plate. The discs were placed in such a manner so that the zone does not overlap and remain in even space. After placing the discs, the plates were turned over and were kept in incubation at 37^{o}C for 16-18hours. After the incubation period, the zones were measured and interpreted.

Chapter 3

Results

3.1: Result of growth on selective media:

Oral swabs collected from the patients' group and control group were streaked in nutrient agar slant and were kept in incubation for 24 hours at 37°C. After 24 hours a loopful of bacterial colony was taken and streaked in selective medias such as Mannitol sugar agar media and KF Streptococcus agar media for isolating Gram-positive bacteria and Eosin methylene blue agar media and Cetrimide agar media for isolating Gram-negative bacteria. Among the 55 samples taken from oral cancer patients, all of those showed positive results in the selective media. Among those 55 patients, 31 patients were pre-operative and 24 patients were post-operative patients. The pre-operative patients were labeled as "PRE" and the post-operative patients were labeled as "PO". Among the 50 control groups, 30 of them showed positive growth on the selective media selected for the growth of gram-positive and gram-negative opportunistic microbes. The control groups were labeled C1-C50 respectively. The appearance and type of growth of the isolates in the selective media are given in Table 3.1 below:

Table 3.1: Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
1	PO1	+ (yellow)	+				Purple	Gram-positive(+ve)
2	PO1		+		Pink/Purple mucoid		Pink	Gram negative(-ve)
3	PO1		+			Green	Pink	Gram negative(-ve)
4	PO2		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
5	PRE3		+		Colorless lush		Pink	Gram negative(-ve)
6	PRE3		+		Green Sheen		Pink	Gram negative(-ve)
7	PRE3		+			Green	Pink	Gram negative(-ve)
8	PO4	+(yellow)	+				Purple	Gram-positive(+ve)
9	PRE5		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
10	PRE5		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
11	PRE6	+(yellow)	+				Purple	Gram-positive(+ve)

Table 3.1: (Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
12	PO7		+			Green	Pink	Gram negative(-ve)
13	PRE8	+(yellow)	+				Purple	Gram positive(+ve)
14	PRE8		+	A pink colony with yellow zone			Purple	Gram positive(+ve)
15	PRE9	+(yellow)	+				Purple	Gram positive(+ve)
16	PO10		+	A pink colony with yellow zone			Purple	Gram positive(+ve)
17	PO10		+			Green	Pink	Gram negative(-ve)
18	PO10		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
19	PRE11		+			Green	Pink	Gram negative(-ve)
20	PRE11		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
21	PRE12		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
22	PRE13	+ (yellow)	+				Purple	Gram positive(+ve)
23	PRE13		+	A pink colony with yellow zone			Purple	Gram positive(+ve)
24	PRE13		+			Green	Pink	Gram negative(-ve)
25	PRE 13		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
26	PRE13		+		Colorless lush		Pink	Gram negative(-ve)
27	PRE14	+ (yellow)	+				Purple	Gram positive(+ve)
28	PRE14		+	A pink colony with yellow zone			Purple	Gram positive(+ve)
29	PRE14		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
30	PRE14		+		Green Sheen		Pink	Gram negative(-ve)
31	PRE15	+ (yellow)	+				Purple	Gram positive(+ve)
32	PRE15		+			Green	Pink	Gram negative(-ve)
33	PRE15		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)

Table 3.1: (Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
34	PRE16		+			Green	Pink	Gram negative(-ve)
35	PRE16		+	Pink/red colony			Purple	Gram-positive(+ve)
36	PRE16		+		Green sheen		Pink	Gram negative(-ve)
37	PO17	+(yellow)	+				Purple	Gram-positive(+ve)
38	PO17		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
39	PO17		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
40	PO17		+			Green	Pink	Gram negative(-ve)
41	PO18	+(yellow)	+				Purple	Gram-positive(+ve)
42	PO18		+	Pink/red colony			Purple	Gram-positive(+ve)
43	PO18		+		Green sheen		Pink	Gram negative(-ve)
44	PO18		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
45	PO19		+			Green	Pink	Gram negative(-ve)
46	PO19		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
47	PO20	+(yellow)	+				Purple	Gram-positive(+ve)
48	PO20		+			Green	Pink	Gram negative(-ve)
49	PO20		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
50	PO20		+		Colorless lush		Pink	Gram negative(-ve)
51	PO21		+			Green	Pink	Gram negative(-ve)
52	PO21		+		Colorless lush		Pink	Gram negative(-ve)
53	PO21		+		Green sheen		Pink	Gram negative(-ve)
54	PO21	+(yellow)	+				Purple	Gram-positive(+ve)

Table 3.1:(Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
55	PO22		+			Green	Pink	Gram negative(-ve)
56	PO22		+		Colorless lush		Purple	Gram-positive(+ve)
57	PO22		+		Green sheen		Pink	Gram negative(-ve)
58	PO22		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
59	PRE23	+(yellow)	+				Purple	Gram-positive(+ve)
60	PRE23		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
61	PRE23		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
62	PO24	+(yellow)					Purple	Gram-positive(+ve)
63	PO24		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
64	PRE25		+	Pink/red colony			Purple	Gram-positive(+ve)
65	PRE25		+		Colorless lush		Pink	Gram negative(-ve)
66	PO26		+			Green	Pink	Gram negative(-ve)
67	PRE27	+(yellow)	+				Purple	Gram-positive(+ve)
68	PRE27		+	Pink/red colony			Purple	Gram-positive(+ve)
69	PRE27		+			Green	Pink	Gram negative(-ve)
70	PRE28		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
71	PRE28		+		Colorless lush		Pink	Gram negative(-ve)
72	PRE28		+			Green	Pink	Gram negative(-ve)
73	PO29		+			Green	Pink	Gram negative(-ve)
74	PO29		+		Green sheen		Pink	Gram negative(-ve)

Table 3.1:(Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
75	PO30		+		Colorless lush		Pink	Gram negative(-ve)
76	PO30		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
77	PO30		+			Green	Pink	Gram negative(-ve)
78	PO30		+	A pink colony with yellow zone			Pink	Gram negative(-ve)
79	PO31		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
80	PO32		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
81	PO32		+			Green	Pink	Gram negative(-ve)
82	PRE33		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
83	PRE33		+			Green	Pink	Gram negative(-ve)
84	PRE34		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
85	PRE35		+			Green	Pink	Gram negative(-ve)
86	PRE36		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
87	PRE36		+			Green	Pink	Gram negative(-ve)
88	PRE37	+(yellow)	+				Purple	Gram-positive(+ve)
89	PRE37		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
90	PRE38	+(yellow)	+				Purple	Gram-positive(+ve)
91	PRE38		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
92	PRE38		+			Green	Purple	Gram-positive(+ve)
93	PRE39	+(yellow)	+				Purple	Gram-positive(+ve)
94	PRE39		+	Pink/red colony			Purple	Gram-positive(+ve)
95	PRE39		+		Colorless lush		Pink	Gram negative(-ve)
96	PRE40		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)

Table 3.1:(Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
97	PO41	+(yellow)	+				Purple	Gram-positive(+ve)
98	PO41		+			Green	Pink	Gram negative(-ve)
99	PO42		+			Green	Pink	Gram negative(-ve)
100	PO42		+		Green sheen		Pink	Gram negative(-ve)
101	PO43	+(yellow)	+				Purple	Gram-positive(+ve)
102	PO43		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
103	PO44	+(yellow)	+				Purple	Gram-positive(+ve)
104	PO44		+	Pink/red colony			Purple	Gram-positive(+ve)
105	PO44		+		Colorless lush		Pink	Gram negative(-ve)
106	PO44		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
107	PRE45	+(yellow)	+				Purple	Gram-positive(+ve)
108	PRE45		+			Green	Pink	Gram negative(-ve)
109	PRE47		+	A pink colony with yellow zone			Purple	Gram-positive(+ve)
110	PRE47		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
111	PRE47		+		Green sheen		Pink	Gram negative(-ve)
112	PRE47		+		Colorless lush		Pink	Gram negative(-ve)
113	PRE48	+(yellow)	+				Purple	Gram-positive(+ve)
114	PRE49		+			Green	Pink	Gram negative(-ve)
115	PO51		+		Colorless lush		Pink	Gram negative(-ve)
116	PO51		+			Green	Pink	Gram negative(-ve)
117	PRE52	+(yellow)	+				Purple	Gram-positive(+ve)
118	PRE52		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)

Table 3.1:(Continued) Growth of isolates in selective media and gram staining results:

Serial no	Specimen number	MSA	NA	KF Streptococcus Agar media	EMB	Cetrimide	Gram Staining	Type of isolates
119	PRE52		+		Green sheen		Pink	Gram negative(-ve)
120	PO53		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
121	PRE54		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
122	PRE54		+		Green sheen		Pink	Gram negative(-ve)
123	PO55	+(yellow)	+				Purple	Gram-positive(+ve)
124	PO55		+		Green sheen		Pink	Gram negative(-ve)
125	PO55		+			Green	Pink	Gram negative(-ve)
126	PO55		+		Pink/ Purple mucoid		Pink	Gram negative(-ve)
127	PO55		+		Colorless lush		Pink	Gram negative(-ve)

3.2 Identification of isolates based on biochemical tests results:

The individual distinct colonies that were found from the selective media were streaked on nutrient agar to observe visual similarities in terms of colony morphology. Fifty-five samples were from infected oral cancer patients, among them, 31 are from pre-operative patients (PRE) and 24 were from post-operative patients (PO). The remaining 30 were from healthy people labeled as C1-C50

In table 3.2 and 3.3 the biochemical test of the found gram-positive and gram-negative isolates of the patient group and control group is given below:

Table 3.2.1: Biochemical characteristics of gram-positive isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1	PO1	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
2	PO4	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
3	PRE5	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
4	PRE6	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
5	PRE8	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
6	PRE8	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
7	PRE9	MSA	-	+	-	-	+	-	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
8	PO10	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	R/Y	+	+	+	+	+	*Streptococcus spp*
9	PRE13	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
10	PRE13	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
11	PRE14	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	R/Y	+	+	+	-	-	*Streptococcus spp*
12	PRE14	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	+	+	*Streptococcus spp*
13	PRE16	KF	-	-	-	-	-	+	+	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*
14	PO17	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
15	PO17	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
16	PO18	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	R/Y	+	+	+	-	-	*Staphylococcus spp*
17	PO18	KF	-	-	-	-	-	+	+	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*
18	PO20	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
19	PO21	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.1: (Continued) Biochemical characteristics of gram-positive isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
20	PRE23	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
21	PRE23	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
22	PO24	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
23	PO24	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
24	PRE25	KF	-	-	-	-	-	+	-	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*
25	PRE27	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
26	PRE27	KF	-	-	-	-	-	+	+	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*
27	PO30	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
28	PRE37	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	R/Y	+	+	+	+	+	*Streptococcus spp*
29	PRE37	MSA	-	+	-	-	+	-	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
30	PRE38	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
31	PRE38	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	R/Y	+	+	+	-	-	*Staphylococcus spp*
32	PRE39	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
33	PRE39	KF	-	-	-	-	-	+	+	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*
34	PO40	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
35	PO41	MSA	-	+	-	-	+	-	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
36	PO43	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	R/Y	+	+	+	-	-	*Staphylococcus spp*
37	PO43	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
28	PO44	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
39	PO44	KF	-	-	-	-	-	+	+	Purple	Cocci	-	Blue	Y/Y	+	+	+	-	-	*Enterococcus spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.1: (Continued) Biochemical characteristics of gram-positive isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
40	PRE45	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
41	PRE47	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
42	PRE48	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
43	PRE52	MSA	-	+	-	-	+	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Staphylococcus spp*
44	PO55	MSA	-	+	-	-	+	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.2: Biochemical tests characteristics of gram-negative isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1	PO1	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
2	PO1	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
3	PO2	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
4	PRE3	EMB	-	+	+	-	+	+	-	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
5	PRE3	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
6	PRE3	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
7	PRE5	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
8	PRE5	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
9	PO7	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R\R	-	-	-	-	+	*Pseudomonas spp*
10	PO10	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
11	PO10	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
12	PRE11	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
13	PRE11	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	Y/Y	-	-	-	-	+	*Pseudomonas spp*
14	PRE12	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
15	PRE13	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
16	PRE13	EMB	-	+	+	-	+	+	-	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
17	PRE13	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
18	PRE14	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
19	PRE14	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.2: (continued) Biochemical characteristics of gram-negative isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
20	PRE15	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
21	PRE15	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
22	PRE16	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
23	PRE16	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
24	PO17	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
25	PO17	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
26	PO18	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
27	PO18	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
28	PRE19	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
29	PRE19	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
30	PO20	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
31	PO20	EMB	-	+	+	-	+	+	-	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
32	PO20	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
33	PRE21	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
34	PRE21	EMB	-	+	+	-	+	+	-	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
35	PRE21	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.2: (continued) Biochemical tests characteristics of gram-negative isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
36	PO22	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
37	PO22	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
38	PO22	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
39	PO22	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
40	PRE23	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
41	PRE25	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
42	PRE25	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
43	PO26	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
44	PRE27	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
45	PRE28	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
46	PRE28	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
47	PRE28	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
48	PO29	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	YY	+	+	+	-	+	*Escherichia spp*
49	PO29	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
50	PO30	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
51	PO30	EMB	-	+	-	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
52	PO30	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
53	PRE31	EMB	-	+	-	-	-	+	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
54	PRE32	EMB	-	+	-	-	-	+	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
55	PRE32	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.2: (continued) Biochemical characteristics of gram-negative isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
56	PRE33	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
57	PRE33	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
58	PRE34	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
59	PRE35	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
60	PRE36	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
61	PRE36	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
62	PRE38	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
63	PRE39	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
64	PO41	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
65	PO42	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
66	PO42	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
67	PRE44	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
68	PRE44	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
69	PRE45	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
70	PRE47	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
71	PRE47	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
72	PRE47	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
73	PRE49	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
74	PO51	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
75	PO51	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.2: (continued) Biochemical tests characteristics of gram-negative isolates (patient group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
76	PRE52	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
77	PO53	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
78	PRE54	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
79	PRE54	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
80	PO55	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
81	PO55	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
82	PO55	EMB	-	+	+	-	+	+	+	Pink	Rod	+	Light Brown	R/Y	+	-	-	+	+	*Proteus spp*
83	PO55	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.3: Biochemical characteristics of gram-positive isolates (control group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1	C6	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
2	C9	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
3	C9	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
4	C10	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
5	C11	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
6	C14	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
7	C15	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
8	C16	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
9	C17	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
10	C18	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
11	C22	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
12	C25	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
13	C25	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
14	C28	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
15	C30	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
16	C35	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*
17	C37	MSA							+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
18	C40	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
19	C46	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
20	C47	MSA	-	+	-	-	+	+	+	Purple	Cocci	+	Golden yellow	Y/Y	+	+	+	-	-	*Staphylococcus spp*
21	C47	KF	-	-	-	-	-	+	-	Purple	Cocci	+	No color	Y/Y	+	+	+	-	-	*Streptococcus spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

Table 3.2.4: Biochemical characteristics of gram-negative isolates (control group)

Serial no.	Isolates no.	Media used for isolation	Oxidase test	Catalase test	MIU			MRVP		Gram Staining		Simmon's citrate	Appearance in HiChrome media	TSI						Probable Organism
					Motility	Indole	Urease	Methyl Red	VogesProskauer	Color	Shape			Slant/ Butt	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1	C5	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
2	C9	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
3	C9	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
4	C13	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
5	C13	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia coli*
6	C14	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
7	C16	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
8	C16	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
9	C19	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
10	C20	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia spp*
11	C21	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
12	C21	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
13	C23	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
14	C26	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
15	C31	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
16	C31	EMB	-	+	+	+	-	+	-	Pink	Rod	-	Purple	Y/Y	+	+	+	-	+	*Escherichia coli*
17	C35	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
18	C39	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
19	C39	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*
20	C42	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
21	C43	EMB	-	+	-	-	+	-	+	Pink	Rod	+	Blue	Y/Y	+	+	+	-	+	*Klebsiella spp*
22	C48	Cet	+	+	+	-	-	-	-	Pink	Rod	+	Green	R/R	-	-	-	-	+	*Pseudomonas spp*

'+'= positive, '- '= negative; 'PRE'= Pre-operative, 'PO' = Post-operative, Y= Yellow, R= Red

3.3 Percentage identity of the identified isolates

After the selection from selective media and the biochemical tests, two types of bacteria were found. One is Gram-positive bacteria and the other is Gram-negative bacteria. Among the Gram-positive bacteria, the probable organism found were *Staphylococcus spp* and *Streptococcus spp*. And among the Gram-negative bacteria, the probable organism found were *Klebsiella spp, Pseudomonas spp, E. coli, and Proteus spp.*
The ratio of selected isolates found is given below.

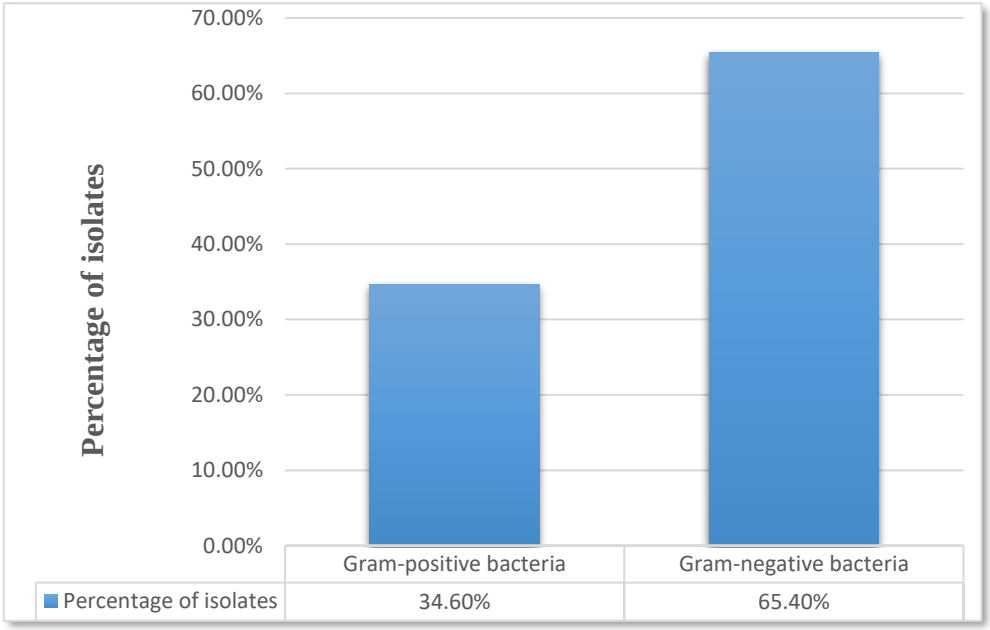


Figure 3.1: Percentage of the Gram positive and Gram-negative bacteria

Figure 3.1 represents that the higher percentage of isolates found were gram-negative 65.4% (83) bacteria and 34.6% (44) percentage of isolates found were gram-positive bacteria.

Table 3.3: Percentage of isolates from cancer patients and control subjects:

Organism	Total no. of isolates in cancer patients	Infection (Post-opt) (%)	Infection (Pre-opt) (%)	Control (%)
Staphylococcus spp	24(43.63%)	11(45.8%)	13(54.2%)	15
Streptococcus spp	14(25.45%)	5(35.71%)	9(64.2%)	6
Enterococcus spp	6(10.9%)	2(33.33%)	4(66.6%)	-
Klebsiella spp	27(49.09%)	10(37.03%)	17(62.96%)	11
Proteus spp	12(21.8%)	6(50%)	6(50%)	-
E.coli	14 (25.45%)	5(35.71%)	9(64.28%)	3
Pseudomonas spp	30(54.54%)	12(41.37%)	18(58.62%)	8

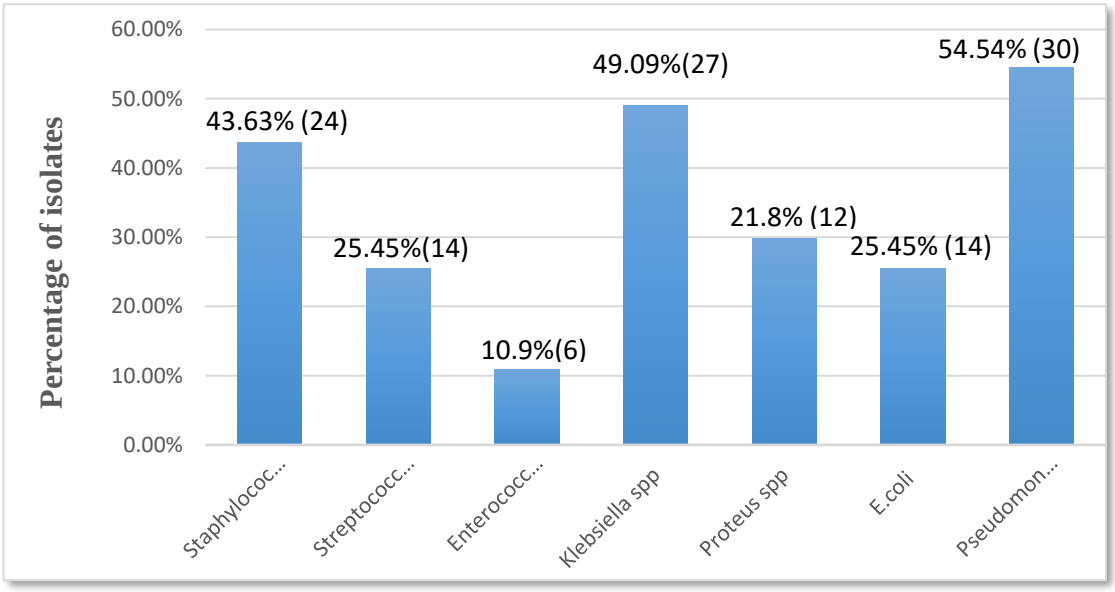


Figure 3.2: Percentage of isolates in cancer patients

Figure 3.2 shows that the highest percentage of isolates found were *Pseudomonas spp* 54.54% (30), followed by *Klebsiella spp* 49.09% (27), *Staphylococcus spp* 43.63%(24), *Streptococcus spp* 25.45%(14) and *E. coli* 25.45%(14) and *Proteus spp* 21.8%(12). The least number of isolates was found was *Enterococcus spp* 10.9% (6).

3.4: Result from antibiotic susceptibility test:

The 127 isolates from cancer patients and 38 isolates from the control group were tested for antibiotic susceptibility with 13 antibiotics from 11 different groups. The results from the AST are given below according to the type of bacteria:

Table 3.4.1: Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients

Zone of inhibition (mm)																													
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**GEN**		**AK**		**IMI**		**CTR**		**VA**		**AMX**		**P**		**AZM**		**AMC**		**CIP**		**NA**		**TE**		**MT**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
1	PO1 (EMB)	*Klebsiella spp*	25	S	22	S	32	S	0	R	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R
2	PO1(Cet)	*Pseudomonas spp.*	23	S	25	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	30	S	0	R	0	R	0	R
3	PO2 (EMB)	*Klebsiella spp*	24	S	23	S	30	S	23	S	0	R	0	R	0	R	0	R	0	R	34	S	0	R	0	R	0	R
4	PRE3(EMB)	*Proteus spp*	22	S	21	S	22	S	24	S	0	R	0	R	0	R	0	R	23	S	25	S	0	R	0	R	0	R
5	PRE3(EMB)	*Escherichia coli li*	22	S	23	S	26	S	34	S	0	R	0	R	0	R	0	R	0	R	27	S	0	R	0	R	0	R
6	PRE3(Cet)	*Pseudomonas spp.*	25	S	21	S	27	S	0	R	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R
7	PRE5(EMB)	*Klebsiella spp*	24	S	26	S	23	S	29	S	0	R	0	R	0	R	0	R	0	R	30	S	0	R	0	R	0	R
8	PRE7(Cet)	*Pseudomonas spp.*	20	S	26	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	37	S	0	R	0	R	0	R
9	PRE10(EMB)	*Klebsiella spp.*	25	S	24	S	24	S	24	R	0	R	0	R	0	R	0	R	23	S	39	S	0	R	0	R	0	R
10	PRE11(EMB)	*Klebsiella spp.*	22	S	23	S	22	S	23	S	0	R	0	R	0	R	0	R	0	R	28	S	0	R	0	R	0	R
11	PRE11(Cet)	*Pseudomonas spp.*	23	S	23	S	28	S	12	R	0	R	0	R	0	R	0	R	0	R	33	S	0	R	0	R	0	R
12	PRE12(EMB)	*Klebsiella spp.*	22	S	23	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	32	S	0	R	0	R	0	R
13	PRE13(EMB)	*Klebsiella spp.*	24	S	23	S	23	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
14	PRE13(EMB)	*Proteus spp.*	0	S	23	S	25	S	23	S	0	R	0	R	0	R	0	R	0	R	14	R	0	R	0	R	0	R
15	PRE13(Cet)	*Pseudomonas spp.*	23	S	23	S	27	S	0	R	0	R	0	R	0	R	0	R	0	R	37	S	0	R	0	R	0	R

PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.1: (continued) Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients

Zone of inhibition (mm)																													
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**GEN**		**AK**		**IMI**		**CTR**		**VA**		**AMX**		**P**		**AZM**		**AMC**		**CIP**		**NA**		**TE**		**MT**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
16	PRE14(EMB)	*Klebsiella spp.*	25	S	24	S	24	S	14	R	0	R	0	R	0	R	13	R	22	S	28	S	0	R	0	R	0	R
17	PRE14(EMB)	*Escherichia coli*	22	S	19	S	27	S	34	S	0	R	0	R	0	R	18	S	25	S	30	S	25	S	24	S	0	R
18	PRE15(EMB)	*Klebsiella spp.*	21	S	22	S	23	S	0	R	0	R	0	R	0	R	0	R	20	S	0	R	20	S	13	R	0	R
19	PRE15 (Cet)	*Pseudomonas spp.*	22	S	23	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	31	S	0	R	0	R	0	R
20	PRE16(EMB)	*Escherichia coli*	19	S	22	S	24	S	22	R	0	R	0	R	0	R	0	R	21	S	36	S	28	S	0	R	0	R
21	PRE16 (Cet)	*Pseudomonas spp.*	20	S	19	S	27	S	0	R	0	R	0	R	0	R	0	R	0	R	36	S	0	R	0	R	0	R
22	PO17 (EMB)	*Klebsiella spp.*	22	S	20	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
23	PO17 (Cet)	*Pseudomonas spp.*	21	S	20	S	23	S	0	R	0	R	0	R	0	R	0	R	0	R	38	S	0	R	0	R	0	R
24	PO18 (EMB)	*Klebsiella spp.*	21	S	19	S	23	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
25	PO18 (EMB)	*Escherichia coli*	20	S	11	R	0	R	13	R	0	R	0	R	0	R	14	R	0	R	0	R	0	R	0	R	0	R
26	PRE19(EMB)	*Klebsiella spp.*	20	S	9	R	0	R	11	R	0	R	0	R	0	R	15	R	0	R	0	R	0	R	0	R	0	R
27	PRE19 (Cet)	*Pseudomonas spp.*	20	S	21	S	27	S	0	R	0	R	0	R	0	R	0	R	0	R	34	S	0	R	0	R	0	R
28	PO20 (EMB)	*Klebsiella spp.*	22	S	20	S	27	S	0	R	0	R	0	R	0	R	0	R	0	R	38	R	0	R	0	S	0	R
29	PO20 (EMB)	*Proteus spp.*	21	S	20	S	23	S	0	R	0	R	0	R	0	R	0	R	0	R	39	S	0	R	0	S	0	R
30	PO20 (Cet)	*Pseudomonas spp.*	24	S	24	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.1: (continued) Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients:

Serial no.	Isolates no.	Antibiotics / Name of organisms	Zone of inhibition (mm)																							
			GEN		AK		IMI 0		CTR		VA		AMX		P		AZM		AMC		CIP		NA		TE		MT	
			ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP
31	PO21 (EMB)	*Escherichia coli*	20	S	9	R	0	R	11	R	0	R	0	R	0	R	16	R	0	R	0	R	0	R	0	R	0	R
32	PO21 (EMB)	*Proteus spp.*	0	R	23	S	20	S	23	R	0	R	0	R	0	R	20	S	0	R	11	R	0	R	0	R	0	R
33	PO21 (Cet)	*Pseudomonas spp.*	22	S	18	S	0	R	0	R	0	R	0	R	0	R	19	S	16	R	34	S	10	R	19	S	0	R
34	PO22 (EMB)	*Klebsiella spp.*	23	S	25	S	23	S	0	R	0	R	0	R	0	R	21	R	21	S	32	S	0	R	0	R	0	R
35	PO22 (EMB)	*Escherichia coli*	21	S	22	S	18	R	0	R	0	R	0	R	0	R	12	R	0	R	0	R	0	R	9	R	0	R
36	PO22 (EMB)	*Proteus spp.*	26	S	24	S	24	S	24	R	0	R	0	R	0	R	0	R	23	S	39	S	0	R	0	R	0	R
37	PO22(Cet)	*Pseudomonas spp*	20	S	20	S	24	S	31	S	0	R	0	R	0	R	13	R	0	R	29	S	19	S	24	S	0	R
38	PRE23(EMB)	*Klebsiella spp.*	28	S	23	S	23	S	23	S	0	R	0	R	0	R	0	R	0	R	28	S	0	R	0	R	0	R
39	PRE25(EMB)	*Proteus spp.*	20	S	13	R	0	R	13	R	0	R	0	R	0	R	15	R	0	R	0	R	0	R	0	R	0	R
40	PRE25 (Cet)	*Pseudomonas spp.*	22	S	23	S	25	S	34	S	0	R	0	R	0	R	12	R	18	S	29	S	0	R	12	R	0	R
41	PO26 (Cet)	*Pseudomonas spp.*	21	S	19	S	25	S	15	R	0	R	0	R	0	R	21	S	23	S	30	S	20	S	21	S	0	R
42	PRE27(Cet)	*Pseudomonas spp.*	20	S	22	S	0	R	25	R	0	R	0	R	0	R	13	R	0	R	30	S	27	S	16	R	0	R
43	PRE28(EMB)	*Klebsiella spp.*	21	S	22	S	20	R	0	R	0	R	0	R	0	R	12	R	0	R	0	R	0	R	9	R	0	R
44	PRE28(EMB)	*Proteus spp.*	26	S	24	S	30	S	22	R	0	R	0	R	0	R	11	R	25	S	38	S	0	R	14	R	0	R
45	PRE28 (Cet)	*Pseudomonas spp.*	22	S	19	S	21	S	10	R	0	R	0	R	0	R	15	R	18	S	27	S	20	S	22	S	0	R
46	PO29 (EMB)	*Escherichia coli*	20	S	21	S	26	S	19	R	0	R	0	R	0	R	0	R	16	R	32	S	10	R	20	S	0	R
47	PO29 (Cet)	*Pseudomonas spp.*	22	S	22	S	21	S	22	R	0	R	0	R	0	R	17	R	19	S	27	S	0	R	0	R	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.1: (continued) Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients:

Zone of inhibition (mm)																													
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**GEN**		**AK**		**IMI**		**CTR**		**VA**		**AMX**		**P**		**AZM**		**AMC**		**CIP**		**NA**		**TE**		**MT**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
48	PO30 (EMB)	*Klebsiella spp.*	22	S	18	S	0	R	0	R	0	R	0	R	0	R	19	S	16	R	34	S	10	R	19	S	0	R
49	PO30 (EMB)	*Proteus spp.*	20	S	20	S	24	S	31	S	0	R	0	R	0	R	15	R	23	R	29	S	19	S	24	S	0	R
50	PO30 (Cet)	*Pseudomonas spp.*	20	S	21	S	25	S	22	R	0	R	0	R	0	R	0	R	20	S	35	S	0	R	25	S	0	R
51	PRE31(EMB)	*Klebsiella spp.*	21	S	19	S	23	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
52	PRE32(EMB)	*Klebsiella spp.*	20	S	13	R	0	R	13	R	0	R	0	R	0	R	15	R	0	R	0	R	0	R	0	R	0	R
53	PRE32 (Cet)	*Pseudomonas spp.*	23	S	21	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	37	S	0	R	0	R	0	R
54	PRE33(EMB)	*Klebsiella spp.*	21	S	22	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
55	PRE33 (Cet)	*Pseudomonas spp.*	21	S	19	S	19	R	25	S	0	R	0	R	0	R	10	R	25	S	28	S	20	S	15	R	0	R
56	PRE34(EMB)	*Klebsiella spp.*	23	S	23	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
57	PRE35 (Cet)	*Pseudomonas spp.*	21	S	26	S	24	S	17	R	0	R	0	R	0	R	18	S	0	R	38	S	12	R	17	R	0	R
58	PRE36(EMB)	*Klebsiella spp.*	22	S	23	S	28	S	22	R	0	R	0	R	0	R	0	R	0	R	39	S	0	R	0	R	0	R
59	PRE36 (Cet)	*Pseudomonas spp.*	19	S	27	S	23	S	20	R	0	R	0	R	0	R	13	R	22	S	24	S	0	R	0	R	0	R
60	PRE38 (Cet)	*Pseudomonas spp.*	20	S	20	S	25	S	31	S	0	R	0	R	0	R	13	R	23	R	29	S	19	S	24	S	0	R
61	PRE39(EMB)	*Proteus spp.*	21	S	20	S	22	S	24	R	0	R	0	R	0	R	0	R	0	R	30	S	0	R	0	R	0	R
62	PO41(Cet)	*Pseudomonas spp.*	24	S	27	S	20	S	0	R	0	R	0	R	0	R	0	R	0	R	38	S	0	R	0	R	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.1: (continued) Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients:

Serial no.	Isolates no.	Antibiotics / Name of organisms	Zone of inhibition (mm)																							
			GEN		AK		IMI		CTR		VA		AMX		P		AZM		AMC		CIP		NA		TE		MT	
			ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP
63	PO42(EMB)	*Escherichia coli*	20	S	21	S	0	R	13	R	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R
64	PO42(Cet)	*Pseudomonas spp.*	25	S	26	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	31	S	0	R	0	R	0	R
65	PRE44(EMB)	*Klebsiella spp.*	26	S	18	S	21	S	0	R	0	R	0	R	0	R	0	R	0	R	38	S	0	R	0	R	0	R
66	PRE44(EMB)	*Proteus spp.*	25	S	24	S	27	S	22	R	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R
67	PRE45(Cet)	*Pseudomonas spp.*	22	S	25	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	34	S	0	R	0	R	0	R
69	PRE47(EMB)	*Escherichia coli*	21	S	22	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	32	S	0	R	0	R	0	R
70	PRE47(EMB)	*Klebsiella spp.*	28	S	26	S	25	S	23	S	0	R	0	R	0	R	0	R	0	R	10	R	0	R	0	R	0	R
71	PRE47(EMB)	*Proteus spp.*	23	S	23	S	23	S	22	R	0	R	0	R	0	R	0	R	0	R	12	R	0	R	0	R	0	R
72	PRE49(Cet)	*Pseudomonas spp.*	20	S	25	S	23	S	25	R	0	R	0	R	0	R	0	R	0	R	27	S	0	R	0	R	0	R
73	PO51(EMB)	*Proteus spp.*	22	S	11	R	22	S	20	R	0	R	0	R	0	R	0	R	0	R	30	S	0	R	0	R	0	R
74	PO51(Cet)	*Pseudomonas spp.*	23	S	28	S	24	S	32	S	0	R	0	R	0	R	0	R	0	R	35	S	0	R	0	R	0	R
75	PRE52(EMB)	*Klebsiella spp.*	26	S	24	S	20	R	0	R	0	R	0	R	0	R	0	R	0	R	29	S	0	R	0	R	0	R
76	PRE52(EMB)	*Klebsiella spp.*	24	S	21	S	25	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
77	PO53(EMB)	*Klebsiella spp.*	23	S	10	R	26	S	15	R	0	R	0	R	0	R	0	R	0	R	34	S	0	R	0	R	0	R
78	PRE54(EMB)	*Klebsiella spp.*	20	S	27	S	28	S	29	S	0	R	0	R	0	R	0	R	0	R	31	S	0	R	0	R	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.1: (continued) Antibiotic sensitivity pattern of gram-negative isolates from oral cancer patients:

Zone of inhibition (mm)																											
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**GEN**		**AK**		**IMI**		**CTR**		**VA**		**AMX**		**P**		**AZM**		**AMC**		**CIP**		**NA**		**TE**		**MT**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
79	PRE54(EMB)	*Escherichia coli*	22	S	25	S	28	S	34	S	0	R	0	R	0	R	0	R	0	R	28	S	0	R	0	R	0	R
80	PO55(EMB)	*Klebsiella spp.*	20	S	24	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
81	PO55(EMB)	*Proteus spp.*	21	S	23	S	24	S	24	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
82	PO55(EMB)	*Escherichia coli*	20	S	9	R	22	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
83	PO55(Cet)	*Pseudomonas spp.*	23	S	19	S	23	S	20	R	0	R	0	R	0	R	0	R	0	R	29	S	0	R	0	R	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.2: Antibiotic sensitivity pattern of gram-positive isolates from oral cancer patients:

Serial no.	Isolates no.	Antibiotics / Name of organisms	Zone of inhibition (mm)																									
			IMI		E		AK		MT		AMX		LZ		NA		GEN		C		COX		OX		CIP		CAZ	
			ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP	ZS	IP
1	PO1 (MSA)	*Staphylococcus spp*	24	S	0	R	20	S	8	R	6	R	25	S	0	R	24	S	20	S	0	R	0	R	22	S	0	R
2	PO4 (MSA)	*Staohylococcus spp*	26	S	12	R	25	S	0	R	18	R	28	S	8	R	24	S	20	S	0	R	0	R	24	S	0	R
3	PRE5(Kf)	*Streptococcus spp*	28	S	0	R	24	S	0	R	0	R	33	S	0	R	25	S	30	S	0	R	0	R	32	S	0	R
4	PRE6 (MSA)	*Staphylococcus spp*	25	S	6	R	22	S	0	R	6	R	25	S	0	R	25	S	21	S	0	R	0	R	22	S	0	R
5	PRE8 (MSA)	*Staphylococcus spp*	24	S	0	R	25	S	0	R	0	R	24	R	0	R	25	S	22	S	0	R	0	R	25	S	0	R
6	PRE8(Kf)	*Streptococcus spp*	25	S	0	R	28	S	0	R	0	R	30	S	0	R	23	S	26	S	0	R	0	R	35	S	0	R
7	PRE9 (MSA)	*Staphylococcus spp*	25	S	0	R	25	S	0	R	0	R	25	S	0	R	25	S	21	S	0	R	0	R	25	S	0	R
8	PO10(Kf)	*Streptococcus spp*	27	S	0	R	23	S	0	R	0	R	35	S	0	R	20	S	28	S	0	R	0	R	40	S	15	R
9	PRE13(MSA)	*Staphylococcus spp*	24	S	0	R	24	S	0	R	5	R	25	S	0	R	24	S	20	S	0	R	0	R	24	S	0	R
10	PRE13(Kf)	*Streptococcus spp*	28	S	0	R	20	S	0	R	0	R	35	S	0	R	26	S	25	S	0	R	0	R	25	S	0	R
11	PRE14(MSA)	*Staphylococcus spp*	45	S	0	R	23	S	0	R	16	R	30	S	14	R	25	S	0	R	0	R	0	R	30	S	0	R
12	PRE14(Kf)	*Streptococcus spp*	33	S	0	R	22	S	0	R	0	R	33	S	0	R	24	S	27	S	0	R	0	R	34	S	0	R
13	PRE16(Kf)	*Enterococcus spp*	28	S	0	R	25	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	17	R
14	PO17 (MSA)	*Staphylococcus spp*	45	S	0	R	23	S	0	R	16	R	30	S	14	R	25	S	0	R	0	R	0	R	30	S	0	R
15	PO17(Kf)	*Streptococcus spp*	23	S	0	R	21	S	0	R	0	R	30	S	0	R	22	S	22	S	0	R	0	R	30	S	13	R
16	PO18(MSA)	*Staphylococcus spp*	23	S	0	R	25	S	0	R	0	R	24	R	0	R	23	S	24	S	0	R	0	R	32	S	0	R
17	PO18(Kf)	*Enterococcus spp*	28	S	0	R	25	S	0	R	0	R	30	S	0	R	25	S	13	R	0	R	0	R	41	S	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.2: (continued) Antibiotic sensitivity pattern of gram-positive isolates from oral cancer patients:

Zone of inhibition (mm)																											
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**IMI**		**E**		**AK**		**MT**		**AMX**		**LZ**		**NA**		**GEN**		**C**		**COX**		**OX**		**CIP**		**CAZ**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
18	PO20 (MSA)	*Staphylocoocus spp*	24	S	0	R	27	S	0	R	0	R	30	S	0	R	25	S	20	S	0	R	0	R	30	S	0	R
19	PO 21(MSA)	*Staphylocoocus spp*	27	S	0	R	22	S	0	R	0	R	0	R	0	R	20	S	30	S	0	R	0	R	33	S	0	R
20	PRE23(MSA)	*Staphylococcus spp*	28	S	0	R	23	S	0	R	0	R	0	R	0	R	25	S	0	R	0	R	0	R	30	S	22	S
21	PRE23(Kf)	*Streptococcus spp*	35	S	0	R	17	R	0	R	0	R	35	S	0	R	20	S	30	S	0	R	0	R	35	S	0	R
22	PO24(MSA)	*Staphylococcus spp*	28	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R	25	S	0	R	0	R	34	S	0	R
23	PO24 (Kf)	*Streptococcus spp*	30	S	0	R	0	R	0	R	0	R	30	S	0	R	0	R	25	S	0	R	0	R	33	S	0	R
24	PRE25(Kf)	*Enterococcus spp*	25	S	0	R	20	S	0	R	0	R	0	R	0	R	23	S	20	S	0	R	0	R	30	S	22	S
25	PRE27(MSA)	*Staphylococcus spp*	30	S	0	R	22	S	0	R	0	R	0	R	22	S	22	S	27	S	0	R	0	R	24	S	0	R
26	PRE27(Kf)	*Enterococcus spp*	25	S	0	R	25	S	0	R	0	R	0	R	0	R	25	S	13	R	0	R	0	R	30	S	13	R
27	PO30(Kf)	*Streptococcus spp*	20	S	0	R	13	R	0	R	0	R	30	R	0	R	17	R	25	S	0	R	0	R	33	S	0	R
28	PRE37(MSA)	*Staphylococcus spp*	32	S	0	R	24	S	0	R	0	R	35	S	0	R	18	R	10	R	0	R	0	R	20	R	0	R
29	PRE37(Kf)	*Streptococcus spp*	35	S	0	R	26	S	0	R	0	R	0	R	0	R	21	S	0	R	0	R	0	R	30	S	0	R
30	PRE38(MSA)	*Staphylococcus spp*	26	S	0	R	20	S	0	R	0	R	0	R	0	R	21	S	15	R	0	R	0	R	38	S	22	S
31	PRE38(Kf)	*Streptococcus spp*	39	S	0	R	19	S	0	R	0	R	35	S	0	R	25	S	28	S	0	R	0	R	25	S	0	R
32	PRE39(MSA)	*Staphylococcus spp*	25	S	0	R	23	S	0	R	0	R	0	R	3	R	22	S	17	R	0	R	0	R	37	S	25	S
33	PRE39(Kf)	*Enterococcus spp*	22	S	23	S	16	R	0	R	0	R	30	S	0	R	22	S	27	S	0	R	0	R	29	S	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.2: (continued) Antibiotic sensitivity pattern of gram-positive isolates from oral cancer patients:

Zone of inhibition (mm)																											
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**IMI**		**E**		**AK**		**MT**		**AMX**		**LZ**		**NA**		**GEN**		**C**		**COX**		**OX**		**CIP**		**CAZ**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
34	PRE40(Kf)	*Streptococcus spp*	20	S	0	R	21	S	0	R	0	R	0	R	0	R	22	S	15	R	0	R	0	R	33	S	20	S
35	PO41(MSA)	*Staphylococcus spp*	25	S	0	R	21	S	0	R	0	R	10	R	24	S	21	S	30	S	0	R	0	R	25	S	14	R
36	PO43(MSA)	*Staphylococcus spp*	32	S	0	R	18	R	0	R	0	R	30	S	0	R	30	S	32	S	0	R	0	R	34	S	0	R
37	PO43(Kf)	*Streptococcus spp*	28	S	0	R	29	S	0	R	0	R	29	S	0	R	28	S	25	S	0	R	0	R	34	S	0	R
38	PO44(MSA)	*Staphylococcus spp*	25	S	0	R	30	S	0	R	0	R	28	S	0	R	24	S	27	S	0	R	0	R	35	S	0	R
39	PO44(Kf)	*Enterococcus spp*	24	S	0	R	27	S	0	R	0	R	33	S	0	R	26	S	30	S	0	R	0	R	28	S	0	R
40	PRE45(MSA)	*Staphylococcus spp*	23	S	0	R	25	S	0	R	0	R	26	S	0	R	22	S	25	S	0	R	0	R	40	S	0	R
41	PRE47(Kf)	*Streptococcus spp*	26	S	0	R	25	S	0	R	0	R	32	S	0	R	27	S	26	S	0	R	0	R	30	S	0	R
42	PRE48(MSA)	*Staphylococcus spp*	30	S	0	R	25	S	0	R	0	R	33	S	0	R	22	S	24	S	0	R	0	R	26	S	0	R
43	PRE52(MSA)	*Staphylococcus spp*	33	S	0	R	20	S	0	R	0	R	0	R	0	R	27	S	33	S	0	R	0	R	19	R	0	R
44	PO55(MSA)	*Staphylococcus spp*	22	S	0	R	15	R	0	R	0	R	30	S	0	R	17	R	23	S	0	R	0	R	30	S	0	R

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.3: Antibiotic sensitivity pattern of gram-negative isolates from the control group

Zone of inhibition (mm)																													
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**GEN**		**AK**		**IMI**		**CTR**		**VA**		**AMX**		**P**		**AZM**		**AMC**		**CIP**		**NA**		**TE**		**MT**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS IP**		**ZS**	**IP**	**ZS**	**IP**
1	C5(EMB)	*Klebsiella spp*	20	S	21	S	35	S	20	S	0	R	0	R	0	R	25	S	25	S	35	S	0	R	22	S	0	R
2	C9(EMB)	*Klebsiella spp*	25	S	26	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	31	S	0	R	20	S	0	R
3	C9(Cet)	*Pseudomonas spp*	26	S	18	S	21	S	0	R	0	R	0	R	0	R	20	S	0	R	38	S	0	R	21	S	0	R
4	C13(EMB)	*Klebsiella spp*	25	S	24	S	27	S	22	R	0	R	0	R	0	R	22	S	25	S	35	S	0	R	0	R	0	R
5	C13(EMB)	*Escherichia coli*	22	S	25	S	25	S	0	R	0	R	0	R	0	R	24	S	0	R	34	S	0	R	0	R	0	R
6	C14(Cet)	*Pseudomonas spp*	21	S	22	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	32	S	0	R	25	S	0	R
7	C16(EMB)	*Klebsiella spp.*	28	S	26	S	25	S	23	S	0	R	0	R	0	R	23	S	20	S	38	S	0	R	23	S	0	R
8	C16(Cet)	*Pseudomonas spp*	23	S	23	S	23	S	22	S	0	R	0	R	0	R	24	S	0	R	40	S	0	R	0	R	0	R
9	C19(Cet)	*Pseudomonas spp*	23	S	23	S	23	S	22	S	0	R	0	R	0	R	24	S	0	R	40	S	0	R	0	R	0	R
10	C20(EMB)	*Escherichia coli*	20	S	25	S	23	S	25	S	0	R	0	R	0	R	0	R	26	S	27	S	0	R	22	S	0	R
11	C21(Cet)	*Pseudomonas spp*	22	S	35	S	22	S	20	S	0	R	0	R	0	R	0	R	25	S	30	S	0	R	0	R	0	R
12	C23(Cet)	*Pseudomonas spp*	21	S	22	S	28	S	0	R	0	R	0	R	0	R	0	R	0	R	32	S	0	R	25	S	0	R
13	C26(EMB)	*Klebsiella spp..*	23	S	28	S	24	S	32	S	0	R	0	R	0	R	0	R	25	S	35	S	0	R	0	R	0	R
14	C31(EMB)	*Klebsiella spp.*	26	S	24	S	20	S	0	R	0	R	0	R	0	R	23	S	0	R	29	S	0	R	24	S	0	R
15	C31(EMB)	*Escherichia coli*	24	S	21	S	25	S	29	S	0	R	0	R	0	R	24	S	24	S	30	S	0	R	0	R	0	R
16	C35(EMB)	*Klebsiella spp.*	23	S	22	S	26	S	15	R	0	R	0	R	0	R	0	R	0	R	34	S	0	R	23	S	0	R
17	C39(EMB)	*Klebsiella spp*	20	S	21	S	35	S	22	S	0	R	0	R	0	R	25	S	25	S	35	S	0	R	0	R	0	R
18	C39(Cet)	*Klebsiella spp*	25	S	26	S	29	S	0	R	0	R	0	R	0	R	0	R	0	R	31	S	0	R	23	S	0	R
19	C42(EMB)	*Pseudomonas spp*	26	S	18	S	21	S	0	R	0	R	0	R	0	R	0	R	0	R	38	S	0	R	0	R	0	R
20	C43(EMB)	*Klebsiella spp*	25	S	24	S	27	S	22	S	0	R	0	R	0	R	23	S	25	S	35	S	0	R	22	S	0	R
21	C48(Cet)	*Pseudomonas spp*	22	S	25	S	25	S	0	R	0	R	0	R	0	R	0	R	0	R	34	S	0	R	0	R	0	R

PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

Table 3.4.4: Antibiotic sensitivity pattern of gram-positive isolates from the control group

Zone of inhibition (mm)																													
Serial no.	**Isolates no.**	**Antibiotics / Name of organisms**	**IMI**		**E**		**AK**		**MT**		**AMX**		**LZ**		**NA**		**GEN**		**C**		**COX**		**OX**		**CIP**		**CAZ**	
			ZS	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**	**ZS**	**IP**
1	C6 (MSA)	*Staphylocoocus spp*	24	S	0	R	27	S	0	R	0	R	24	S	0	R	25	S	20	S	0	R	0	R	30	S	0	R
2	C9(MSA)	*Staphylocoocus spp*	27	S	0	R	22	S	0	R	0	R	27	S	0	R	20	S	30	S	0	R	0	R	33	S	0	R
3	C9(KF)	*Streptococcus spp*	28	S	25	S	23	S	0	R	0	R	28	S	0	R	25	S	25	S	0	R	0	R	30	S	22	S
4	C10(MSA)	*Staphylocoocus spp*	35	S	26	S	25	S	0	R	0	R	35	S	0	R	20	S	30	S	0	R	0	R	35	S	0	R
5	C11(MSA)	*Staphylococcus spp*	28	S	0	R	30	S	0	R	0	R	28	S	0	R	25	S	25	S	0	R	0	R	34	S	25	S
6	C14(MSA)	*Staphylocoocus spp*	30	S	0	R	32	S	0	R	0	R	30	S	0	R	24	S	25	S	0	R	0	R	33	S	30	S
7	C15(KF)	*Streptococcus spp*	25	S	28	S	20	S	0	R	0	R	25	S	0	R	23	S	20	S	0	R	0	R	30	S	25	S
8	C16(MSA)	*Staphylococcus spp*	30	S	21	S	22	S	0	R	0	R	30	S	22	S	22	S	27	S	0	R	0	R	24	S	20	S
9	C17(MSA)	*Staphylocoocus spp*	25	S	0	R	25	S	0	R	0	R	25	S	0	R	25	S	13	R	0	R	0	R	30	S	13	R
10	C18(MSA)	*Staphylocoocus spp*	20	S	26	S	30	S	0	R	0	R	20	S	0	R	26	S	25	S	0	R	0	R	33	S	0	R
11	C22(MSA)	*Staphylococcus spp*	32	S	0	R	24	S	0	R	0	R	32	S	0	R	28	S	10	R	0	R	0	R	20	S	0	R
12	C25(MSA)	*Staphylocoocus spp*	35	S	22	S	26	S	0	R	0	R	35	S	0	R	21	S	0	R	0	R	0	R	30	S	0	R
13	C25(KF)	*Streptococcus spp*	26	S	28	S	20	S	0	R	0	R	26	S	0	R	21	S	15	R	0	R	0	R	38	S	22	S
14	C28 (KF)	*Streptococcus spp*	26	S	28	S	20	S	0	R	0	R	26	S	0	R	21	S	15	R	0	R	0	R	38	S	22	S
15	C30(MSA)	*Staphylocoocus spp*	39	S	0	R	19	S	0	R	0	R	39	S	0	R	25	S	28	S	0	R	0	R	25	S	0	R
16	C35(KF)	*Streptococcus spp*	25	S	26	S	23	S	0	R	0	R	25	S	3	R	22	S	17	R	0	R	0	R	37	S	25	S
17	C37(MSA)	*Staphylocoocus spp*	35	S	22	S	26	S	0	R	0	R	35	S	0	R	21	S	0	R	0	R	0	R	30	S	0	R
18	C40(MSA)	*Staphylocoocus spp*	35	S	22	S	26	S	0	R	0	R	35	S	0	R	21	S	0	R	0	R	0	R	30	S	0	R
19	C46(MSA)	*Staphylocoocus spp*	22	S	23	S	30	S	0	R	0	R	22	S	0	R	22	S	27	S	0	R	0	R	29	S	0	R
20	C47(MSA)	*Staphylocoocus spp*	32	S	0	R	31	S	0	R	0	R	32	S	0	R	28	S	0	R	0	R	0	R	32	S	0	R
21	C47(KF)	*Streptococcus spp*	25	S	23	S	35	S	0	R	0	R	25	S	0	R	28	S	0	R	0	R	0	R	30	S	26	S

'PRE'= Pre-operative, 'PO= Post-operative, ZS= Zone size, IP= Interpretation, S= Sensitive, R= Resistant

The tables (3.4.1- 3.4.4) include the zone sizes of the different isolates from the patients and the control group when those were tested for their susceptibility against various antibiotics. This table also includes the zone size interpretation. Zone sizes were interpreted as “Resistant” or “Sensitive” as per the Clinical & Laboratory Standard Institute (CLSI) guidelines.

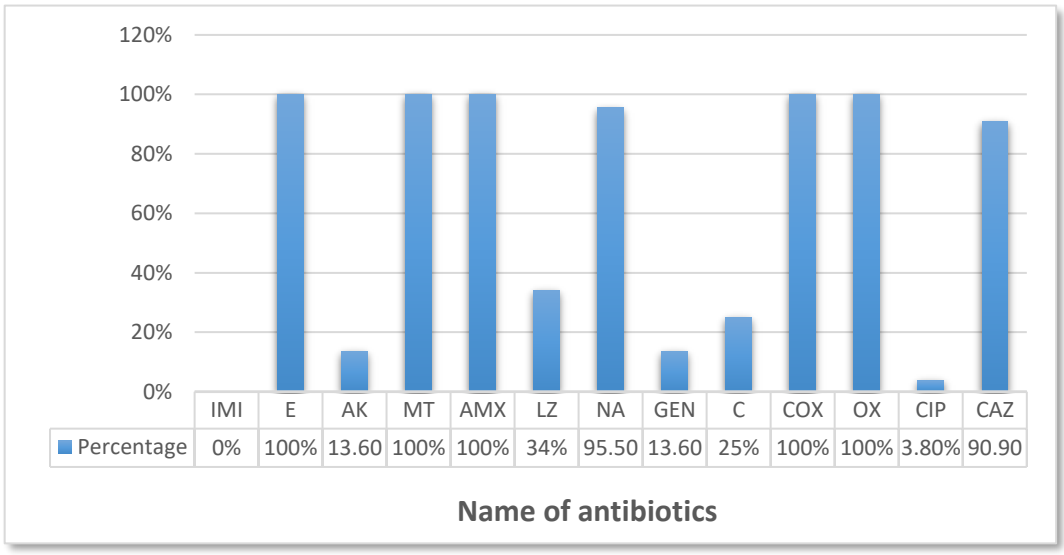


Figure 3.3: Antimicrobial resistance pattern of Gram-positive bacteria isolated from cancer patients

Here, most of the isolates from cancer patients were 100% resistant to metronidazole, erythromycin, amoxicillin, cloxacillin, oxacillin. The resistance of isolates was followed by nalidixic acid with the percentage of 95.5% resistance and ceftazidime with 90.9% resistance. Imipenem showed no resistance against the isolated Gram-positive isolates from oral cancer patients.

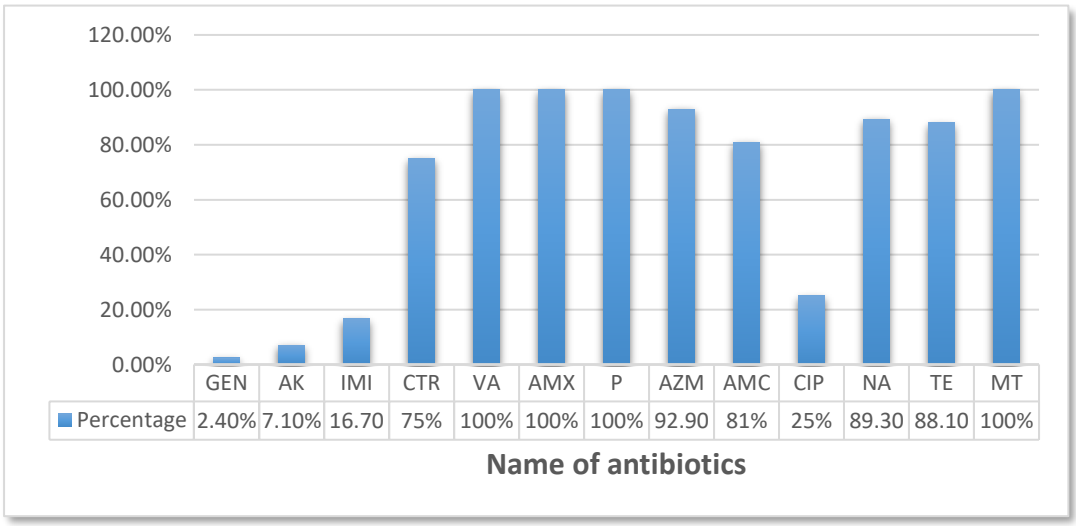


Figure 3.4: Antimicrobial resistance pattern of Gram-negative bacteria isolated from cancer patients

Here, also most of the isolates from cancer patients were 100% resistant to metronidazole, vancomycin, amoxicillin, penicillin. The resistance of isolates was followed by azithromycin with 92.9% resistance, nalidixic acid with 89.3% resistance, tetracycline 88.1% resistance, and amoxyclav 81% resistance. Isolates showed 16.7% resistance to Imipenem, 7.1% to amikacin, and minimum resistance of 2.4% to gentamicin.

The control group had 50 specimen samples from which only 38 isolates could be isolated, and among these 19 isolates were gram-negative bacteria and 18 were gram-positive bacteria. The result of the antibiotic susceptibility test for the isolates of the control group are given below:

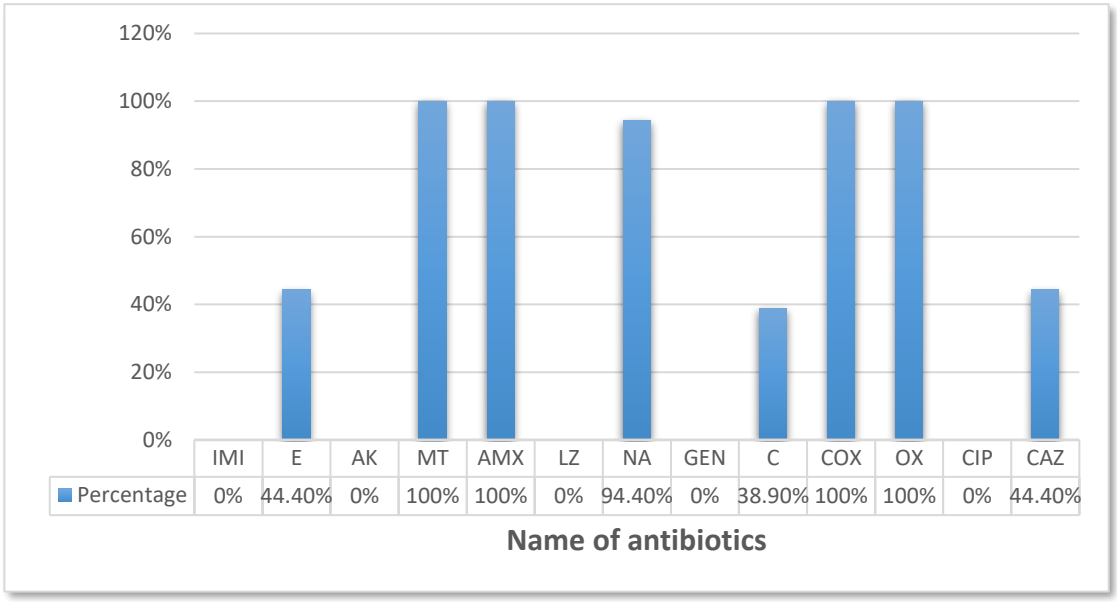


Figure 3.5: Antimicrobial resistance pattern of Gram-positive bacterial isolates (control group)

Here, it is seen that all the isolates showed 100% resistance to metronidazole, amoxicillin, cloxacillin and oxacillin antibiotics. No resistance was found in the case of imipenem, amikacin, gentamicin, ciprofloxacin and linezolid antibiotic.

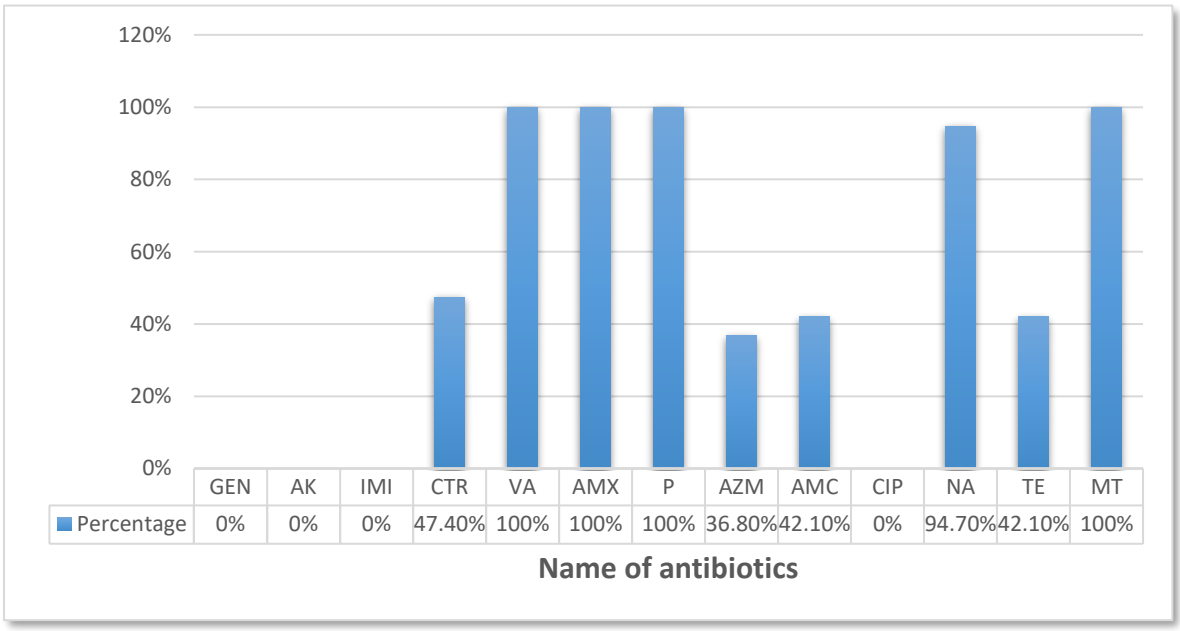


Figure 3.6: Antimicrobial resistance pattern of Gram-negative bacterial isolates (control group)

Here, it is seen that all the isolates showed 100% resistance to vancomycin, metronidazole, amoxicillin, penicillin. No resistance was found in the case of amikacin, gentamicin, and ciprofloxacin antibiotic.

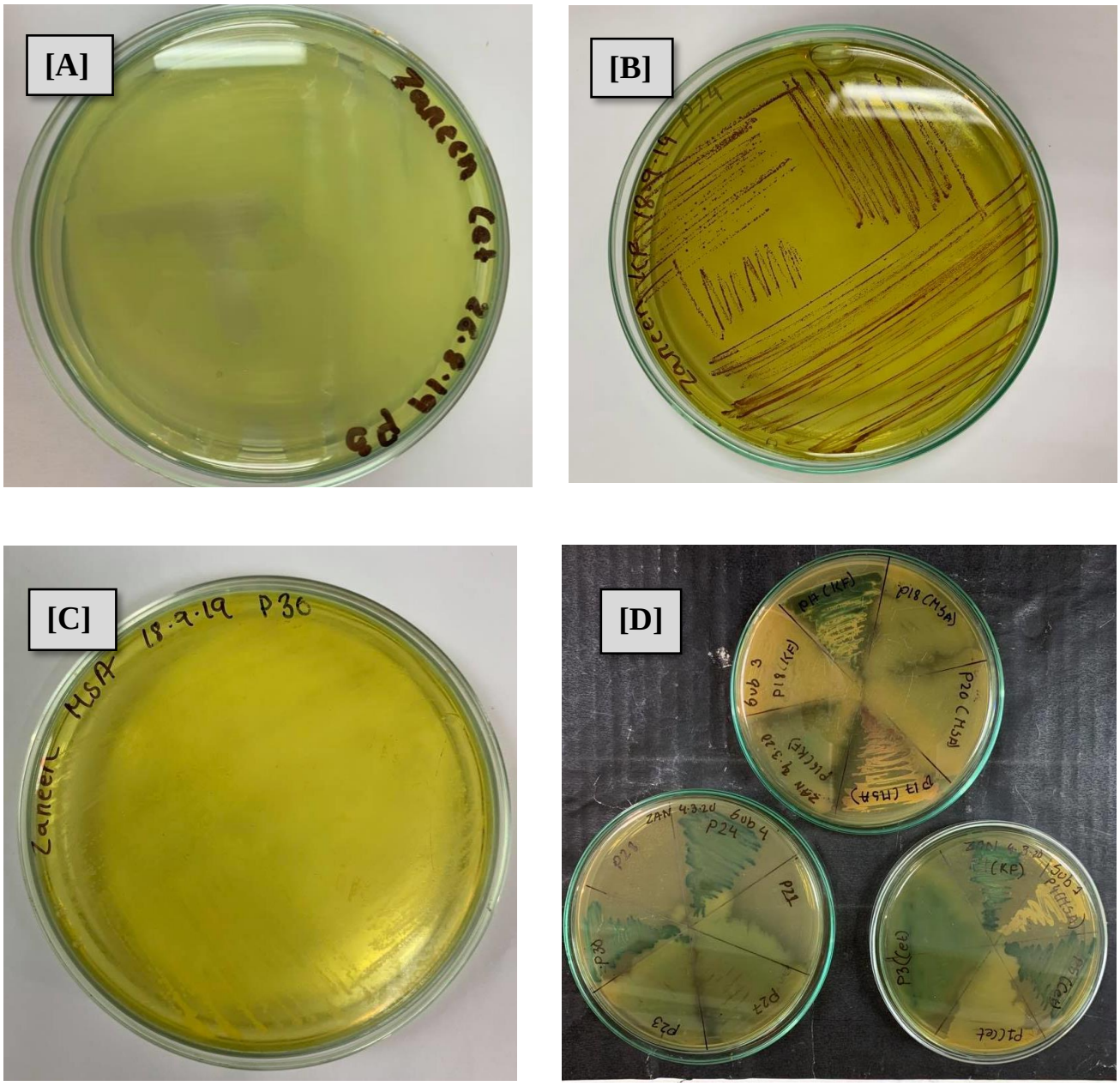


Figure 3.7: [A] *Pseudomonas* species grown in cetrimide media. [B] *Streptococcus* species grown on KF streptococcus agar media. [C] *Staphylococcus* species grown in MSA media. [D] *Staphylococcus, Pseudomonas, Streptococcus* species grown in HiChrome media.

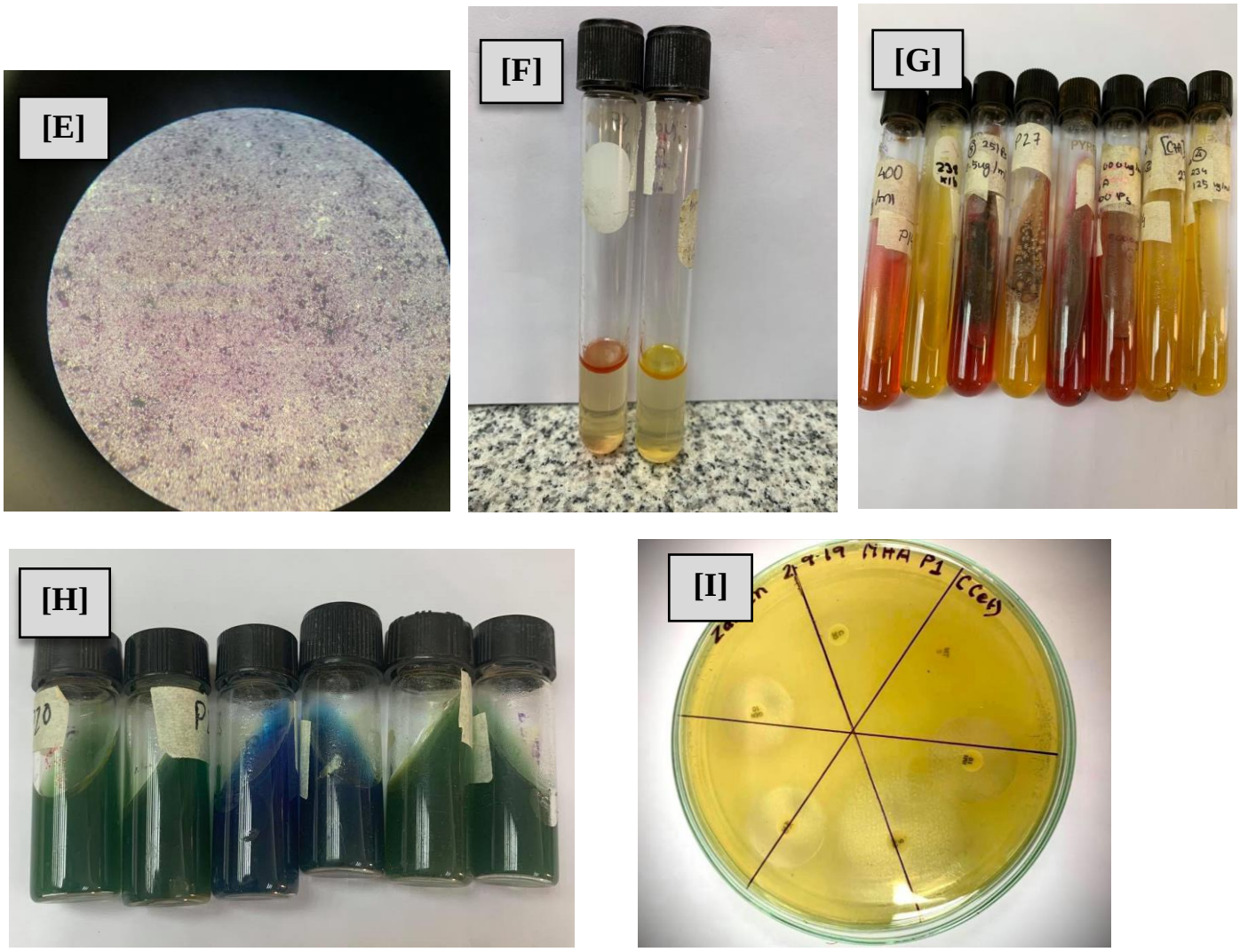


Figure 3.8: [E] Microscopic observation of gram-positive bacteria. [F] Indole test positive (left) negative) right. [G] TSI slant [H] Citrate test negative (green) positive (blue). [I] Antibiotic susceptibility test with antibiotic discs.

3.5: Analysis of the survey in terms of Questionnaire:

A statistical analysis was done with the data collected from oral cancer patients. The survey was done in terms of gender, age, type of patients, habits of the patients, treatment facilities they are taking.

3.5.1: Gender distribution in the survey:

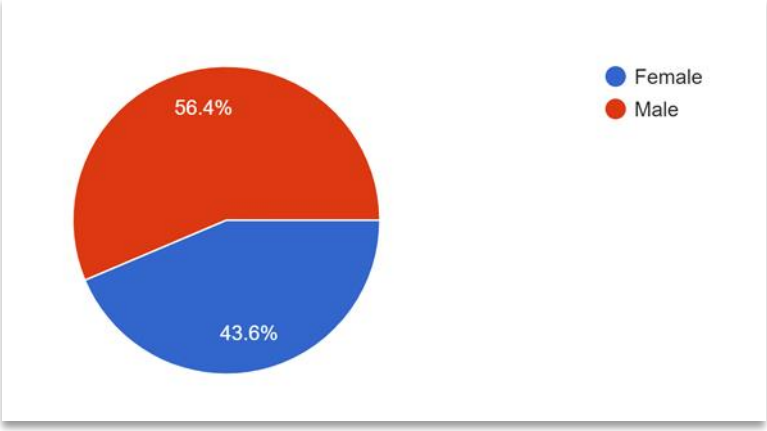


Figure 3.9: Pie chart of gender distribution

The Pie chart shows that how oral cancer is distributed among the genders. Here the majority of the patients are male with a percentage of 56.4%. Affected female patients are 43.6%.

3.5.2: Age group distribution of cancer patients:

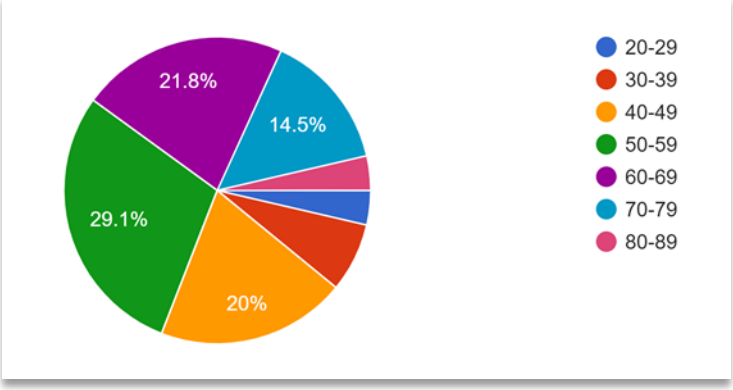


Figure 3.10: Pie chart of age group distribution of cancer patients

In the Pie chart, the age group distribution of oral cancer patients is given. From the survey, it was seen that the number of oral cancer patients was more for the people from 50-59 age group. The second-highest number of patients was in 60-69 age group. And the least number of patients was in 80-89 and 20-29 age group. So, from the pie chart, it is seen that oral cancer occurs more in people of the 40-70 age group.

3.5.3: Distribution of post-operative and pre-operative oral cancer patients:

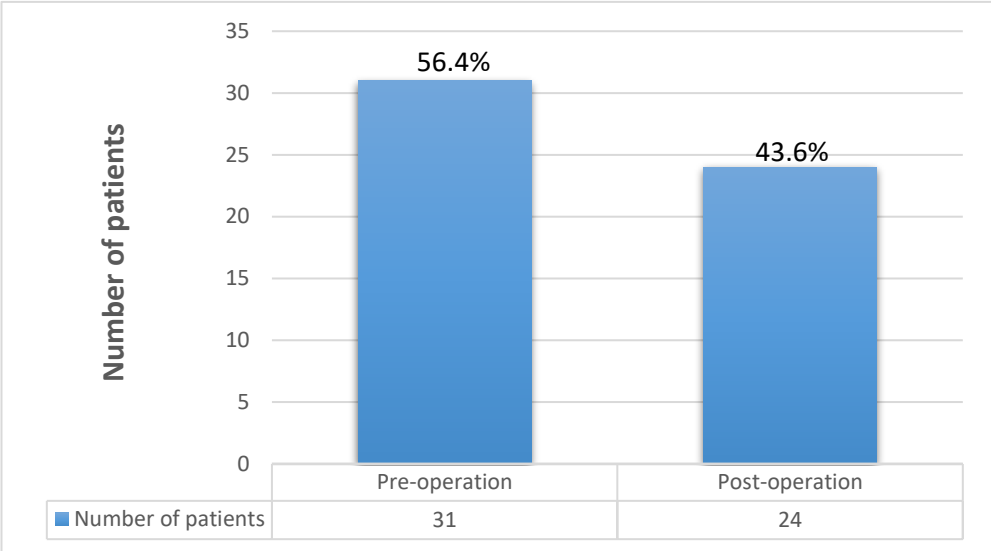


Figure 3.11: Number of pre-operative and post-operative oral cancer patients

Figure 3.11 shows the distribution of post-operative and pre-operative oral cancer patients collected from the survey. Among the 55 oral cancer patients, the majority that is 31(56.4%) patients were pre-operative and 24(43.6%) patients were post-operative.

3.5.4: Distribution of the predisposing factors of oral cancer:

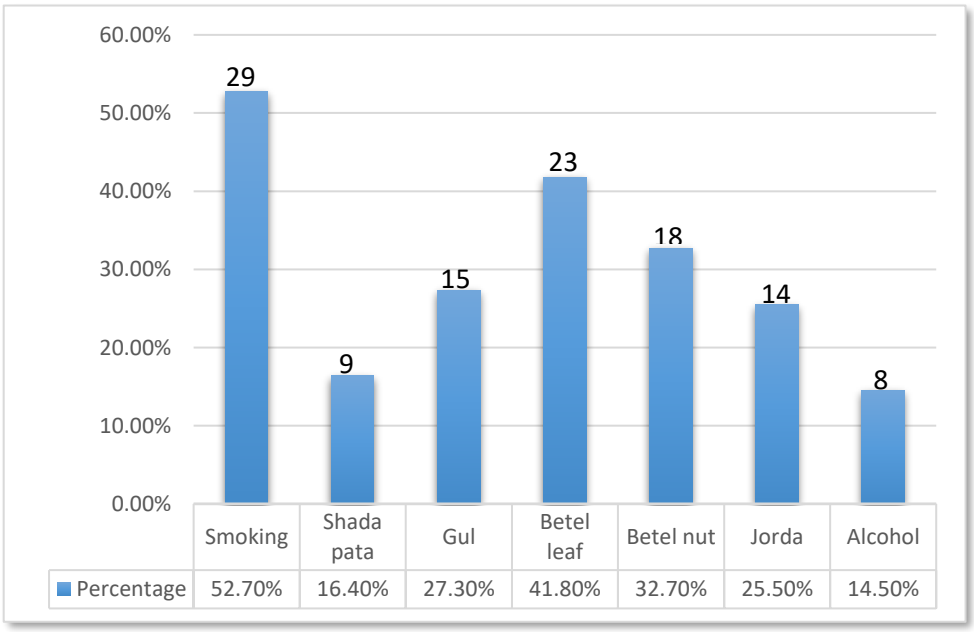


Figure 3.12: Predisposing factors of oral cancer

Figure 3.12 shows a representation of the predisposing factors of oral cancer. Nicotine, betel nut, betel leaf, and other intoxicant leaves are considered as predisposing factors of oral cancer. From the survey, it was found that the majority of oral cancer patients have the habit to consume nicotine. Second was the consumption of betel leaf and betel nut.

Chapter 4

Discussion

4: Discussion:

The oral pit possesses a blend of microbial species with their own dietary and physicochemical necessities. Bacteria are the most predominant microorganisms present, though fungi, viruses, and protozoa are also additionally found. The salivation in oral cavity and spit may contain around 100 million of these microscopic organisms for every milliliter. (Wade, 2013)

A microbiological study was done to identify and isolate opportunistic bacteria from the buccal cavity of oral cancer infected patients and also from healthy adults. Oral cancer patients usually develop ulcers in their oral cavity. Opportunistic bacteria develop and infect that ulcer area. For this the healing process of these kinds of patients becomes long and complex. The study clearly shows the presence of opportunistic bacteria in the oral cavity of both oral cancers infected patients and also in healthy adults and also resistance patterns of different kinds of antibiotics.

The aim of this study was to develop a protocol for assessing risk factors related to oral cancer infection. The oral cavity is interlinked with the respiratory tract and digestive tract so organisms that reside there can infect and colonize in the mouth. In contrast, several other studies have reported qualitative changes in oral flora during chemotherapy. This is also a risk factor for immunocompromised patients. (Whitmore, 2014). This can lead to pneumonia, bacteremia, and other health hazards.

It is imperative to have information on the sort of pathogens that dwells in the oral cavity in arrange to anticipate dental maladies as well as the related systematic complications caused by them (Philip *et al*., 2009). Oral cancer patients undergoing chemotherapy and radiotherapy lacks immunity. Drug-resistant opportunistic infections cause health problems in this immunocompromised host (Yamashita, 2013), thus it creates various complexities in oral cancer patients. Gram-positive and Gram-negative bacteria prone threats to these weak immune system patients. In a previous study, 61(63.54%) Gram-positive bacteria and 41(42.7%) Gram-negative bacteria were isolated, with 28(29%) *Streptococcus spp* being the most prevalent (Kanadan *el al.,* 2020). But in the present study, 83(65.4%) Gram-negative bacteria and 44(34.6%) Gram-positive bacteria were isolated, with 30(54.54%) *Pseudomonas spp* being the most prevalent one. Gram-positive bacteria cause great problems, but gram-negative bacteria develop dangerous resistance to antibiotics and are classified as a more serious threat. They don't absorb the toxin inside. Their ability to resist traditional antibiotics makes them more dangerous in weaker immune system patients.

Because of the thin peptidoglycan layer, Gram-negative bacteria do not absorb any foreign materials surrounding it. The thick peptidoglycan layer of Gram-positive bacteria absorbs antibiotics easily. That's why gram-negative bacteria are more harmful than gram-positive bacteria.

The statistical study has represented that males between the ages of 50-59 were more affected by oral cancer than females. It is seen that 56.4% of the male was more affected by this disease. Poor oral hygiene and use of alcohol and tobacco increases the risk of oral cancers (Oji *et al.*, 2012) Tobacco and alcohol consumption are considered as the primary risk factor of oral cancer (Gaonkar *el at.*, 2018). In the present study, a higher percentage of smokers has proved this theory. After smoking consumption of betel leaf and betel nut also have a major role in the etiology of oral cancer.

Among the Gram-positive bacteria, the most predominant bacteria were *Staphylococcus spp* 24(43.63%) followed by *Streptococcus spp* 14(25.45%) and *Enterococcus spp* 6(10.90%). Among 44 Gram-positive isolates, 24 were of *Staphylococcus spp*. In pre-operative patients, the number of *Staphyloccocus spp* was more. In a study conducted earlier, only 7 *Staphyloccocus spp* were prevalent. In that study, 28 *Streptococcus spp* were isolated, which was the most prevalent one (Kanadan *el al.*, 2020). In the present conducted study, the most prevalent Gram-negative bacteria were *Pseudomonas spp* 30(54.54%) followed by *Klebsiella spp* 27(49.09%), *E.coli* 14(25.45%), and *Proteus spp* 12(21.81%). In a recent two studies, it was seen that the most isolated Gram-negative bacteria were *Klebsiella spp* 13(13.5%) (Kanadan *el al.*, 2020) and 37(45%) (Ashreen *el al.*, 2020). So indeed, with the change of time and place the type of bacteria causing infection has changed. In the present study, the percentage of *Pseudomonas spp* were also higher in pre-operative patients than in post-operative patients. *P. aeruginosa* has been responsible for many nosocomial infections and a major cause of pneumonia (Gaynes& Edwards, 2005). However, in this regard to isolates from pre-operative patients, the percentage of *Pseudomonas* was higher. In several conducted studies, 3%-7% of nosocomial infections were because of this species (Horan *et al*., 1988). Among immunosuppressed patients such as cancer patients, the rate of pneumonia infection has been increased (Carpenter, 1990). However, to comment on whether this high level of prevalence is due to oral cancer or just the hospital, more studies have to be conducted with patients who received surgical treatment there

Again, in the present study, it was also observed that the maximum number of isolates were found in pre-operative 31(56.4%) patients than in post-operative 24(43.6%) patients. This proves that most of the patients in our country do not maintain proper oral hygiene. That's why bacteria can develop in their ulcer site even before going into any kind of operation. Due to significant change in the oral environment, the balance of oral microbes gets disturbed which in turn leads to infection.

There is a considerable disease burden attributed to inadequate water, sanitation, and hygiene facilities and practices, particularly in low-income countries (Cairncross *et al.,* 2010). It has also been hypothesized that poor dental health facilitates the conversion of ethanol to the mutagenic acetaldehyde through the metabolic activity of bacterial enzymes which, in turn, is linked to oral cancer (Gaonkar *et al.,* 2018). A significant amount of infection was also found in post-operative patients too. This indicates a heavy presence of the bacteria in the hospital's vicinity. This also might be due to wound exposure during and after the operation, when microorganisms infect oral regions, oropharynx, nasal cavity, and paranasal sinuses areas. In a study conducted last year, it was seen that infection in post-operative patients were more than in pre-operative patients (Ashreen *et al.,* 2020). Infections that are found in oral cancer patients after surgical excision of the tumor as evidence shows hospital-associated infections are often spread by the hands of health care workers or contaminated medical devices (Gupta A, 2002). But at present evidence shows that the living environment and oral hygiene are deteriorating much more, which in turn is causing infection even before any kind of surgery is being done.

The microbiome in the oral cavity of cancerous patients appears to differ from healthy people. In comparison, 50 swab samples were taken from the control group from where 38 bacteria could be isolated. Over there, the highest percentage was seen in *Staphylococcus spp.* (34%), the second highest was *Klebsiella spp.* (29%) followed by *Pseudomonas spp* (15%), *Streptococcus spp* (13%), and *E. coli* (7.8%). The presence of these microbes in the control group can be due to an infection in their oral cavity or poor oral hygiene. Dental plaques can act as a reservoir of many Gram-negative bacilli (Ali *et al*., 2006). The bacterial isolates obtained from the control group could be a source to the dental plaque (Rocio, 2015). Although, only cheek swabs were taken and any sort of deep swab was avoided to prevent contamination by the throat microbes. Nevertheless, the major difference in percentage between the prevalence of bacteria in cancer patients to the bacteria in the control group shows the role of immune-suppression being a catalyst in allowing pathogenic microbes to grow (Minah *et al*., 1985).

The present study also focused on the antibiotic susceptibility pattern of the opportunistic microbes. The antibiotic susceptibility test was done by taking 13 different antibiotics of 11 different groups those were selected based on Gram-negative and Gram-positive bacteria. After the antibiotic susceptibility test, it was observed that all the Gram-positive bacteria showed 100% resistance to the antibiotics of Penicillin groups such as amoxicillin, cloxacillin and oxacillin, metronidazole, and Macrolide group which includes erythromycin. In a study, it was found that *Staphylococcus spp* showed 69.2% resistance to antibiotic oxacillin (Yamashita, 2013). Thus, now the resistance has increased a lot. The Gram-positive microbes of the present study showed 95.5% resistance to the antibiotic of the Quinolones group which is nalidixic acid. Minimum resistance was observed for amikacin, gentamicin antibiotics, and no resistance for imipenem antibiotic. *S. aureus* showed 100% susceptibility to amikacin in a previous study (Kanadan el al., 2020). But in present study, it has shown 86.4% susceptibility which proves that resistance of this species against the strongest antibiotics is also increasing.

The majority of the microbes that could be isolated were Gram-negative bacteria. The Gram-negative bacteria showed 100% resistance to the antibiotics of Penicillin groups which included penicillin-G, amoxicillin. Similar results were observed for the antibiotics from Glycopeptide (vancomycin) group and metronidazole. Most Gram-negative bacteria are regarded as intrinsically resistant to vancomycin because of their outer membranes and different cell wall structure which is impermeable for large glycopeptides molecules. Virtually all the anaerobic Gram-negative rods are known to be susceptible to metronidazole (Dhand & Snydman, 2009). This emerging resistance of Gram-negative bacteria against metronidazole can be defined by the occurrence of specific resistance genes which code for an alternative set of enzymes that can convert activated forms of metronidazole into non-toxic derivatives (Leiros *et al.*, 2004) The resistance of these Gram-negative microbes was followed in azithromycin with 92.9% resistance, nalidixic acid with 89.3% resistance, tetracycline with 88.1% resistance and amoxyclav with 81% resistance. It showed the minimum resistance to amikacin, gentamicin, and imipenem. A previous study has shown 100% susceptibility to Carbapenem group antibiotic which includes imipenem (Kanadan *el al.*, 2020). But the present study has shown that the Gram-negative bacteria gave a percentage of 83.4 of susceptibility towards this antibiotic imipenem. The increase in carbapenem-resistant Gram-negative bacteria, across the globe is a matter of great concern (Patel G, 2013).

In this study, both the Gram-positive and gram-negative isolates showed maximum resistance to antibiotic metronidazole, amoxicillin, nalidixic acid. It has shown the minimum resistance to antibiotic amikacin, gentamicin, imipenem, and ciprofloxacin. Previously a study showed that all the bacteria have shown 80% sensitivity to the antibiotics used (Kanadan *el al.,* 2020). But in this study, the rate of sensitivity is very low. Rather the microbes have shown a huge percentage of resistance towards the antibiotics used. Thus, the rise of antibiotic resistance is already evident worldwide (Bud, 2007). This can be explained by the fact that antibiotic resistance varies with the type of population studied and also because of geographic and lifestyle change.

Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives. Antibiotic resistance is one of the biggest threats to global health, food security, and development today. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. Nowadays bacteria are becoming more drug-resistant due to exposure to these various antibiotics. Moreover, hospital bacteria can also infect the patient which is usually multidrug resistance (Ohara, 2013). Cancer patients also go through many chemotherapies, radiotherapy which can be a reason for being drug-resistant of bacteria. Many oral cancer patients do not complete the antibiotic courses and some of them do not maintain proper hygiene. The incident of penicillin resistance is not a new case. Since, the 1950s there have been reports of penicillin resistance (Spellberg & Gilbert, 2015).

Regarding the antibiotic resistance, combination therapy of antibiotics can be a suitable alternative to treat the opportunistic gram-negative bacteria. Moreover, the genes responsible for the resistance should be investigated via molecular techniques. Most importantly, that would allow faster detection of antibiotic resistance. Hence, it will provide a quicker administration of the most suitable drug.

In the present study, it was also observed that the microbes isolated from healthy people were more susceptible to the antibiotics used. The isolated Gram-positive isolates showed maximum resistance to metronidazole, amoxicillin. cloxacillin and oxacillin bacteria. It showed a good percentage of susceptibility for chloramphenicol (61.1%), ceftazidime (44.4%), and erythromycin (44.4%). These isolated Gram-positive bacteria were 100% susceptible to imipenem, amikacin, gentamicin, ciprofloxacin, and linezolid. Whereas, the Gram-positive bacteria that were isolated from cancer patients showed 13.6% resistance to amikacin and gentamicin, 3.8% resistance to ciprofloxacin and 34% resistance to linezolid. Even these bacteria showed 100% resistance to erythromycin and 90.9% resistance to ceftazidime. Again, the isolated Gram-negative bacteria of control group exhibited 100% resistance to metronidazole, amoxicillin, penicillin, and vancomycin. These Gram-negative bacteria didn't exhibit any resistance to imipenem, amikacin, gentamicin, and ciprofloxacin**.** But the Gram-negative bacteria that were isolated from patients showed 25% resistance to ciprofloxacin, 16.7% to imipenem, 7.10% to amikacin, 88.1% to tetracycline, 75% to Ceftriaxone, 92.9% to azithromycin and 81% in amoxicillin. These percentage of resistance is much higher than the percentage of resistance got from the Gram-negative bacteria isolated from healthy people (control group). Such a difference in resistance of isolated microbes between the patient group and control group can be due to a difference in their genes. But from this, it can be interpreted that the microbes of healthy people are less harmful compared to the microbes of the oral cancer patients.

A large difference between these healthy people and oral cancer patients were also observed at the time of isolating opportunistic bacteria. Out of 50 samples collected from healthy people, only 30 samples formed bacterial colonies of which only 38 isolates were collected. But from the cancer patients the number of isolates collected were huge. Out of 55 samples, 55 samples formed bacterial colonies and from which 127 isolates were collected. From this difference it can be interpreted that due to having a high immunity response and maintaining proper oral hygiene the growth of opportunistic bacteria in the oral cavity of healthy people are less than those of the oral cancer patients.

This study revealed risk factor and life-threatening effect of the group of opportunistic microbes such as *Staphylococcus, Streptococcus spp, and Enterococcus spp, Pseudomonas spp, Proteus spp, Klebsiella spp,* and *E. coli*. A significant difference was also found in the study between the numbers of isolates collected from different groups of the sample. The successful management of bacteria in infection is of great importance. However, it is still a complex issue. Therefore, the study evaluates the current situation of commonly used antibiotics. This is mostly helpful to the clinicians involved because it can make them aware of the real circumstances that they are dealing with presently. Knowing the prevalent type of microorganisms present in infected wounds and their resistance pattern is pertinent to choose the adequate treatment. The data presented here together with the discussion carried out can be useful to improve the management of oral cancer infection.

References

5 References:

1. Ahmed, F., & Islam, K. M. (1990). Site predilection of oral cancer and its correlation with chewing and smoking habit--a study of 103 cases. *Bangladesh Medical Research Council Bulletin*, *16*(1), 17-25
2. Albert, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. Molecular Biology of the Cell, (Garland Science, New York, 2002). *Google Scholar*.
3. Ali A., Pineda, L. A., &Saliba, R. G. (2006). Effect of oral decontamination with chlorhexidine on the incidence of nosocomial pneumonia: a meta-analysis. Critical Care, 10(1), R35.
4. Amini, K., &Mubasseri, P. (2017). Detection Rate of Metallo-β-Lactamase-Expressing Genes; blaVIM-1, blaVIM-2 and blaSPM-1 in Pseudomonas aeruginosa Isolates. *International Journal of Basic Science in Medicine*, *2*(1),41-45.
5. Arlet, G., Sanson-le Pors, M. J., Rouveau, M., Fournier, G., Marie, O., Schlemmer, B., &Philippon, A. (1990). Outbreak of nosocomial infections due to Klebsiella pneumoniae producing SHV-4 beta-lactamase. European Journal of Clinical Microbiology and Infectious Diseases, 9(11), 797-803.
6. Ashreen, Samiha & Ahmed, Akash & Hasan, Nadimul & Akhtar, Waheed & Hossain, M. (2020). Isolation and Identification of Gram-Negative Bacteria from Oral Cancer Site Infections and Study of Their Antibiotic Resistance Pattern. Bangladesh Journal of Microbiology. 36. 85-90. 10.3329/bjm.v36i2.45533.
7. Bessa, L. J., Fazii, P., Di Giulio, M., & Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International wound journal, 12(1), 47-52
8. Boucher, B. J., &Mannan, N. (2002). Metabolic effects of the consumption of Areca catechu. Addiction Biology, 7(1),103-110.
9. Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K.,&Tannergård, P. (1994). Mutation in the DNA mismatch repair gene homologue hMLH 1 is associated with hereditary non-polyposis colon cancer. Nature, 368(6468), 258.
10. Bud, R. (2007). Penicillin: triumph and tragedy. Oxford University Press on Demand.
11. Carpenter, C. B. (1990). Immunosuppression in organ transplantation

12. Cloke, D. J., Green, J. E., Khan, A. L., Hodgkinson, P. D., & McLean, N. R (2004). Factors influencing the development of wound infection following free-flap reconstruction for intra-oral cancer. British journal of plastic surgery, 57(6), 556-560
13. Davison, J. (1999). Genetic exchange between bacteria in the environment. Plasmid, 42(2), 73-91.
14. Dhand A and Snydman DR. 2009. Mechanism of resistance in metronidazole. In Antimicrobial Drug Resistance. Infectious Disease (Mayers DL ed). Humana Press, New York.
15. Dunlop, M. G., Farrington, S. M., Carothers, A. D., Wyllie, A. H., Sharp, L., Burn, J., ...& Vogelstein, B. (1997). Cancer risk associated with germline DNA mismatch repair gene mutations. *Human molecular genetics*, *6*(1), 105-110.
16. Fanidi, A., Harrington, E. A., & Evan, G. I. (1994). Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature, 359(6395), 554.
17. Fainstein V, Rodriguez V, Turck M, Hermann G, Rosenbaum B, Bodey GP (1981) Patterns of oropharyngeal and fecal flora in patients with acute leukemia. J Infect Dis 144, 10-18. 24.
18. Gabrilove, J. L., Jakubowski, A., Scher, H., Sternberg, C., Wong, G., Grous, J.,...&Oettgen, H F. (1988). Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. New England Journal of Medicine, 318(22), 1414-1422.
19. Gaynes, R., Weinstein, R. A., Edwards, J. R., & National Nosocomial Infections Surveillance System. (2005). Overview of nosocomial infections caused by gram-negative bacilli. Clinical infectious diseases, 41(6),848-854.
20. Gazin, M., Paasch, F., Goossens, H., & Malhotra-Kumar, S. (2012). Current trends in culture- based and molecular detection of extended-spectrum-β-lactamase-harboring and carbapenem-resistant Enterobacteriaceae. Journal of clinical microbiology, 50(4), 1140-1146.
21. Gaonkar, PratyushaPrakash & Patankar, SangeetaR & Tripathi, Nidhi & Sridharan, Gokul. (2018). Oral bacterial flora and oral cancer: The possible link?. Journal of Oral and MaxillofacialPathology.22. 234. 10.4103/jomfp.JOMFP_89_16.

22. Gupta, N., Gupta, R., Acharya, A. K., Patthi, B., Goud, V., Reddy, S., ...&Singla, (2016). Changing Trends in oral cancer-a global scenario. Nepal journal of epidemiology, 6(4),613.74
23. Hancock, R. E. (1998). Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. Clinical Infectious Diseases, 27 (Supplement_1), S93-S99
24. Hassett, D. J., Cuppoletti, J., Trapnell, B., Lymar, S. V., Rowe, J. J., Yoon, S. S., ... & Hwang, S. H. (2002). Anaerobic metabolism and quorum sensing by Pseudomonas aeruginosa biofilms in chronically infected cystic fibrosis airways: rethinking antibiotic treatment strategies and drug targets. *Advanced drug delivery reviews*, *54*(11),1425-1443.
25. Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., &Fridkin, S K. (2008). Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control & Hospital Epidemiology*, *29*(11), 996-1011.
26. Horan, T. C., Garner, J. S., Jarvis, W. R., Emori, T. G., & Hughes, J. M. (1988). CDC definitions for nosocomial infections, 1988. *American journal of infection control*, *16*(3),128-140.
27. Hussain, S. M. A. (2013). Comprehensive update on cancer scenario of Bangladesh. *South Asian journal of cancer*, *2*(4), 279.
28. Jemal, A., Zou, Z, Tiwari, R. C., & Ward, E. (2015). A New Method of Estimating United States and State-level Cancer Incidence Counts for the Current Calendar Year. *Cancer Journal*, *57*(1),30-42.
29. Kalman, D., &Barriere, S. L. (1990). Review of the pharmacology, pharmacokinetics, and clinical use of cephalosporins. *Texas Heart Institute Journal*, *17*(3), 203.
30. Kanadan, Anjali & Bhagwath, ArunAnatapadma & Sebastian, BastitianThattil & Rudrapathy, Parthiban & Manickam, Selvamani. (2020). Study on oral microbial flora and antibiotic sensitivity pattern among oral cancer patients in a tertiary cancer care center. 11. 93. 10.4103/jofs.jofs_68_19.
31. Lau, G. W., Hassett, D. J., Ran, H., & Kong, F. (2004). The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends in molecular medicine*, *10*(12), 599-606.

32. Levine, A. J., &Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science, 330(6009), 1340-1344.
33. Leiros HK, Kozielski-Stuhrmann S, Kapp U, Terradot L, Leonard GA and McSweeney SM. 2004.Structural basis of 5-nitroimidazole antibiotic resistance: The crystal structure of NimA from Deinococcus radiodurans. J Biol Chem. 279: 55840-55849.
34. Livermore, D. M., &Hawkey, P. M. (2005). CTX-M: changing the face of ESBLs in the UK.
35. Minah, G. E., Solomon, E.S., & Chu, K. (1985). The association between dietary sucrose consumption and microbial population shifts at six oral sites in man. Archives of oral biology, 30(5), 397-401
36. Minah GE, Rednor JL, Peterson DE, Overholser CD, DePaola LG, Suzuki JB (1986) Oral succession of gram-negative bacilli in myelosuppressed cancer patients. J Clin Microbiol 24, 210-213. 25.
37. Mingeot-Leclercq, M. P., Glupczynski, Y., &Tulkens, P. M. (1999). Aminoglycosides: activity and resistance. Antimicrobial agents and chemotherapy, 43(4), 727-737.
38. Mordi, R. M., & Momoh, M. I. (2009). Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. African Journal of Biotechnology, 8(5).
39. Nagy KN, Sonkodi I, Szöke I, Nagy E, Newman HN. The micro□ora associated with human oral carcinomas. Oral Oncol 1998;34:304-8
40. Normark, B. H., &Normark, S. (2002). Evolution and spread of antibiotic resistance. Journal of internal medicine, 252(2), 91-106.
41. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase producing Enterobacteriaceae. Emerg Infect Dis 2011;17: 1791-8
42. Olivier, M., Hussain, S. P., de Fromentel Caron, C., Hainaut, P., & Harris, C. C. (2004). TP53 mutation spectra and load: a tool for generating hypotheses on the etiology of cancer. IARC scientific publications, (157),247-270
43. Oji C, Chukwuneke F. Poor oral hygiene may be the sole cause of oral cancer. J Oral Maxillofac Surg 2012;11:379-83

44. Pasqualucci, L., Neumeister, P., Goossens, T., Nanjangud, G., Chaganti, R. S. K., Küppers, R., & Dalla-Favera, R. (2001). Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature, 412(6844), 341.
45. Patel G, Bonomo RA. "Stormy waters ahead": Global emergence of carbapenemases. Front Microbiol 2013;4:48.
46. Pillai DR, Mc Geer A, Low DE. New Delhi metallo-b-lactamase-1in Enterobacteriaceae: Emerging resistance. CMAJ 2011; 183:59-64.
47. Peleg, A. Y., & Hooper, D. C. (2011). Hospital-acquired infections due to gram negative bacteria New England Journal of Medicine, 362(19), 1804-1813.
48. Perera, M., Al-hebshi, N. N., Speicher, D. J., Perera, I., & Johnson, N. W. (2016). Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio- pathogenic bacteria. Journal of oral microbiology, 8(1), 32762.
49. Petitjean, A., Achatz, M. I. W., Borresen-Dale, A. L., Hainaut, P., & Olivier, M. (2007). TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene, 26(15),2157
50. Pitout, J. D., & Laupland, K. B. (2008). Extended-spectrum β-lactamase-producing Enterobactericeae: an emerging public-health concern. The Lancet infections disease, 8(3), 159-166
51. Philip K, Teoh WY, Muniandy S and Yaakob H. 2009. Pathogenic bacteria predominate in the oral cavity of Malaysian subjects. J Biol Sci. 9: 438-444.
52. Poirel, L., Naas, T., Nicolas, D., Collet, L., Bellais, S., Cavallo, J. D., &Nordmann, P. (2000). Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid-and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. Antimicrobial agents and chemotherapy, 44(4), 891-897.
53. Poudyal, S., Bhatta, D. R., Shakya, G., Upadhyaya, B., Dumre, S. P., Buda, G., &Kandel, B. P. (2011). Extended spectrum â-lactamase producing multidrug resistant clinical bacterial isolates at National Public Health Laboratory, Nepal. Nepal Med Coll J, 13(1),34-38.

54. Rocio, R. C., Florence L. G., Krista R, Gilles, R., Michèle, V., Stephanie, G., Geneviève, H.,Georges, B., & Sylvie B. (2015). Pseudomonas aeruginosa and periodontal pathogens in the oral cavity and lungs of cystic fibrosis patients: a case control study. Journal of clinical microbiology, JCM-00368.
55. Rodrigues, V. C., Moss, S. M., &Tuomainen, H. (1998). Oral cancer in the UK: to screen or not to screen. Oral oncology, 34(6), 454-465.
56. Ruiz, J., Gómez, J., Navia, M. M., Ribera, A., Sierra, J. M., Marco, F., ...& Vila, J. (2001). High prevalence of nalidixic acid resistant, ciprofloxacin susceptible phenotype among clinical isolates of Escherichia coli and other Enterobacteriaceae. Diagnostic microbiology and infectious disease, 42(4),257-261.
57. Rutvij, A. P., Ashwinirani, S. R., Ajay, N., Girish, S., Kamala, K. A., Sande Abhijeet, S., & Santosh, N. R. (2018). Oral cancer prevalence in Western population of Maharashtra, India, for a period of 5 years. Journal of Oral Research and Review, 10(1), 11.
58. Singh, G & Singh, P. (2017). Awareness and knowledge of oral cancer inrural population. International Journal of Community Health and Medical Research, 3(1), 72-76.
59. Sixou JL, De Medeiros-Batista O, Gandemer V, BonnaureMallet M (1998) The effect of chemotherapy on the supragingival plaque of pediatric cancer patients. Oral Oncol 34, 476-483
60. Smith A. 2018. Metronidazole resistance: A hidden epidemic? BDJ. 224: 403-404
61. Souli, M., Galani, I. &Giamarellou, H. (2008). Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. Eurosurveillance, 13(47), pii:19045.
62. Soussi, T., &Béroud, C. (2001). Assessing TP53 status in human tumours to evaluate clinical outcome. Nature Reviews Cancer, 1(3), 233
63. Torre, L. A., Bray, F., Siegel, L. R., &Jemal, A. (2012). Global Cancer statistics. Cancer Journal, 65(2), 87-108.
64. Ubeda, C., &Pamer, E. G. (2012). Antibiotics, microbiota, and immune defense. Trends in immunology, 33(9), 459-466.

65. Vogelstein, B., &Kinzler, K. W. (2004). Cancer genes and the pathways they control. Nature medicine, 10(8), 789.
66. Whitmore SE, Lamont RJ. Oral bacteria and cancer. PLoS Pathog 2014;10:e1003933
67. Yamashita, K., Ohara, M., Kojima, T., Nishimura, R., Ogawa, T., Hino, T., … Sugiyama, M. (2013). Prevalence of drug-resistant opportunistic microorganisms in oral cavity after treatment for oral cancer. Journal of Oral Science, 55(2), 145–155.
68. Wade WG. Characterisation of the human oral microbiome. J Oral Bio sci 2013;55:143-148
69. Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A. M., Härkönen, T., 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 medicine, 8(343), 343ra81-343ra81.
70. Yehia M, Thomas M, Pilmore H, Van Der Merwe W, Dittmer I (2004). Subcutaneous black fungus (phaeohyphomycosis) infection in renal transplant recipients: three cases. Transplantation, 77: 140-142.
71. Zhang, T., Zhang, X. X., & Ye, L. (2011). Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. PloS one, 6(10), e26041.
72. Zhao, H., Chu, M., Huang, Z., Yang, X., Ran, S., Hu, B., ...& Liang, J. (2017). Variations in oral microbiota associated with oral cancer. Scientific reports, 7(1), 11773

.