Screening of Gram-positive Opportunistic Bacteria from Oral Cancer Infection and Their Antibiotic Resistance Pattern

Submitted by
Farzana Rahman Eva
Student ID: 13326022

Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University
Dhaka, Bangladesh

Certification Statement

This is to certify that the project titled "Screening of gram-positive opportunistic bacteria from oral cancer infection and their antibiotic resistance pattern." submitted for the partial fulfillment of the requirements for the degree of Bachelor of science in Microbiology from the Department of Mathematics and Natural Sciences, BRAC University constitutes my own work under the supervision of **Dr. M. MahboobHossain**, Professor, Microbiology program, Department of Mathematics and Natural Sciences, BRAC University that appropriate credit is given where I have used the language, ideas or writings of another.

(FarzanaRahman Eva)

Candidate

Certified by

(Dr. M. MahboobHossain)

Supervisor

Professor

Microbiology Program Department of Mathematics and Natural Sciences

BRAC University, Dhaka

Dedicated

To

My Parents

Acknowledgment

Praise is to Allah whose blessing and compassion have guided me in my studies and my works throughout my academic career including this very project. This research could also not have been completed without the support of many people who are gratefully accredited here.

I acknowledge my gratitude to Professor **A F M Yusuf Haider**, Chairperson of MNS Department of BRAC University, for giving me the opportunity to complete my undergraduate thesis. I am really grateful to Professor late **A. A. Ziauddin Ahmad**, former Chairperson of MNS department of BRAC University.

I would like to express my earnest gratitude and appreciation to my most venerable supervisor **Dr. M. MahboobHossain** (Professor, Department of Mathematics and Natural Sciences,and BRAC University) without whose inspiration my commitment to work on certain crucial matters would be amiss. I would like to thank my thesis partner Maria Kibtiafor her constants support and encouragement throughout the research work.

Finally, I would like to extend a token of gratitude towards my family memberswithout whose support and prayers, I would not have made as far as I have.

Abstract

The immune compromised patients after treatment of oral cancer may have a chance of infection by drug-resistant opportunistic microbes. This study investigated the gram-positive opportunistic bacteria found in the oral cancer patient with infection and their resistance profile with the common antibiotics used. Total 100 swab sample were collected from oral cancer patients. Of them 21 had post-operative infection, 47 had pre-operative infection and 32 patients had no infection but tumor. Total 40 swab samples were taken from a group of healthy volunteers (control). After screening and identifying the isolates the gram-positive bacteria found were *Staphylococcusspp*(24%), *Streptococcus*spp(5%), *Enterococcusspp*(5%). Following statistical analysis there was a significant difference found between oral cancer patients with infection and control group. The highest number of pathogens found in the post-operative infection. The most effective antibiotics against these pathogens are linezolid, levofloxacin and ciprofloxacin and pathogens were more resistant against amoxicillin and ampicillin. Relatively the post-operative infection pathogens were more resistant.

Contents

List of Figures

List of tables

Chapter: 1

Introduction

1.1 Oral cancer and Bangladesh

Oral cancer is a very disastrous disease that affects many people each year all over the world and it is the sixth most dominant cancer in the world according to the World Health Organization (WHO). (Olajuyigbe & Ajele, 2002)The WHO predicts a continuing worldwide increase in the number of patients with oral cancer, extending this trend well into the next several decades. InBangladesh oral cancer is one of the prevalent cancer. More than 7000 people are newly diagnosed and among them, 6.6% people die every year.The percentage of incident and mortality are increasing every year.Oral cancerappears as a growth or sore in the mouth that does not go away. Oral cancer, which includes cancers of the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx (throat), can be life threatening if not diagnosed and treated early.

1.2 Types of oral cancer:

Oral cancers are part of a group of cancers commonly referred to as head and neck cancers, and of all head and neck cancers, they comprise 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 are squamous cell carcinoma. Squamous cell carcinoma means that some squamous cells are abnormal. More than 90 percent patient falls into this category.

Verrucous carcinoma: About 5 percent of all oral cavity tumors are verrucous carcinoma. This is a very slow-growing type cancer made up of squamous cells and rarely spreads to other parts of the body. But can 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 develop in the lymph tissue which is a part of immune system. The tonsils and base of the tongue both contain lymphoid tissue

Benign oral cavity and oropharyngeal tumors: Several types of non-cancerous tumors and tumor-like conditions can arise in the oral cavity and oropharynx. Sometimes, these conditionsmay 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)t. In leukoplakia, a white area can be seen, and inerythroplakia, there is a red area, flat or slightly raised, that often bleeds when scraped. to determine whether the cells are cancerousa biopsy or other test is done.(Whitmore & Lamont, 2014)

1.3 Risk factors of oral cancer:

Significant agents involved in the etiology of oral cancer such as age, gender, food habit, race, tobacco uses, consumption of alcohol. Ageis 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. About two thirds of people with oral cancer 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 human papilloma virus and a subset of head and neck cancers has been noted. (Khode, Dwivedi, Rhys-Evans, & Kazi, 2014).Others factors include Poor nutrition, especially a diet low in fruits and vegetables, prolonged sun exposure, Long-term irritation caused by ill-fitting dentures.

1.4 Treatment:

Treatment of oral cancer continues to improve over time. The past five decades have brought needed refinements in radiation therapy and surgical interventions, and the introduction of chemotherapy has been helpful in fighting the spread of the oral cancer to sites elsewhere in the body. For the first time, scientists also know enough about the molecular defects in oral cancer cells to begin to design targeted molecular therapies to kill the cancer cells with greater precision and efficiency.

1.5 Oral cancer infection:

In most of the cases, infections are commonly found in oral cancerpatients after surgical excision of the tumor (Senpukuet al., 2003). This might be due to exposure of wound during and after the operation. Oral cavity interlinks with respiratory tracts and digestive tracts. After surgery in these tracts, aerobic and anaerobic bacteria frequently induce operative wound infections in teeth, periodontal and supporting tissues of the teeth and tonsils (Pan & Jiang, 2014). Micro-organisms may also infect oral regions, oropharynx, nasal cavity, and paranasal sinuses areas. Itis found that these infected areas generally havebeneficial environment for bacterial proliferation; suitable temperature and humidity, which account for the frequent infections.

1.6 Opportunistic bacterial infection and risk factor:

Oral infection caused by opportunistic bacteria that take advantage of a weakened immune system of the host. Many of those pathogens do not cause disease in a healthy host that has a normal immune system. Opportunistic infections are associated with individuals inpoor health (referred to as an immunocompromisedhost or an opportunistic situation) and are caused by several

Different microorganisms. Among those that originate inthe oral cavity, representative microorganisms include*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candidaalbicans. Khebsiallespp,protius* Most of these organismshave become drug-resistant, which has resulted in difficulties in curing the related infectious diseases. Patients after oral cavity surgery often appear to have complications after bacterial infections, as well. After hospital discharge, attending physicians check not only for cancer recurrence but also for woundhealing, local infection, and oral mucositis. Clinicians are also aware that their patients may become carriersof drug-resistant microorganisms and may spread themto others. Colonization of pathogenic bacteria in oral cavity is thought to increase the risk of infections such as pneumonia and bacteremia (Costerton et al., 1999; Gosney et al, 1999).

1.7 *Staphylococcus* spp:

S. aureus causes a high number of both human and animal infections,it is gram-positive, salt tolerant and commonly found on skin. However, some of its strains are pathogenic as they can produce harmful toxins. *S. aureus* is one of the major causative agents of a wide range of pyogenic infections, e.g. skin and soft tissue infections, endocarditis, osteomyelitis, pneumonia and sepsis, and toxin-related syndromes. A recent European survey reported *S. aureus* in up to 27.5% of bloodstream infections and in up to 19.3% of pneumonias 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*bacteraemia and other invasive infections

1.8 *Streptococcus* spp:

Streptococcus is gram-positive bacteria that cause many types of bacteria. There are many different types of Streptococci and infections vary in severity from mild throat infections to pneumonia.*Streptococci* are divided into many groups based on their hemolytic activity. Main two groups are alpha (α)-hemolytic Streptococci and beta (β)-hemolytic*Streptococci*.*Streptococcus pneumonia* is a (α)-haemolytic streptococci that is usually found on the surface of the skin and inside the throat. In an aetiological study, the most common organisms isolated from patients with severe community-acquired pneumonia were *Streptococcus Pneumonia, Streptococcus pyogenes* is a (β)-hemolytic Streptococci that cause strep throat, impetigo and scarlet fever.

1.9 *Enterococcus*spp

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

infection. Although enterococci have been considered a low-virulent pathogen, during the last decades they have become an important cause of a variety of infections that primarily affect debilitated and immunocompromised patients and are mainly hospital-acquired or healthcare associated infections.

1.8 Antibiotics:

A wide number of antibiotics are used that is commonly used to treat against gram positive bacteria. These are:

Aminoglycosides: Aminoglycosides exert their bactericidal effect by inhibiting protein synthesis in bacteria and compromising the structure of the wall. Amikacin, gentamicin are used from this group.

Quinolons: Quinolones are bacteriocidal drugs, meaning that they kill bacteria. These antibiotic drugs inhibit the bacterial DNA gyrase enzyme which is necessary for DNA replication. Since a copy of DNA must be made each time a cell divides, interfering with replication makes it difficult for bacteria to multiply. Ciprofloxacinis used from this group.

Cephalosporin:cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. Peptidoglycan is a strong structural molecule specific to the cells walls of bacteria. With the cell wall structure compromised, the bactericidal result is lysis and death of the cell. cefepime, ceftriaxone,ceftazidime, cefuroxime used from this group

Carbapenems: carbapenems are broad spectrum antibiotic that inhibit bacteria cell wall synthesis. imipenem is used from this group

Penicillin: penicillin, and other beta-lactam antibiotics, work by interfering with interpeptide linking of peptidoglycan, a strong, structural molecule found specifically bacterial cell walls. Cell walls without intact peptidoglycan cross-links are structurally weak, prone to collapse and disintegrate when the bacteria attempt to divide. Since the eukaryotic cells of humans do not have cell walls, our cells are not damaged by penicillin, ampicillin, and amoxicillin. Are used in this experiment.

1.9 Antibiotic resistance:

Different antibiotics are used to treat the infection. But antibiotics are failing to cure the infection because of antibiotic resistant microbes.Antibiotic resistance occurs when microorganisms alter when they are exposed to antibiotics. Other environmental factors are responsible of antibiotic resistance of microorganisms. Moreover, they can spread easily. This is an alarming issue worldwide. Antibioticresistant microbes are prevalent in oral cancer infection.as a result oral cancer patients suffer a lot after removal of the tumor. 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 carriersof drug-resistant microorganisms and may spread them to others.

1.10 Objectives:

The aims of this thesis work were to identify and screening of gram positive bacteria from oral cancer infection patients. Those results are compared with isolates number of healthy people. Due to 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. Our futures objectives are molecular identifications of most drug-resistant bacteria. Detecting the respective resistant gene.The use of biostatistics to find the best effective antibiotics. Collect epidemiological data and compare it with the result.

Chapter: 2

Methods and materials:

2.1 Working area:

This thesis work was done with the collaboration of National Institute of Cancer Research and Hospital, Mohakhali, Dhaka. All the samples were collected from oral cancer patients of the cancer hospital. The lab work was conducted at the Microbiology Research Laboratory of the Department of Mathematics and Natural Sciences of BRAC University.

2.2 Sample collection:

Total 100 swab samples were collected from the infected area of oral patients by autoclaved sterilized cotton swab. The samples were carried inearly preparednutrient broth. Then 50 swab samples were collected from random healthy person. They were BRAC university students and stuffs. All the samples were labeled properly.

2.3 Microbial culture of the sample:

Initially samples were inoculated in nutrient broth and then incubated 24 hours at 37^0C for growth. Next day after incubation those samples were spread in nutrient agar plate.

2.4 Screening of gram positive bacteria from sample:

these Bacterial samples were isolated then identified using the biochemical tests and put into the selective media for re-confirmation of the organism.

2.5 Inoculation on mannitol salt agar:

Mannitol salt agar is a selectivemedium used for the isolation of pathogenic staphylococci. The medium contains mannitol, a phenol red indicator, and 7.5%sodium chloride. The high salt concentration inhibits the growth of most bacteria other than staphylococci. On MSA, pathogenic *Staphylococcusaureus*produces small colonies surrounded by yellow zones. The reason for this change in color is that*S. aureus* ferments the mannitol, producing an acid, which, in turn, changes the indicator from red to yellow. The growth of other types of bacteria is generally inhibited.

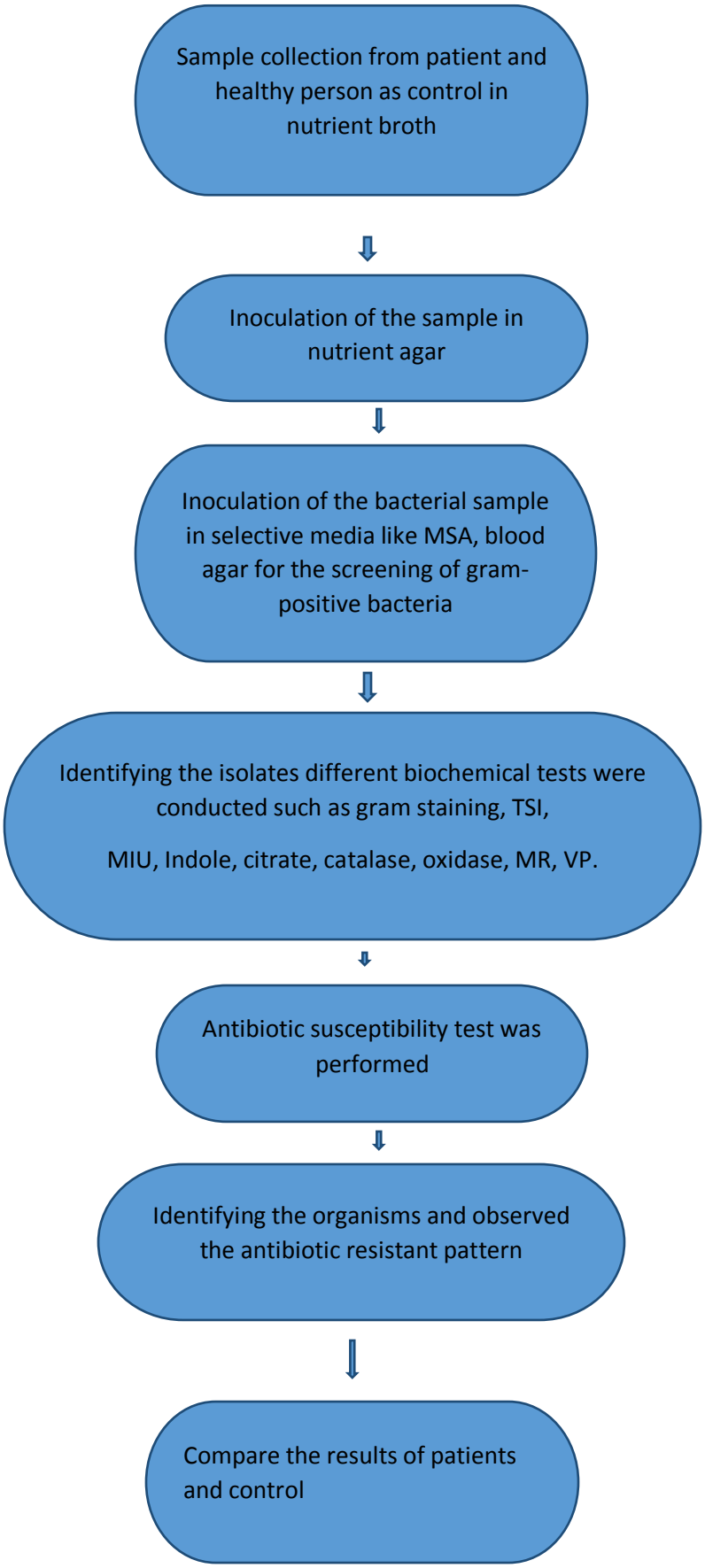
2.6 Inoculation on blood agar:

Blood Agar (BA) is an enrichedmedium used to culture pathogens that do not grow easily. It is also adifferential medium allowing the detection of hemolysis (destruction of the RBC).This media is used to see the lysis of red blood cells by the organisms. Usually three types of hemolysis are found including alpha hemolysis, beta hemolysis and gamma hemolysis. Hemolysis is determined by observing the clear zones around the bacterial growth. Hemolysins are extracellular enzymes that can be detected by their ability lyse red blood cells. Gram-positivebacteria like streptococcus, spp. Staphylococcus spp have the ability to producehemolysins.

2.7 Inoculation of Hi-chrome 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 *Klebsiellapneumonia,Enterococcus fecalis,Staphylococcusaureus,Proteus mirabilis*, *E. coli*, *Pseudomonas aeruginosa*and they produce distinctive different colors on media. *E. coli* gives pink-purple colonies, *Staphylococcus aureus* gives golden yellow colonies, *Proteusspp.*give brown colonies, *Enterococcus faecalis* produce blue colonies, *Klebsiellapneumonia*produce blue mucoid colonies and *Pseudomonasspp.*give colorless colonies on Hi-Chrome agar.

2.8 Experimental work flow:



2.9 Biochemical tests:

The gram-positive isolates are thought to be staphylococcus and enterococcus. a set of biochemical tests were performed to confirm their identification. The biochemical tests are

- 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

2.10 Gram staining:

Gram staining is a common technique that is used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between gram-positive and Gram-negative groups. The morphology of the bacteria can also be checkedusing this method

2.11 Triple sugar iron test:

Triple sugar iron agar is a differential medium used to determine H_2S production and the type of carbohydrate fermentation. Gas from carbohydrate metabolism can also be detected. To conduct the test, an isolated colony was inoculated in the TSI medium. The results were observed after 24 hours of incubation at 37°C.

2.12Motility Indole Urease test:

In laboratory Motility testing using semi-solid medium is commonly used for the identification of gram positive bacteria. MIU test was donefor determining the motility of bacteria, indole production and urea degradation by means of the enzyme urease. Using an inoculating needle,

acolony from a 24-hour fresh bacterial culture was picked up and inoculated in the medium. The test tubes were incubated at 37°C for 24 hours. The appearance and colour of the media was observed after incubation (Cappuccino and Sherman, 2005).

2.13 Catalase test:

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide. A sterile microscopic slide was placed on a petri dish and a small amount of organism picked using a sterile inoculating loop. Then 1 drop of 3% $H_2O_2$was placed on the organism on the microscopic slide by using a dropper. Finally, the positive result indicates the presence of bubbles of oxygen gas. If there was no bubble formation that means result was negative. (Cappuccino and Sherman, 2005).

2.14 Oxidase test:

The oxidase test detects bacteria that produce cytochrome c oxidase, which is an enzyme of the bacterial transport system. In positive cases, a deep blue or purple stain appears within 5-10 seconds. In this procedure, Kovacs Oxidase Reagent was used (Cappuccino and Sherman, 2005).

2.15 Indole test:

Indole production test was done to determine the production of indole by pathogens. Only some pathogens have the ability to produce indole. For the indole test, tryptophan broth was inoculated with bacterial culture to observe the production of indole and incubated at 37°C for 24 hours. Then Kovac's reagent was added to the broth culture to observe the production of indole by observing the colour changes to determine whether the result is positive (cheery red ring) or negative (yellow) (Cappuccino and Sherman, 2005).

2.16Methyl Red (MR) test:

Methyl red test was applied to analyze the bacterial ability to produce stable acid end products. Bacterial cultures were inoculated MR broth in clean test tubes and incubated overnight at a 37°C. Then methyl red reagent was added and the medium was observed for the immediate development of colour. Appearance of a red colour indicates a positive result (Cappuccino and Sherman, 2005

2.17 Voges–Proskauer (VP) test:

The Voges-Proskauer test determines the capability of producing non-acidic or neutral end products. Bacterial cultures were inoculated VP broth in clean test tubes and incubated overnight at 37°C. Then Barrit's reagent A and Barrit's reagent B was added. The tube was then allowed to remain still for 10-15mins and the solution was observed for colour changes to determine whether the result is positive (pink-red) or negative (yellow) (Cappuccino and Sherman, 2005).

2.18 Antibiotic Susceptibility Test:

Kirby-Bauer antibiotic testing test was done to detect antimicrobial susceptibility in the bacterial isolates.

Inoculation on Muller Hinton agar:

Muller Hinton agar plates were prepared to make a lawn culture. A sterile cotton swab was taken and was dipped into the broth culture of the organism. The swab was later spreadonto the MHA plate tomake a lawn culture and to ensure that the cotton swab is touched entirely on the agar surface.After the streaking was complete, the plate was allowed to dry for 5 minutes.

Placement of the Antibiotic disc:

Sterilized forceps were used to place the antibiotic discs. After taking the discs, the discs weregently pressed onto the surface of the MHA agar plates. Once all the discs were properly placed,

the MHA plates were inverted and incubated at 37^0C for 24 hours.

Measurement of the zone of inhibition:

After incubation, the zone of inhibition for each of the antibiotics was observed on theMHA plate. The size of zones for each antibiotic was measured carefully in millimeters (mm).All the measurements were taken viewing the back of the Petri dish. The zone size was recordedon the recording sheet in a chart

2.19 List of antibiotics:

Table 1: These antibiotics were used to conduct the experiment

Aminoglycosides	Quinolones	Cephalosporins	Carbapenems	Penicillin	Others
Gentamicin (GEN)	Ciprofloxacin (CIP	Cephalexin (CF)	Imipenem (IMI)	Ampicillin (AMP)	Linezolid (LD)
Amikacin (AK)	Nalidixic Acid (ND)	Ceftazidime (CAZ)		Amoxicillin (AMX)	Metronidazole (MT)

Table 2: Disk Diffusion Zone Diameter Chart

Name of antibiotic	Code	Disc potency	Staphylococcus			Enterococcus			Streptococcus		
			resist	inter	sus	resist	inter	sus	resist	inter	sus
Amikacin	AK	30	<14	15-16	>17	<		>	<		>
Gentamicin	GEN	10	<12	13-14	>15	<		>	<		>
Ciprofloxacin	CIP	5	<15	16-20	>21	<15	16-20	>21	<		>
Vancomycin	VA	30	-	-	-	<14	15-16	>17	-	-	>17
Levofloxacin	LV	20/10	<13	15-16	>19	<17	14-16	>13	<13	14-16	>17
Ceftazidime	CAZ	30	<		>	<		>	<		>
Cefuroxime		30	<		>	<		>	<		>
Imipenem	IMI	10	<13	14-16	>16	<		>	<		>
Ampicillin	AMP	10	<28		>29	<16		>17	<		>
Amoxicillin	AMX		<19		>20	<		>	<		>
Linezolid	LZD	30	<20		>21	<20	21-22	>23	<		>21
Metronidazole	MT		<		>	<		>	<		>

2.20 Preparation of Stock Sample:

Three ml of T1N1 media were prepared into sterile vials. Colonies from the cultures to bepreserved were touched by a needle from nutrient agar plates and stabbed onto thebutt of the vials. Then the vials were incubated at 37^0 C for 6 hours. After the incubation period was over, 200µl of paraffin oil was added into the surface of the medium contained in each of the vials. All the vials were carefully labeled and stored at room temperature. Result

Chapter: 3

Results

3.1Number of isolates from patients:

Total 100 samples were collected from surgical scar, saliva, gingiva, and palate of 100 oral cancer patient. From then 62 had infection on the site.in this 62 patients with infection 21 had post-operative the infection and 46 were pre-operative. The specimens from post-operative patients were labeled as Po1-21. While the specimens from pre-operative patients were labeled as Pr1-47. Other 38 were cancer patients without infection they were labeled as N 1-38, Controlwas labeled as C1-C120

These samples were streaked onto various selective, differential and nutrient agar media to identify organisms present in each sample. From these sampletotal isolatesinclude gram positive and Gram-negative bacteria were found.

3.2 Result of gram positiveisolates found from oral cancer patient:

After individual distinct colonies were marked, these were streak on selective media. These media were Mannitol Salt Agar (MSA) and hi-chrome ager for gram-positive bacteria identification. Total 69 probable gram-positive isolates were found.

Table 3: Growth of the isolates in different selective media

Serial no	Specimen number	MSA	NA	HI-crome Agar	Blood agar	Probable Organism
1	Po1	_	+	Blue color colony	+	*Enterococcus*
2	Po1	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
3	Po3	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
4	Po4	+(yellow)	+	Golden yellow	+	*Staphylococcus*
5	Po6	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
6	Po7	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
7	Po8	+	+	No specific colour	+	*Streptococcus*
8	Po8	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
9	Po10	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
10	Po11	+	+	Blue color colony	+	*Enterococcus*
11	Po12	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
12	Po3	-	+	No specific colour	+	*Streptococcus*
13	Po12	+(yellow)	+	Golden yellow	+	*Staphylococcus*
14	Po13	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
15	Po17	-	+	No specific colour	+	*Streptococcus*
16	Po16	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
17	Po17	-	+	No specific colour	+	*Streptococcus*
18	Po18	-	+	Blue colour colony	+	*Enterococcus*
19	Po20	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
20	Po20	+(yellow)	+	Golden yellow	+	*Staphylococcus*
21	Po21	-	+	No specific color	+	*Streptococcus*
22	Pr1	+(yellow)	+	Golden yellow	+	*Staphylococcus*
23	Pr2	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
24	Pr4	-	+	No specific colour	+	*Streptococcus*
25	Pr5	-	+	Blue colour colony	+	*Enterococcus*
26	Pr5	+ (yellow)	+	-	+	*Staphylococcus*
27	Pr6	-	+	Blue colour colony	+	*Enterococcus*
28	Pr7	+ (yellow Colony)	+	No specific colour	+	*Streptococcus*
29	Pr9	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
30	Pr11	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
31	Pr12	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
32	Pr15	-	+	Blue colour colony	+	*Enterococcus*
33	Pr16	+ (yellow)	+		+	*Staphylococcus*
34	Pr18		+	No specific colour	+	*Streptococcus*
35	Pr19		+	No specific colour	+	*Streptococcus*

Table 3 (Continued): Growth of the isolates in different selective media

36	Pr20	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
37	Pr24	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
38	Pr27	-	+	Blue colour colony	+	*Enterococcus*
39	Pr30	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
40	Pr32	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
41	Pr36	-	+	Blue colour colony	+	*Enterococcus*
42	Pr38	-	+	Blue colour colony	+	*Enterococcus*
43	Pr39	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
44	Pr41	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
45	Pr42	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
46	Pr45	-	+	No specific	+	*Streptococcus*
47	Pr45	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
48	Pr46	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
49	Pr47	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
50	N5		+	No specific colour	+	*Streptococcus*
51	N11	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
52	N14	-	+	Blue colour colony	+	*Enterococcus*
53	N19	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
54	N24	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
55	N26	-	+	No specific colour	+	*Streptococcus*
56	N25	-	+		+	*Enterococcus*
57	N29	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
58	N36	-	+		+	*Streptococcus*
59	C3	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
60	C8	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
61	C12	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
62	C18	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
63	C20	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
64	C22	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
65	C25	-	+	Blue colour colony	+	*Enterococcus*
66	C28	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
67	C33	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
68	C36	+ (yellow)	+	Golden yellow	+	*Staphylococcus*
69	C38	+ (yellow)	+	Golden yellow	+	*Staphylococcus*

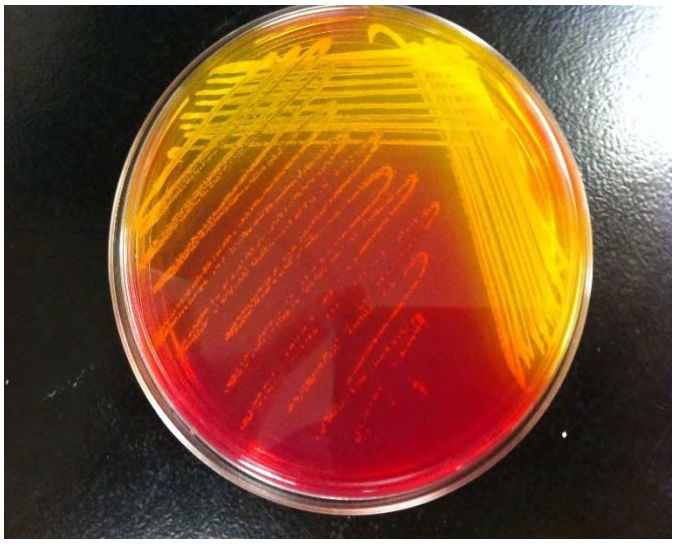


Figure 1: *Staphylococcus*growth onManital Salt Agar

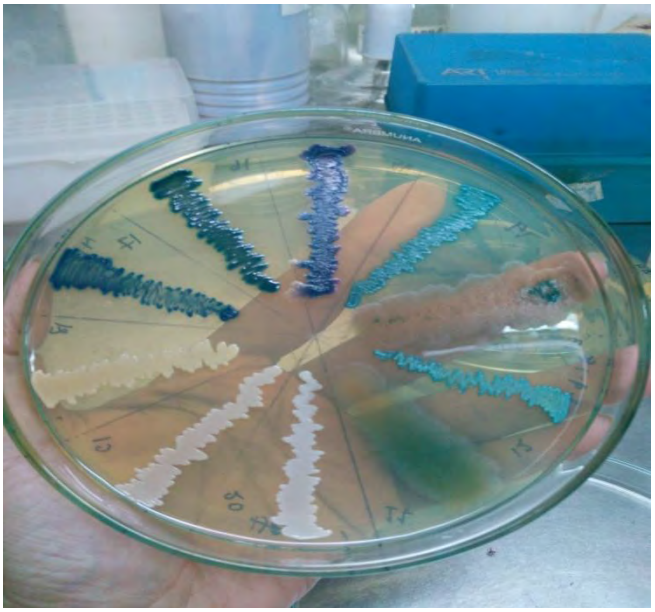


Figure 2:Different isolates growth on Hi-chrome agar

3.3 Results of biochemical tests for the identification of bacteria

		MRVP				MIU			Gram staining		TSI						Probable organism
Serial Number	Sample number	Methyl red	Vogesproskeur	Catalase	Citrate	Motility	Urease	Indole	Colour	Shape	Slant/butt colour	Glucose	Lactose	Sucrose	H_2S production	Gas production	
1	.Po1	+	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Enterococcus*
2	Po1	+	-	-	+	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
3	Po3	+	-	-	-	-	-	-	Purple	Cocci	Y/Y	-	+	-	-	-	*Staphylococcus*
4	Po4	+	-	-	+	+	-	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Staphylococcus*
5	Po6	+	+		-	+	+	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Staphylococcus*
6	Po7	+	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	-	-	-	-	*Staphylococcus*
7	Po8	+	+	-	+	+	+	-	Purple	Cocci	Y/Y	-	-	-	-	-	*Streptococcus*
8	Po8	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
9	Po10	-	-	-	+	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Staphylococcus*
10	Po11	-	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Enterococcus*
11	Po12	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
12	Po3	-	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Streptococcus*
13	Po12	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
14	Po13	+	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Staphylococcus*
15	Po16	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Streptococcus*
16	Po17	+	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
17	Po17	+	-	-	-	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Streptococcus*
18	Po18	+	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Enterococcus*
29	Po20	+	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
20	Po20	+	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*

3.3 (continued) Results of biochemical tests for the identification of bacteria

Serial Number	Sample number	MRVP		Catalase	Citrate	MIU			Gram staining		TSI						Probable organism
		Methyl red	Vogesproskeur			Motility	Urease	Indole	Colour	Shape	Slant/butt colour	Glucose	Lactose	Sucrose	H_2S production	Gas production	
21	Po21	+	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Streptococcus*
22	Pr1	+	-	-	+	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
23	Pr2	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
24	Pr4	-	-	-	+	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Streptococcus*
25	Pr5	+	-	-	-	-	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Enterococcus*
26	Pr5	+	-	+	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
27	Pr6	+	-	+	-	-	-	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Enterococcus*
28	Pr7	+	-	+	+	-	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Streptococcus*
29	Pr9	+	-	+	-	-	+	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Staphylococcus*
30	Pr11	+	-	-	-	-	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
31	Pr12	-	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Staphylococcus*
32	Pr15	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Enterococcus*
33	Pr16	-	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
34	Pr18	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Streptococcus*
35	Pr19	+	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Staphylococcus*
36	Pr20	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
37	Pr24	+	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
38	Pr27	+	-	-	-	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Enterococcus*
39	Pr30	+	-	-	-	-	-	-	Purple	Cocci	Y/Y	+	-	+	-	-	*Staphylococcus*
40	Pr32	+	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*

3.3 (continued) Results of biochemical tests for the identification of bacteria

Serial Number	Sample number	MRVP		Catalase	Citrate	MIU			Gram staining		TSI						Probable organism
		Methyl red	Vogesproskeur			Motility	Urease	Indole	Colour	Shape	Slant/butt colour	Glucose	Lactose	Sucrose	H_2S production	Gas production	
41	Pr36	+	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Enterococcus*
42	Pr38	+	-	-	+	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Enterococcus*
43	Pr39	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
44	Pr41	-	-	-	+	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Staphylococcus*
45	Pr42	-	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Staphylococcus*
46	Pr45	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Streptococcus*
47	Pr45	-	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
48	, Pr46	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
49	Pr47	+	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Staphylococcus*
50	N5	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Streptococcus*
51	N11	+	+	-	-	-	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Enterococcus*
52	N14	+	+	-	-	-	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
53	N19	+	+	-	-	-	+	-	Purple	Cocci	Y/Y	-	+	+	-	-	*Staphylococcus*
54	N24	+	+	+	-	-	-	-	Purple	Cocci	Y/Y	+	+	+	+	-	*Staphylococcus*
55	N26	+	+	-	+	-	-	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Streptococcus*
56	N25	+	+	+	-	-	-	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Enterococcus*
57	N29	+	+	-	+	-	-	-	Purple	Cocci	Y/Y	+	+	+	+	-	*Staphylococcus*
58	N36	+	+	+	+	-	-	-	Purple	Cocci	Y/Y	+	+	-	-	-	*Staphylococcus*
59	C3	+	+	+		-	-	-	Purple	Cocci	Y/Y	+	-	-	-	-	*Staphylococcus*
60	C8	+	+	-	+	-	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Staphylococcus*

3.3 (continued) Results of biochemical tests for the identification of bacteria

Serial Number	Sample number	MRVP		Catalase	Citrate	MIU			Gram staining		TSI						Probable organism
		Methyl red	Vogesproskeur			Motility	Urease	Indole	Colour	Shape	Slant/butt colour	Glucose	Lactose	Sucrose	H_2S production	Gas production	
61	C12	+	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
62	C18	+	-	-	+	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
63	C20	-	-	-	-	+	-	-	Purple	cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
64	C22	-	-	-	+	+	-	-	Purple	cocci	R/Y	+	-	-	-	-	*Staphylococcus*
65	C25	-	-	-	-	+	-	-	Purple	Cocci	R/Y	+	-	-	-	-	*Enterococcus*
66	C28	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
67	C33	-	-	-	-	+	+	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
68	C36	-	-	-	-	+	-	-	Purple	Cocci	Y/Y	+	+	+	-	-	*Staphylococcus*
69	C38	+	+	+	+	-	+	-	Purple	Cocci	R/Y	+	+	+	-	-	*Staphylococcus*

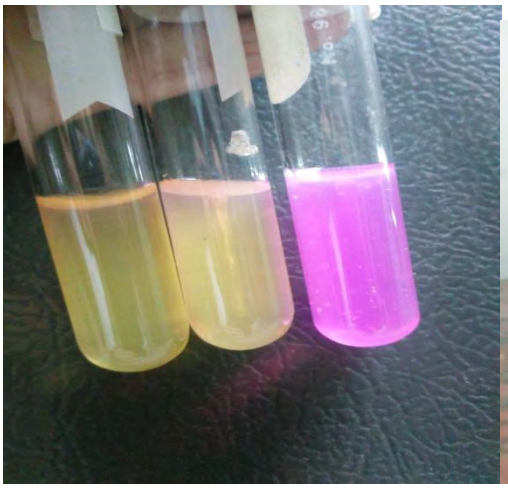


Figure 3: MIU TestFigure 4: Citrate Utilization Test

Figure 5: TSI Test **Figure 6: Indole Test**

Figure 7: Catalase Test.

3.4 Comparisons of isolates number between infectious patients and control:

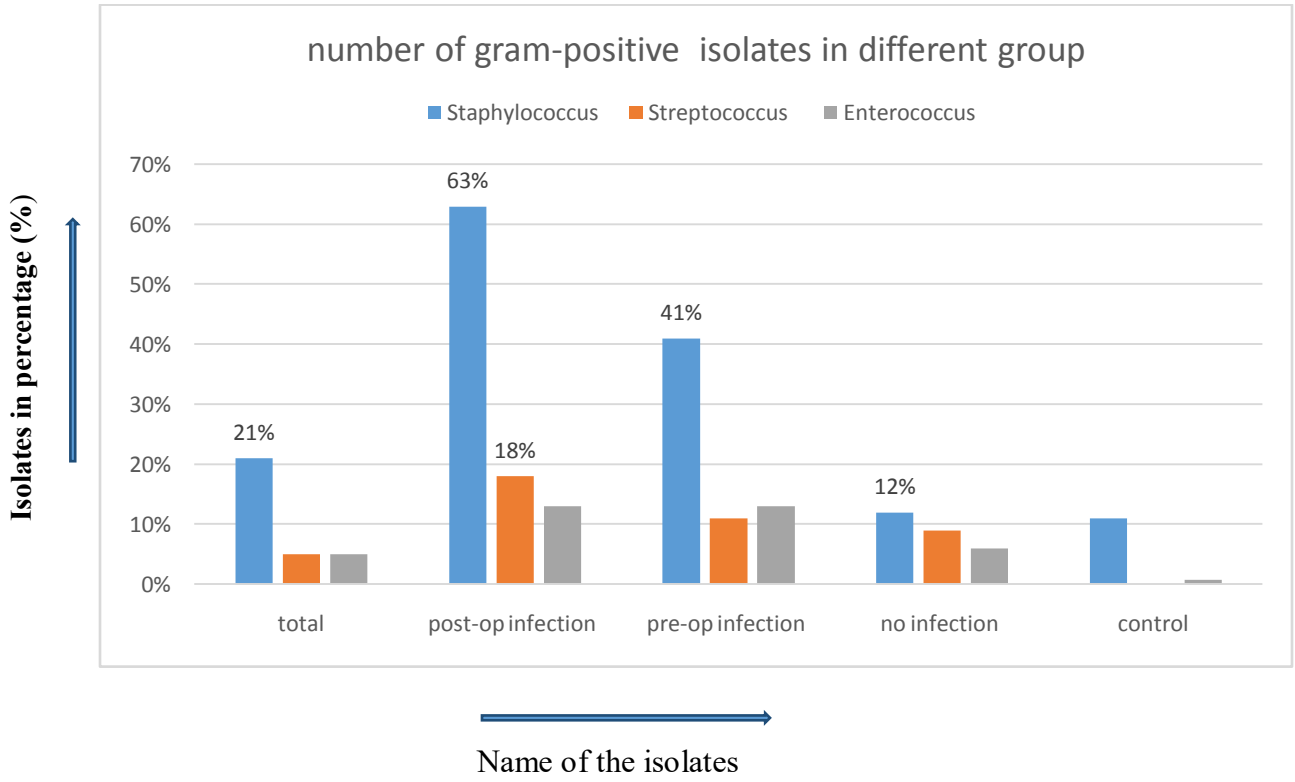


Figure 8: **Comparisons of isolates number between infectious patients and control**

Table 4: Comparison among different infectious patients and control

Name of the isolates	Total isolates	Post-operative isolates	Pre-operative isolates	No infection	Control
staphylococcus	48(21%)	14(63%)	19(41%)	4(12%)	11(9%)
streptococcus	12(5%)	4(18%)	5(11%)	3(9%)	0(0%)
enterococcus	11(5%)	3(13%)	6(13%)	2(6%)	1(.8%)
	69	21	30	9	12

3.5 Antibiotic susceptibility test:

Isolates	AK	GEN	CIP	VA	LV	CAZ	AMP	AMX	LZD	Name of the isolates
1	S	S	R	R	S	R	R	R	S	*Enterococcus*
2	R	R	S	R	S	R	R	R	S	*Staphylococcus*
3	R	S	S	R	S	S	R	R	R	*Staphylococcus*
4	S	S	S	R	S	R	R	R	S	*Staphylococcus*
5	S	R	S	R	R	R	R	R	S	*Staphylococcus*
6	S	R	S	S	S	R	R	R	S	*Staphylococcus*
7	R	S	S	S	S	S	R	R	S	*Streptococcus*
8	S	S	S	S	S	S	R	R	S	*Staphylococcus*
9	S	S	R	S	S	R	R	R	S	*Staphylococcus*
10	S	R	S	R	S	S	R	R	R	*Enterococcus*
11	S	S	S	R	R	R	R	R	S	*Staphylococcus*
12	S	S	R	S	S	S	R	R	S	*Streptococcus*
13	S	R	S	S	S	S	R	R	S	*Staphylococcus*
14	S	S	S	S	S	R	R	R	S	*Staphylococcus*
15	R	S	S	S	S	S	R	R	S	*Streptococcus*
16	R	R	S	R	S	S	R	R	S	*Staphylococcus*
17	R	S	S	R	R	R	R	R	S	*Streptococcus*
18	S	S	R	S	S	S	R	R	R	*Enterococcus*
19	S	S	R	S	S	S	R	R	S	*Staphylococcus*
20	S	S	S	S	R	R	R	R	S	*Staphylococcus*
21	S	R	R	S	S	R	R	R	S	*Streptococcus*
22	S	S	S	S	S	S	R	R	S	*Staphylococcus*
23	R	S	S	R	S	S	R	R	S	*Staphylococcus*
24	S	R	S	R	S	S	R	R	S	*Streptococcus*
25	S	S	S	R	S	R	R	R	S	*Enterococcus*
26	R	S	R	R	S	R	R	R	S	*Staphylococcus*
27	S	R	S	S	S	R	R	R	R	*Enterococcus*
28	S	S	R	S	S	R	R	R	S	*Streptococcus*
29	R	S	S	S	R	S	R	R	S	*Staphylococcus*
30	S	S	S	S	S	R	R	R	S	*Staphylococcus*
31	R	S	S	S	S	R	R	R	S	*Staphylococcus*
32	S	R	S	R	S	S	R	R	S	*Enterococcus*
33	R	S	S	S	S	R	R	R	S	*Staphylococcus*
34	S	S	S	R	S	S	R	R	S	*Streptococcus*
35	S	R	S	S	S	S	R	R	S	*Streptococcus*
36	S	S	S	S	S	S	R	R	R	*Staphylococcus*
37	R	R	R	S	S	S	R	R	S	*Staphylococcus*
38	R	S	S	R	S	S	R	R	S	*Enterococcus*
39	S	R	S	S	S	R	R	R	S	*Staphylococcus*

3.5 Antibiotic susceptibility test (Continued):

	AK	GEN	CIP	VA	LV	CAZ	AMP	AMX	LZD	MT
40	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**S**	*Staphylococcus*
41	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	*Enterococcus*
42	**S**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	**S**	*Enterococcus*
43	**S**	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
44	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**R**	*Staphylococcus*
45	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	*Staphylococcus*
46	**R**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	*Streptococcus*
47	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
48	**R**	**R**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
49	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
50	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Streptococcus*
51	**S**	**S**	**R**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
52	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**R**	*Enterococcus*
53	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**R**	**S**	*Staphylococcus*
54	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
55	**S**	**R**	**S**	**S**	**R**	**R**	**R**	**R**	**S**	*Streptococcus*
56	**R**	**S**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	*Enterococcus*
57	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
58	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Streptococcus*
59	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
60	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	*Staphylococcus*
61	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
62	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
63	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
64	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	*Staphylococcus*
65	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Enterococcus*
66	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**S**	*Staphylococcus*
67	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	*Staphylococcus*
68	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*
69	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**S**	*Staphylococcus*

3.6: Comparisons of antibiotic sensitivity between patients and control group

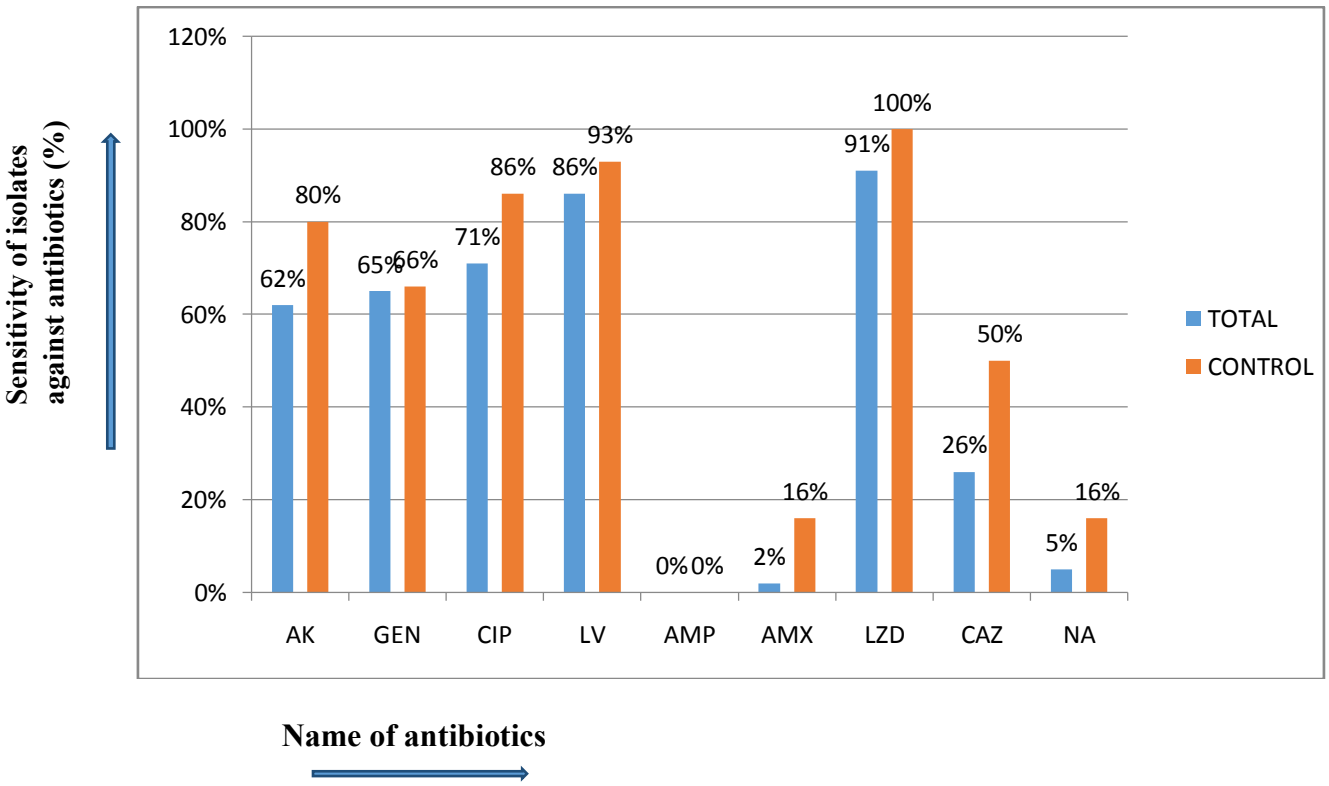


Figure 9: Comparisons of antibiotic sensitivity between patients and control group

Table 5: Comparison of antibiotic sensitivity between isolates from patient and isolates from control

ANTIBIOTICS	Isolates from patient	Isolates from control
AK	62%	80%
GEN	65%	66%
CIP	71%	86%
LV	86%	93%
AMP	0%	0%
AMX	2%	16%
LZD	91%	100%
CAZ	26%	50%
NA	5%	16%

DISCUSSION

A microbiological study was performed on major opportunistic gram-positive microorganisms of healthy volunteer and adults with oral cancer infection. In the analysis, the most common gram-positive bacteria found was *Staphylococcus*that was 21%. *Streptococcus* (5%) and *Enterococcus* (5%) also found in similar amount. We observed a significant difference of the number of isolates between oral cancer patients with infection and healthy control. Oral cancer patients also sub-divided into groups such as oral cancer with infection, oral cancer without infection, patients with post-operative infection and pre-operative infection, so that the isolates number can be compared and their response to antibiotics can be compared.

The aim of this is to develop a protocol for assessing risk factors related to oral cancerinfection. Oral cavity is interlinked with respiratory track and digestive track so organisms that reside there can infect and colonize in mouth. This is a risk factor for immunocompromised patients.(Whitmore, 2014) This can lead pneumonia, bacteremia and other health hazard. The highest number microbial species identified staphylococcus. The height number of this bacteria found in post-operative infection.

This is the reason that patient has to come to the hospital after discharge from the hospital. The reason behind this is bacteria become more drug resistance after exposure into various antibiotics. Moreover hospital bacteria can also infect the patient which are usually multidrug resistance.(Ohara, 2013) Cancer patient also go through many chemotherapy, radiotherapy which can be a reason of being drug resistance of bacteria. Many of oral cancer patient do not complete the antibiotic courses and some of them do not maintain proper hygiene. These facts play important role in infection

In this study after revealingthe high percentage of bacteria in post-operative infection. We focused in their resistance pattern. The isolates were already identified. The antibiotics that are common in clinical practice were taken for the study. In the result it was observed that linezolid has the highest sensitivity for isolated pathogens. Levofloxacin and ciprofloxacin were also

sensitive to a widerange. The pathogens were most resistant to ampicillin, amoxicillin and nalidixicacid. The most resistance isolates found in post-optative infection.

Proper prescription of antibiotic in very important as it reduce the sufferings of patients and recover the infection in a short time. Penicillin group antibiotics are not effect in infection management for a long time. Therefore, it is important to find out the most effective antibiotics for microbes responsible for this type of infection.

Several evidence support our view that that there is a possibility of drug-resistant microbes present in immunocompromised patients, particularly after the treatment of oral cancer. In general, infections are commonlyfound in oral cancer patients after surgical excision of the tumor.(Li et al., 2014)This might be due to wound exposure during and after the operation, when microorganisms may infect oral regions, oropharynx, nasal cavity, and paranasal sinuses areas. Patients after oral cavity surgery often appear to have complications after bacterial infections, as well. Colonization of pathogenic bacteria in oral cavity is thought to increase the risk of infections such as pneumonia and bacteremia.

Conclusion:

This study revealed the group of gram-positive opportunistic bacteria such as *Staphylococcus*, *Streptococcus,* and *Enterococcus*. These opportunistic bacteria have serious risk factor and can be life threating. A significant differenceis also found the study between the numbers of isolates in different group of sample. Result indicates that the post-operativepatients with infection has maximum number of isolates and healthy person has the lowest number of isolates in their mouth. It is also found that isolates of post-operative infection are more resistant than any other groups. The successful management of bacteria in infection is of great importance. However, it is still a complex issue. Therefore, our study evaluates the current situation of commonly used antibiotics.Which 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 clearly 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.

Reference:

Belusic-Gobic M, Car M, Juretic M, Cerovic R, Gobic D, Golubovic V (2007). Risk factors for Wound infection after oral cancer surgery.Oral Oncol. 43(1): 77-81.

Bennett MW, O'Connell J, O'Sullivan GC, Brady C, Roche D, Collins JK, Shanahan F (1996). The Fas counterattack in vivo: apoptotic depletion of tumor-infiltrating lymphocytes associated with Fas ligand expression by human esophageal carcinoma. J. Immunol. 160: 5669-5675

Camp BJ, Dyhrman ST, Memoli VA, Mott LA, Barth RJ (1996). In situ cytokine production by breast cancer tumor-infiltrating lymphocytes. Ann. Surg. Oncol. 3:176-184.

Cloke DJ, Green JE, Khan AL, Hodgkinson PD (2004). McLean NR. Factors influencing the development of wound infection following free-flap reconstruction for intra-oral cancer.

Br. J. Plast. Surg. 57:556-60.

Costerton JW, Stewart PS, Greenberg EP (1999). Bacterial biofilms: a common cause of persistent infections. Science, 284: 1318-22.

Gimmi CD, Morrison BW, Mainprice BA, Gribben JG, Boussiotis VA, Freeman GJ, Park SY, Watanabe M, Gong J, Hayes DF, Kufe DW, Nadler LM (1996). Breast cancer-associated

antigen, DF3/MUC1,induces apoptosis of activated human T cells. Nat Med. 2: 1367-1370.

Gosney MA, Preston AJ, Corkhill J, Millns B, Martin MV (1999).Pseudomonas aeruginosa septicaemia from an oral source. Br. Dent.J. 187: 639-40.

Jewett A, Head C, Cacalano NA (2006). Emerging mechanisms of immunosuppression in oral cancers. J. Dent. Res. 85(12): 1061-1073

Khode, S. R., Dwivedi, R. C., Rhys-Evans, P., & Kazi, R. (2014). Exploring the link between human papilloma virus and oral and oropharyngeal cancers. *Journal of Cancer Research and Therapeutics*, *10*(3), 492–498. https://doi.org/10.4103/0973-1482.138213

Li, G., Lai, R., Duan, G., Lyu, L.-B., Zhang, Z.-Y., Liu, H., & Xiang, X. (2014). Isolation and identification of symbiotic bacteria from the skin, mouth, and rectum of wild and captive tree shrews. *Zoological Research*, *35*(6), 492–499. https://doi.org/10.13918/j.issn.2095-8137.2014.6.492

Olajuyigbe, F. M., & Ajele, J. O. (2002). African journal of biotechnology. *African Journal of Biotechnology*, *4*(8), 776–779. https://doi.org/10.5897/AJB10.2700

Pan, J., & Jiang, N. (2014). Oral cavity infection: an adverse effect after the treatment of oral cancer in aged individuals. *Journal of Applied Oral Science : Revista FOB*, *22*(4), 261–267. https://doi.org/10.1590/1678-775720130546

Whitmore, S. E., & Lamont, R. J. (2014). Oral Bacteria and Cancer. *PLoS Pathogens*, *10*(3), 1–3. https://doi.org/10.1371/journal.ppat.1003933

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. https://doi.org/10.2334/josnusd.55.145

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.