SURVEY ON ORAL CANCER WITH PREVALENCE AND ANTIBIOTIC RESISTANCE STUDIES OF OPPORTUNISTIC GRAM NEGATIVE BACILLI FROM CANCER SITE INFECTIONS

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIALFULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OFBACHELOR OF SCIENCE IN BIOTECHNOLOGY

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For those who have gone
And may return never
Have bestowed us with the duty of
Remembering them forever

Dedicated to my late father,
Md. Mamtaz Uddin
& my role model
Professor Dr. A. A.Ziauddin Ahmad

Declaration

I hereby declare that the thesis project titled "Evaluation of the prevalence of opportunistic gram negative bacilli from the infections of oral cancer sites in comparison with non-cancer patients and assessment of the antibiotic resistance" has been written and submitted by me, Maria Kibtia and has been carried out under the supervision of M Mahboob Hossain, Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka. It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Abstract

One of the most lethal diseases, cancer, affects millions of people worldwide every year. The nature of cancer makes it extremely difficult to treat and it can occur at any part of the human body. In addition to genetic elements, carcinogens influence the occurrence of the disease. Similarly, due to many factors, Bangladesh has an increasing number of cancer patients with a significant portion suffering from Oral cancer. Development of infections in cancer sites is also quite common among oral cancer patients. Gram negative opportunistic bacilli can dwell in these cancer ulcer sites and prolong the infection. The presented study was undertaken to find the most prevalent gram negative bacilli. One hundred swab samples from 100 patients were taken to check presence of the organisms. Among the isolates, the most prevalent organism was *Pseudomonas* species (46%), followed by *Proteus* species (33%) and *Klebsiella* species (20%). The least prevalent was *Escherichia coli* (14%). While, in the control group set with people with no cancer, the prevalence was as follows: *Pseudomonas* species (7%), *Escherichia coli* (7%) and *Klebsiella* species (3%). The isolates were all taken for antibiotic sensitivity testing against 17 antibiotics used in hospitals. Results of the AST are as follows: 100% of the isolates from cancer patients were resistant to nalidixic acid, metronidazole, ampicillin, and amoxicillin and penicillin-G. The least resistance was seen against Imipenem (11%), followed by amikacin and gentamicin both having 17%. In terms of the control group, the highest level of resistance was seen against metronidazole (67%) and lowest was seen with linezolid, colistin, levofloxacin and ceftriaxone with a percentage of 5. The study also revealed presence of *vim*-2 gene from the isolates via molecular detection with PCR method. A substantial amount of qualitative data on the medical history, clinical examination and treatment etc. was documented and presented in this study. Statistical tests were also performed to find out significant associations from the data.

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Chapter 1

Introduction and literature review

1.1 Cancer and its development

Cancer is one of the most lethal diseases in the world. It can be defined as the uncontrolled growth of cells that can be spread throughout the body. Worldwide, cancer affects 1 out of 6 people (Jemal et al., 2009). In 2015, 8.8 million people were diagnosed with cancer. In the United States about 1.7 million people were expected to develop cancer and more than six hundred thousand were expected to die (Siegel et al., 2017). While, in Asian countries such as India, Indonesia, Philippines, Vietnam and China more than 3 million people are expected to be diagnosed with cancer each year (Torre et al., 2015).

As mentioned before, the development of cancer begins from the uncontrolled division of damaged cells. Some of these cells can proliferate and give rise to a mass of cells which is referred to as a tumor. These tumors can be either benign or malignant. Malignant tumors are called cancer. Moreover, these have the ability to metastasize. Metastasis is the process through which the clump of cells ignores the cellular signals to stop proliferation and invade nearby tissues, mainly blood and lymphatic tissues. On the molecular level, progression of cancer occurs through a multistep process. Cells go through several rounds of mutations and eventually become transformed into immortal metastasized cells (Weinberg, 2014).

Even though a vast array of genes interacts to develop into cancer, the main groups of genes that hold the major responsibility are tumor suppressor genes and oncogenes. While the name “oncogene” clearly suggests a link to cancer formation; contrary to their name, tumor suppressor genes are also capable of causing cancer. The answer to these genes being liable for causing cancers is due to mutations. Mutation in either of these groups of genes can initiate cancer progression (Alberts et al., 2002).

Tumor suppressor genes have numerous essential functions in the healthy cell. They code for proteins that can inhibit abnormal cell growth, keep check on the number of cell cycles, repair damaged DNA, halt division of cells with mutated DNA and even repress metastasis. One of the prominent genes in this group is the TP53 gene that codes for p53 protein (Vogelstein &Kinzler, 2004). This protein plays a major role in preventing irregular cell growth as it can stop the cell cycle at even G1 phase, along with initiating apoptosis, and even assuring necessary telomere shortening. Similarly, by disregulation of these vital processes, the mutated gene for this protein plays a major role in causing cancer (Petitjean, 2007).

Exposure to carcinogens and mutagens that include tobacco, UV, certain chemicals such as asbestos, benzene, cyclamide and many others can impair the DNA. Any deterioration of the DNA stimulates p53 production (Soussi&Beroud, 2001). As a result, an over production of p53 can stress the TP53 gene. Moreover, cellular exposure to mutagens and carcinogens can directly mutate this gene. Mutated p53 fails to control cell division that often leads to aneuploidy (Soussi*et al*., 2000). Furthermore, malfunction of p53 production subsequently falters the normal prevention of cell growth in those cells with damaged DNA. With no regulatory substance to hold back uncontrolled division, these faulty cells continue to proliferate into a mass of undefined cells that can invade and damage other parts of the body (Olivier *et al*., 2004)

Other well-known tumor suppressor genes of note are *MSH2*, *MSH6*, *PMS1*, *mutL*and*mutH*among numerous others. All of these genes produce different proteins that can repair mismatched DNA (Bronner*et al*., 1994). Occurrence of any damage in these genes let cells with mutated DNA go unchecked and divide continuously. In some of the very common cancers like colon, head and neck, and stomach cancers, a lower or improper level of expression these genes were seen (Dunlop *et al*. 1997).

In terms of oncogenes, the most studied and significant one is the *ras*gene that codes for Ras protein. Some other oncogenes include myc gene, ERK and TRK. The oncogenes stay in the form of proto-oncogenes. Most of the proto-oncogenes are related to various, important signal transduction pathways (Fanidi*et al*., 1994). It is to be mentioned that the proto-oncogenes do not directly cause cancer; similar to tumor suppressor genes, they initiate cancer only upon mutation (Pasqualucci*et al*., 2001). Additionally, over expression of the proteins these genes code for can also lead to cancer (Levine &Puzio-Kuter, 2010).

Cancers can be of many types. However, on the broader aspect, they can be divided into 3 major classes: carcinoma, sarcoma and lymphoma (Cooper, 2000). Carcinomas are the most common type of cancers where the epithelial cells are affected. While sarcoma is the cancer of connective tissues, lymphoma is related to blood and immune cells. In regards of specific cancers, one of the most common cancers irrespective of gender is lung cancer. Although breast cancer is the most prevalent cancer in women and prostate cancer is the most common in males, the incidence of a certain cancer types can also depend on geographical location. In South East Asian countries, the frequency of different cancer occurrences is slightly dissimilar to western countries. While lung cancer is still one of the most prevalent cancers

overall, in South East Asian countries like India, Bangladesh, Pakistan and Sri Lanka, oral cancer, esophageal cancer, and stomach cancers are also very common.

1.2 Oral Cancer

Oral cancer is one of the most common cancers in south Asian countries. Worldwide, it is the 6th most prevalent cancer. In Southeast Asia, about 40% of the cancers are of the oral cavity (Rodrigues *et al*., 1998). The cancer can take place in the anterior tongue, gingival, buccal mucosa, retromolartrigone, hard palate, salivary glands and even tonsil glands (Ahmed & Islam, 1990).

Oral cancer primarily starts as lesions that are hyperplasic growth. In presence of external carcinogenic stimuli and internal absence of cell regulation mechanisms by tumor repressor genes, the hyperplasia can turn into metaplasia and anaplasia that leads to malignant invasion. The external factors that set off DNA inside the cells are use of tobacco, alcohol, betel leaf and catechu. Biological factors such as infection with herpes virus, human papilloma virus, *candida albicans*, *treponema pallidum* and even poor oral hygiene can increase the risk of developing oral cancer (Cawson, 1969). In some studies, leukoplakia, the white lining that grows inside the oral cavity, is deemed as one of the risk factors of oral cancer (Brad *et al*., 2009).

While investigating the molecular basis of oral cancer progression, it was found that the increased expression of epidermal growth factor receptor (EGFR), K-ras, c-myc, int-2, Parathyroid adenomatosis 1 (PRAD-1) and B-cell lymphoma (bcl) like oncogenes are found in the progression of oral cancer, as well as damaged p53 production. Additionally, loss of chromosome 17p is seen in lesions in the oral cavity that ultimately led to cancer. Despite many studies and extensive research, the exact cause and mechanism of oral cancer development is still not clearly understood and requires much more intensive analysis (Cooper, 2002).

1.3 Oral cancer and Bangladesh

In Bangladesh, every year more than 7000 people are diagnosed with oral cancer and many more remain undiagnosed. It is estimated that 6.6% of the patients who are diagnosed with cancer, face mortality. However, the majority of the oral cancer patients belong to the

underreported rural community (Hussain, 2013). The relatively high rate of oral cancer in this country can be traced back to over consumption of tobacco with betel leaf. Regular smoking habit also increases risk of developing oral cancer along with esophageal cancer. In addition, the habit of adding catechu and betel nut with betel leaf increases oral cancer risk further. Arecoline is a compound found in catechu that has known carcinogenicity (Boucher &Mannan, 2002). To add to this, poor oral hygiene in the rural community and improper dental treatment also worsens the situation.

Despite being one of the most prevalent cancers in Bangladesh, the treatment for oral cancer is still not well managed and accessible to all. Due to being a developing country, proper cancer treatment is still not available to the rural communities. The situation aggravates as the patients only come to realize about the cancer when it becomes metastasized. Even though this scenario is common worldwide, in rural underdeveloped areas of this country, it is more prominent. Moreover, some diagnosed patients cannot even attain cancer treatment due to their financial instability (Singh & Singh, 2017).

1.4 Risks associated with oral cancer

One of the major risks of oral cancer is metastasis into the lymphatic zone. Due to being in a close proximity to the oral cavity, lymph glands surrounding the neck can be easily invaded by the malignant cancer cells. Invasion into the lymphatic system can spread the cancer throughout the body and increases the mortality rate by many folds. Moreover, it complicates the treatment procedure that requires surgical action.

Apart from the risk of increased metastasis, the oral cancer sites are also susceptible to infection by a vast number of opportunistic pathogens. The infection can take place during the cancer progression, and also after surgery by nosocomial infections (Cloke*et al*., 2004). These infections decrease the rate of patient recovery. It prolongs healing time and can rapidly spread to other organs like the lungs, esophagus, stomach and blood. Most importantly, the cancer patients often become immune suppressed after the radiotherapy and chemotherapy treatments which reduce the number of viable white blood cells (Gabrilove*et al*., 1998). Lower level of immunity and neutropenia due to radiation therapy makes the person more susceptible to infectious diseases.

1.5 Opportunistic microbes in the oral ulcer of oral cancer patients

There has been a role of microbes in connection with oral cancer. As previously mentioned, some microbes such as *Treponema pallidum,* HPV and *Candida albicans*are risk factors of oral cancer. In some other studies different species of *Streptococcus* was seen in the oral cancer sites.

Moreover, in other studies, there have been indications of a few bacterial species such as *Porphyromonasgingivalis*and*Fusobacteriumnucleatum*having carcinogenic properties (Perera*et al*., 2016). However, these microbes were found in the tumor or in the saliva of the cancer patient and were present before or during the development of the cancer. In terms of microbes present in the oral cavity of immune compromised patients, there have been reports of microbes like *Pseudomonas* species, *Fusobacteriumnucleatum*and gram negative bacilli- namely *Klebsiella, Enterobacter*and*Proteus* (Minah*et al*., 1985).

Here, *Pseudomonas*, *Escherichia coli* and *Proteus* species are some of the opportunistic pathogens that can infect immunocompromised patients. Apart from the hampered immune system due to the radiation and chemical treatments, most of the oral cancer patients are also aged. These factors allow the opportunistic pathogens to harbor in the ulcers of the oral cavity. Additionally, after surgical treatment, nosocomial infections also take place on the site where surgical operation was applied, which are again caused by *Pseudomonas* and *Klebsiella*(Green *et al*., 1973). Alarmingly, these microbes can cause a myriad of infections in the body that can be even lethal.

1.5.1 *Pseudomonas* species

Pseudomonas species is distinguished in the world of microbiology due to its large genomic size and being a prolific, opportunistic human pathogen. These are Gram negative, rod shaped, motile and aerobic in character. Additionally, they are oxidase and catalase positive (Tortora, 1982). Being oxidase positive is a distinguishing feature of *Pseudomonas*. Another distinguishing feature is their ability to produce pyocyanin which is a yellowish-green pigment siderophore (Lau *et al*., 2004). They also release exopolysaccharide alginate, Psl and Pe which are used in the formation of biofilms. This biofilm adds up to their pathogenic attributes as it becomes more difficult to treat their infections. Moreover, the exopolysaccharide also help the bacteria to avoid phagocytosis.

Other than biofilm formation however, these bacteria pose a great risk to human health due to being resistant to major antibiotic classes (Hasset*et al*., 2002). Especially those found in nosocomial infections are typically highly resistant forms. There have been reports of *Pseudomonas* being resistant to beta-lactam antibiotics and penicillin group. The antibiotic resistance may be as a result of their large genomes, porin channels that facilitates efflux pumps and once again, biofilm formation (Cornelis, 2008).

1.5.2 *Klebsiella*species

Another opportunistic pathogen that is responsible for nosocomial infections is *Klebsiella.* Rod shaped gram negative *Klebsiella*species are facultative anaerobes and have a slimy capsule layer outside it (Tortora, 1982). These bacteria are capable of causing many severe diseases that include septicemia, pneumonia, meningitis, urinary tract infection and more. Normally, they are a regular part of human nasal, oral and gastrointestinal floras. However, when a patient is immunocompromised or has an infection where the bacteria are present, they can turn pathogenic (Bagley, 1985). Cancer patients with oral ulcer who have weakened immunity are highly vulnerable to *Klebsiella*infections. Additionally, there is no available vaccine against these bacteria and their resistance to regularly used antibiotics makes them far more dangerous (Ogawa, 2005).

1.5.3 *Escherichia coli*

Normally a part of the gut flora, *Escherichia coli* can be found in the oral cavity as well. *E. coli* is gram negative, rod shaped and motile with no capsule (Tortora, 1982). Especially by way of the fecal-oral pathway, *E. coli* can inhabit inside the human mouth. While not all the strains of *E. coli* are pathogenic, the ones that are can cause major infections. As well as severe diarrhea, *E. coli* is capable of causing septicemia, gastrointestinal infection and unitary tract infection. In addition to that, *E. coli* often prolongs the infection healing period. Likewise to many other gram negative bacteria, these are also showing antibiotic resistance.

1.5.4 *Proteus* species

Another gram negative opportunistic pathogenic bacterium is *Proteus*. The swarming colony it produces is a distinguishing feature of its major species *P. mirabilis*. It is rod shaped with peritrichous flagella that gives it swarming motility. Additionally, it also exhibit urease activity (Tortora, 1982). Ubiquitous in soil and water, these bacteria can reside in human infection sites.

These are capable of causing nosocomial infections that include septicemia and pneumonia.

1.6 Antibiotic resistance

Although a more recent term than cancer, antibiotic resistance has been appearing to be destructive in equal magnitude. Since the emergence of antibiotics as a means to treat bacterial infections, the global health scenario has improved multi-fold. However, with the rise of microbes which are no longer susceptible to commonly used antibiotics (Nikaido, 2010). Some bacteria could evade the deadly effect of antibiotics by acquiring mutations. Then, by the process of natural selection, those bacteria may carry on and pass the resistant genes into the remaining gene pool (Davison, 1999). There are 3 major ways by which bacteria can show antibiotic resistance. These involve enzymatic degradation of the antibiotic agent, alteration of the site where the antibiotic would have initially worked and lastly by pumping out the agent out of the cell. Efflux pumping that *Pseudomonas* undertakes is an example of the latter (Hancock, 1998).

Initially, antibiotic resistance was seen in the Gram-positive *Staphylococcus aureues*(Kumarasamy*et al*., 2010); however, recently, it is the gram negative organisms which are showing more resistance to antibiotics. Due to horizontal gene transfer, antibiotic resistance is being spread out. The gene transfer is taking place mainly through plasmid (Zhang *et al*, 2011) and chromosomal DNA that can include mobile elements such as transposons, integrons and R-plasmid (Hooper, 2000).

As mentioned before, cancer patients often lose their natural immune responses due to the treatment regimen. In such situations, they are highly at risk of various microbial infections. Especially, those patients which already have infections in their oral cancer sites fall under an even greater risk. However, determination of the most suitable antibiotic is also important as all classes of antibiotics have a degree of side effects that can be lethal to cancer patients. Therefore, it becomes crucial to administrate the most suitable antibiotic. Moreover, antibiotic usage affects the normal flora of the body (Yassour*et al*, 2016) which can later aggravate the patients. By conducting antibiotic susceptibility testing, the effects of various antibiotics on a certain bacterial isolate can be tested.

1.7 Antibiotics used for treating cancer patients

For treating cancer patients, not all antibiotics can be used. The groups of antibiotics that can be used are cephalosporins, aminoglycosides, quinolones, carbapenems, penicillin and

several other antibiotic classes (Ubeda&Pamer, 2012). Sometimes, a mixture of antibiotics is given to cover both gram positive and gram negative bacterial pathogens, as immediate susceptibility testing cannot be conducted some of the cancer facilities.

Penicillin

Penicillin is a group of antibiotics that contain a beta-lactam ring which is capable of destroying the peptidoglycan layer, which is a major constituent of bacterial cell walls (Green, 2002). The antibiotics that fall into this vast group are penicillin-G, penicillin-V, ampicillin, amoxicillin, cloxacillin and many more. As time is proceeding, most bacterial species are showing resistance against the penicillin group. The enzyme beta-lactamase that certain gram negative bacteria can produce degrades the active beta-lactam ring. Hence, the antibiotics can no longer work. Even though, these are therefore not very effective, they are administered in combination with other antibiotics to the general and cancer patients.

Cephalosporin

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

Carbapenems

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

Aminoglycosides

This group of antibiotics is mostly effective against gram negative bacteria. They work by inhibiting protein synthesis. The antibiotics that fall into this group are tobramycin, kanamycin, gentamicin and amikacin (Mingeot-Leclercq*et al*, 1999). There have been reports of genes such as aac, aan and aph that can inhibit the effect of aminoglycosides by enzymatic modification. The bacteria harboring these genes can therefore become resistant to this group of antibiotics.

Quinolones

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

Others

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

1.9 Objectives

Oral cancer is one of the most common cancers in Bangladesh. Due to increased exposure to carcinogens, the risk of developing cancers is increasing alarming. Oral cancer can be treated effectively if detected early. However, it most often remains undiagnosed. By analyzing the risk factors and demographical distribution, the population at risk can be inferred and then can be screened for later. Worryingly, death of many patients occurs from not only the cancer itself but also from infections via bacteria that no longer respond to antibiotic treatment. Thus, it is very necessary to evaluate the status of opportunistic bacteria harbored in the ulcers of oral cancer sites. Additionally, their antibiotic susceptibility should also be studied

to determine the most effective antibiotic. These lead to the objective of the study that includes:

1. Evaluation of the prevalence of the gram negative opportunistic pathogens namely Pseudomonas, Klebsiella, Proteus and Escherichia coli and their antibiotic susceptibility pattern.

2. Detection of antibiotic resistance gene *vim*-2.

3. Estimation of the epidemiological, etiological and socio-economic status of oral cancer patients in Bangladesh.

Chapter 2

Materials and methods

2.1 Study Place

This study was conducted in the BRAC University. The laboratory work needed for the successful completion of the research was done in the Biotechnology and Microbiology laboratory of Mathematics and Natural Sciences Department in BRAC University.

2.2 Study Duration

The duration of the study was from February, 2018 to July, 2018.

2.3 Study Population

The study involved collection of both data through a questionnaire and swab sample from the oral cavity. Data was collected from 100 oral cancer patients who were taking treatment from National Institute of Cancer Research and Hospital, Bangladesh. They were checked for the presence of infection on their cancer site. The patients who did not have any infection on their oral cancer site were included in the control group. Another control group of 80 people was set with normal people who did not exhibit any sign of cancer. This group included people from various part of Dhaka city aged above 18.

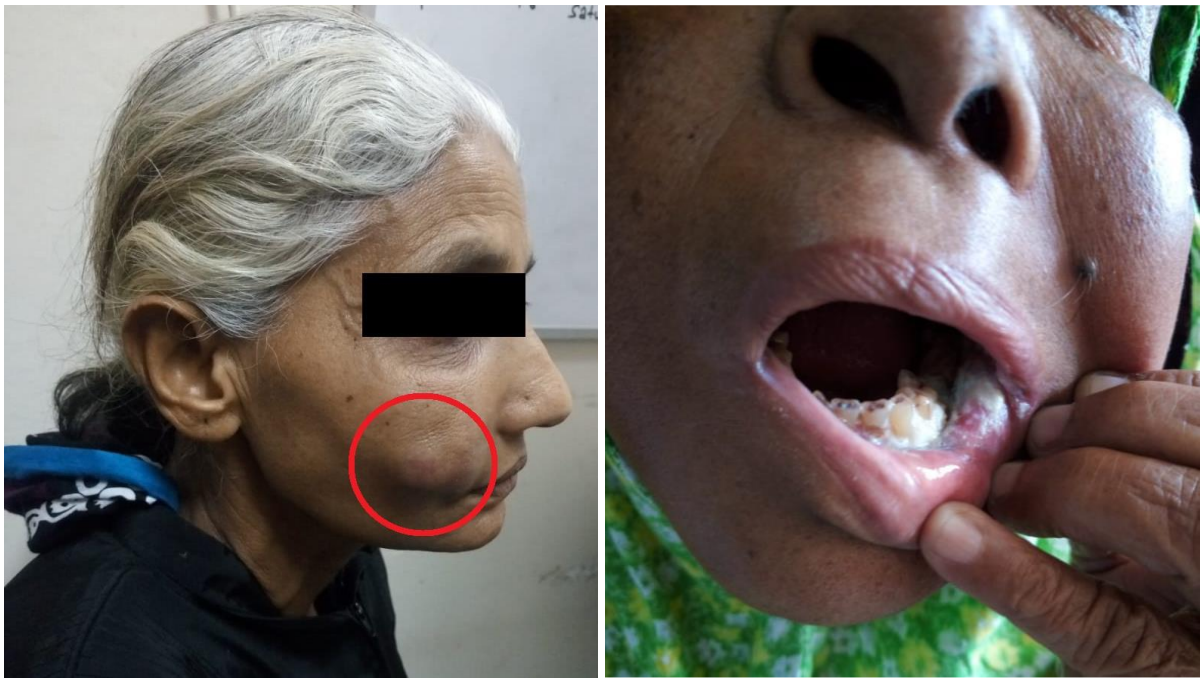


Figure 1: Cancer patients with their cancer sites

All the data and samples were collected with informed consent.

2.4 Sample Collection

2.4.1 Bacterial Sample Collection

With sterile autoclaved cotton swab, the puss from the infection site in the oral cavity of oral cancer patients were taken and quickly transferred to sterile test tubes containing nutrient broth. The tubes were then taken into the laboratory and kept overnight in the incubator for isolation of microbes.

For the control group who were diagnosed with oral cancer but showed no infection in the cancer site, similar process was done.

While for the control group with no oral cancer symptoms, the sterile cotton was rubbed inside the cheeks, gum area and under the tongue.

All the participants had cleaned mouth during and had eaten at least 2 hours prior to the sample collection.

2.4.2 Data Collection

To investigate etiological, demographical and socio-economic aspect of oral cancer patients in Bangladesh, a survey was also done. The contents of the questionnaire are given below in the following table 1.

Table 2.1: Survey questionnaire for oral cancer patients

Name	Question
Particular of the patient	• Name of the patient • Address • Contact information • Age • Sex
Medical History	History of cancer in any family member History of oral cancer in any family member History of non-infectious disease History of infectious disease History of mental illness
Lifestyle	Hygiene practice Consumption of tobacco/ betel nut/ betel leaf/ alcohol
Clinical examination of the cancer	Duration of the cancer Size Location Tenderness Infection Treatment status
Socioeconomic	Education Income Availability of the finance for treatment

2.5 Apparatus

The list of apparatus used is given below

- 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)

- Centrifuge machine
- Glasswares, Laboratory distillation apparatus- fractional distillatory set up, Microscope,
- Petri-dishes, Test-tubes, Micro-pipettes, Bunsen burner, Electric balance, etc.

2.5 Isolation of Microbes

Here, opportunistic gram negative microbes were screened. The isolation process involved overnight incubation of the nutrient broth containing specimen in shaker incubator. Shaker incubator assured even growth of the microbes. After that, by four quadrant method, the broth was streaked onto selected agar media designated for the microbes that were being investigated. Growth of microbes on respective media indicated positive result. Later, the single colonies were streaked on nutrient agar for subculture and taken for further analysis.

2.6 Bacterial Culture Media used for isolation

2.6.1 Nutrient Agar

Nutrient Agar is a basal growth medium for non-fastidious bacteria. It is used for isolation and subculture of various bacteria. It was prepared by weighing 28g of its powder and dissolving in 1 litre of distilled water in a conical flask. After stirring on heat it was boiled. Later, the flask was covered in aluminum foil and kept in autoclave for sterilization. After the liquid was sterile and lukewarm, it was plated into Petri dishes.

2.6.2Cetrimide Agar

Cetrimide Agar is used for the isolation of *Pseudomonas* species. The substance cetrimide is normally toxic to microbes, while *Pseudomonas* species is unaffected due to its membrane efflux pump. Moreover, it can produce pyocyanin, which gives the distinctive greenish hue (Leoboffe and Pierce, 2011).

It was prepared by mixing 46.7g of the laboratory grade powder with 1 litre of distilled water. After dissolving the powder in the water through heating, it was sealed in aluminum foil and autoclaved. Later, the liquid media was poured into dry sterilized Petri dishes. The dishes were later used for bacterial culture.

2.6.3 Eosin Methylene Blue Agar

This is a selective and differential medium that can be used to isolate fecal coliform. It inhibits growth of gram positive bacteria as it contains eosin and methylene blue dyes. Additionally, it also has lactose which acts as the differential element. Some coliforms, mostly *Escherichia coli* can produce a large amount of lactose which lowers the pH of the medium and the dyes react with the acid to give a green metallic sheen. Non lactose fermenters or slow lactose fermenters fail to give the distinctive green sheen due to lack of acid production.

The preparation of eosin methylene blue agar involved dissolving 35.96g powder into 1 litre 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.

2.6.4 HiChrome Agar

HiChrome agar is used to differentiate among many opportunistic pathogens that cause nosocomial and urinary tract diseases. For consisting several dyes and compounds that different bacteria break with their unique enzymes, this is called a chromogenic dye.

The preparation of HiChromeincluded mixing 56.8g powder into conical flask containing 1 litre distilled water. Later boiling and autoclaving followed by pouring into Petri dishes.

2.7 Biochemical Tests

Biochemical tests were done to deduce about the identity of the isolated organisms.

2.7.1 Methyl Red

In sterile peptone water loopful of bacterial colony was inoculated. Later it was mixed into fine suspension. Afterwards, it was incubated overnight at 37 2-3 drops of methyl red were added, and it was kept still for 5 minutes. The presence of dark red color indicated positive result. While, orange or yellow color indicated a negative result. After the incubation, The suspension was kept in an incubator for another overnight growth.

2.6.2 VogesProskeur

Young cultures of the isolates were inoculated into peptone water likewise to the Methyl Red test. After overnight incubation, it was brought out and 2-3 drops of Barritte’s A reagent were

added. Later, 2-3 drops of Barritte's B reagent were added and kept for 15 minutes. A pink colour indicated positive result, no colour change meant negative result.

2.6.3 Citrate

Small vials containing kept at a slanted position and later streaked with young culure of bacteria after hardening. On the next day, the slants were observed for colour change. Blue colour showed positive result; green colour indicated negative result.

2.6.4 Indole

Peptone water was inoculated with a loopful of overnight culture of the organism and mixed into fine suspension. The suspension was kept in the incubator. On the next day, 2-3 drops of Kovac's reagent was added to each test tube. After keeping that for 2 minutes, it was taken to observe any colour change. Red colour meant positive result; yellow colour meant negative result.

2.6.5 Catalase

Catalase test was done by making 3% hydrogen peroxide solution and then placing it on a glass slide where a loopful of fresh culture was added. Bubble formation indicated positive result.

2.6.6 Motility Indole Urease

The base of Motility Indole Urease powder was added to distilled water and boiled on burner. While the medium remained liquid, it was poured in test tubes, and put in autoclave. After bringing out of the autoclave, sterile 40% urea solution was added to each tube at a 10% volume of the total volume. After the medium was hardened, fresh culture of the organism was stabbed on the agar with the help of a needle.

2.6.7 Triple Sugar Iron

The base powder of the Triple sugar iron was added to distilled water and boiled, and poured into test tubes. Afterwards, it was autoclaved. While it remained warm, it was put on a angled position and hardened into slants. With an inoculating needle, fresh culture was first stabbed and then streaked on the slant. It was observed for sugar fermentation and gas formation.

2.6.8 Gram Staining

Gram staining allows bacteria to be differentiated in terms of their cell wall composition. From overnight culture of the organism, loopful of bacteria is smeared onto a sterile glass slide. After heat fixing it was allowed to dry. A few drops of crystal violet were placed on the

smear and washed off after 1 minute. Later, Gram's iodine was put on the smear for another minute. After washing the Gram's iodine, the smear was again washed with 95% ethanol to remove residual dye. Then, a few drops of safranin was placed on the smear for 30 to 45 seconds. After washing this last dye off, the slide was allowed to be dried. At the end, it was observed under the microscope for viewing the cell.

2.8 Antibiotic Susceptibility test

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

2.8.1 Mueller Hinton Agar

By adding 38g of Mueller Hinton agar powder in 1 litre distilled water and boiling while stirring, MHA was prepared. The opening of the flask containing the mixture was wrapped in aluminum foil and autoclaved for sterilization. After sterilization, the liquid was poured into sterile Petri dishes.

2.8.2 Bacterial Suspension Preparation

With a sterile loop, bacterial colony was taken and mixed with sterile 0.9% saline. The concentration was kept at 0.5 McFarland Standard solutions.

2.8.3 List of Antibiotics Used

The antibiotics used in the susceptibility testing were selected on the criteria of being regularly used in hospital for controlling the infection and prophylaxis purpose. These antibiotics are divided into 6 six groups and given below in table 2.

Table 2.2: List of antibiotics used

Antibiotic Group	Antibiotic	Abbreviation
Amynoglycosides	Amikacin	AK
	Gentamicin	GEN
Quinolones	Ciprofloxacin	CIP
	Nalidixic acid	ND
	Levofloxacin	LV
Cephalosporins	Cefepime	CFM
	Ceftazidime	CAZ
	Ceftriaxone	CTR
	Cephalexin	CL
	Cefuroxime	CXM
Carbapenems	Imipenem	IMI
Penicillin	Ampicilin	AMP
	Amoxicilin	AMX
	Penicilin-G	P
Others	Colistin	CL
	Linezolid	LD
	Metronidazole	MT

2.8.4 Inoculation and Disc Diffusion

The Mueller Hinton plates were inoculated with bacterial suspension by sterile cotton swab. During the inoculation, the entire plate surface was covered and allowed to dry. After the plates dried up, with the aid of sterile forceps, antibiotic discs were placed on those. The discs were placed in a manner that the zones would not have overlapped and remained evenly spaced. After placing the discs, the plates were turned over and kept in incubator set at 37^{o}C overnight. On the next day, the zones were measured and noted to be interpreted later on.

2.9 Storage in T1N1

For preservation, the isolates were stored in T1N1 vials. The preparation of T1N1 involved addition of 1gm tryptone casein digest, 1gm NaCl and 0.6gm agar powder. After boiling the mixture it was poured into glass vials. Later, the vials were autoclaved and allowed to solidify. After solidification, bacterial inoculum was taken on a sterile needle and stabbed on the media. It was kept in the incubator for overnight. On the following day, 300ul of sterile paraffin oil was added on top of the agar. After capping the vials tightly and wrapping the junction between the cap and vials, these were stored in room temperature.

2.10 Identification of antibiotic resistant gene

Here, the presence of antibiotic resistant gene *vim*-2 was checked. This was done by polymerase chain reaction with DNA isolated from samples which were resistant towards imipenem antibiotic discs. Later, the PCR samples were passed through gel electrophoresis to check the presence of the gene in investigation.

2.10.1 DNA extraction

The DNA of the *Pseudomonas* isolates which were resistant to imipenem was extracted by the following protocol:

- Luria Bertanni medium was prepared, sterilized and poured into conical flasks. A single colony was picked from each isolates and added to their respective flasks. The flasks were vortexed for even suspension and left overnight.
- From the overnight culture, 1.5 ml was taken into Eppendorf tubes and centrifuged at 13,500 rpm for 3 minutes to pellet the cells.
- The supernatant was carefully discarded.
- The cell pellet was then resuspended in 600 µl lysis buffer and vortexed to resuspend completely
- Later, theresuspended cell pellet was then incubated for 1 hour at 37 °C
- After incubation, 750 µl of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and mixed by inverting the tubes until the phases were completely mixed.
- After the second round of centrifugation for 5 minutes, three distinct layers were visible: bottom layer of phenol: chloroform: isoamyl alcohol, intermediate layer of proteins, and the top aqueous layer of nucleic acids
- The top layer was carefully transferred to a new tube
- An equal volume of chloroform was added to the aqueous layer to remove phenol. The tube was again inverted to mix well.
- The tubes were centrifuged at 13,500 rpm for 5 minutes
- Approximately 200 µl of the upper aqueous layer containing DNA was transferred to new tubes.
- For precipitation of the DNA, 3 volumes of cold ethanol was added and mixed gently
- The tubes were incubated at -20°C for 30 minutes.

- It was followed by centrifugation at 13,500 rpm for 15 minutes
- The supernatant containing ethanol was discarded and the DNA pellet rinsed with 1 ml 70% ethanol
- Centrifugation was done once again at 13,500 rpm for 2 minutes
- The supernatant was discarded and the DNA pellet was air-dried
- The DNA was resuspended in 50 µl TE buffer for storage.

2.10.2 Polymerase Chain Reaction

Polymerase chain reaction technique is a process to amplify genes (Kary Mullis, 1990). Heat is applied to denature DNA template where DNA primers specific to a sequence binds. Later, with the aid of DNA polymerase, nucleotides are added to form new strands. This process can be used for gene detection if the primer specific for that gene is added. Here, primers of the *vim*-2 gene are used.

The protocol and primers are given below (Amini&Mobasseri, 2017).

- In sterile PCR tubes, 2ul DNA template was added.
- With micropipette, 23ul of PCR master mix was added.
- The PCR temperature and timing parameter were set in the machine and the tubes were taken there for amplification.
- After completion, the tubes were taken inside -20°C refrigerator until further use.

Table 2.3.1: PCR reaction mixture for *vim*-2 gene.

Forward Primer	1 µl
Reverse Primer	1µl
Taq Polymerase	1µl
dNTPs	2µl
Template DNA*	2µl
10x Reaction buffer	3.5µl
Nuclease free water	14.5 µl
Total	25

Table 2.3.2: Forward and reverse primers for *vim*-2 gene.

Forward primer	5′- AAAGTTATGCCGCACTCACC- 3′
Reverse primer	5′- TGCAACTTCATGTTATGCCG- 3′

Table 2.3.3: Cycling parameter for *vim*-2 gene.

PCR step	Temperature	Time
Initial denaturation	94°C	3 minutes
Denaturation*	94°C	1 minute
Annealing*	55°C	1 seconds
Extension*	72°C	2 minutes
Final extension	72°C	7 minutes

***35 cycles each**

2.10.3. Gel electrophoresis

The DNA samples which were amplified by the PCR process were passed through agarose gel in presence of electronic current. As DNA is negative in electric nature, it moves along with electric charge. However, the movement of the DNA depends on its size and charge. The larger ones move slower and vice versa. This allows a segregation of different sized DNA molecules. A DNA ladder allows comparison of the moved DNA with the expected size.

The agarose gel was prepared at 1% concentration and the DNA was run at 80volts for 30 minutes in the gel electrophoresis apparatus.

2.11. Statistical analysis

For any research, statistical analyses backs up the insights found from data in an academically appreciated manner. In the current research, the data collected from patients consisted of a large number of qualitative ones. The major ones were: Location where the patient comes from, gender, diabetic status, cardiac problem, major infections, hygiene status, habit of: smoking, shadapata, gul, jorda,betel leaf, betel nut; financing sources, differentiation of cancer cells, site of cancer, presence of infection at that site, treatment procedures: surgery, chemo therapy and radiotherapy. Other than these, income, age, treatment cost and time gap before seeking treatment were also noted.

With the qualitative data, the chi square test was used. Chi square test applies expected value of frequency got from cross tabulation based on two categorical divisions, to find out if an association is present between the categorical divisions. For the quantitative data, independent samples T test or ANOVA was carried out. The T test allows us to find out whether the means of quantitative variables are equal for two different qualitative segments. For more than two categories, ANOVA was used. All the tests were run by SPSS version 16 software according to the standard procedures.

Chapter 3

Results

3.1 Results from the growth on selective media

The nutrient broth test tubes that were inoculated with oral swabs were streaked on 3 selective media namely, cetrimide agar, eosin methylene blue agar and hiChrome agar. From the 100 patients that participated in the study, 68 patients were positive for infection in their cancer site. Among these 68 patients, twenty one patients were post-operative patients and remaining 46 were pre-operative patients. All the specimens from both pre-operative and post-operative patients showed positive result on at least one of the selective media. However, among the 32 patients that showed no prior visible infection, 9 of the patient's swab specimen showed growth on the used selective media. Of the 80 control samples, only 18 of their specimen's showed positive results on the selective media used for the isolation of selected gram negative opportunistic pathogens. Their isolate numbers are labeled from C1-18.

Colonies with green or light green color were considered positive result in cetrimide agar. While, colonies with mucoid pink/purple color, green sheen and colorless lush appearance were considered positive results. The summary of the positive isolates is given below. Po-Green- 20 NG-5 LG-3 PM- 16 NG- 9 GS-3

Table 3.1: Summary of growth of the isolates in selective media

Patient status	Media name						
	Cetrimide			EMB			
	Colony appearance			Colony appearance			
	Green	Light green	No growth	Purple/pink mucoid	Green sheen	Colorless lush	No growth
Post-op	20	3	5	16	3	-	9
Pre-op	22	6	18	13	13	5	15
No-infection	3	-	29	3	8	-	21
Control group	7	-	113	3	8	-	109

3.2 Result from biochemical tests

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. A total of 95 different bacterial colonies were taken from the positive cultures of which 86 belonged to cancer patients with infection and 9 belonged to cancer patients with no visible infections. These were labeled as 1-95. Later, biochemical tests were done for further deduction of the organism's identity. The isolates from the control group were labeled as C1-C18.

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity

Isolate number	Media used for selection	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
1	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
2	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
3	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
4	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
5	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
6	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
7	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
8	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
9	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
10	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
11	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
12	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species

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

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity (cont.)

Isolate number	Selective media used for isolation	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
13	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
14	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
15	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
16	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
17	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
18	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
19	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
20	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
21	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
22	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
23	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
24	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
25	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
26	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
27	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
28	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species

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

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity (cont.)

Isolate number		MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
29	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
30	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
31	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
32	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
33	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
34	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	*Proteus* species
35	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
36	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
37	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	Proteus species
38	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
39	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
40	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
41	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
42	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
43	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
44	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
45	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
46	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*

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

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity (cont.)

Isolate number	Selective media used for isolation	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	MIU			Gram staining		TSI						Probable organism
		Methyl red	Vogesprosk eur					Motility	Urease	Indole	Colour	Shape	Slant/butt colour	Glucose	Lactose	Sucrose	H_2S production	Gas production	
47	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
48	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
49	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
50	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
51	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
52	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
53	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
54	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
55	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
56	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	*Proteus* species
57	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
58	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
59	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
60	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	*Proteus* species
61	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
62	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
63	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
64	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
65	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
66	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*

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

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity (cont.)

Isolate number	Selective media used for isolation	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
67	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	*Proteus* species
68	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
69	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
70	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
71	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
72	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
73	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
74	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
75	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
76	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
77	EMB	+	-	+	-	+	Light brown	+	+	-	Pink	Rob	R/Y	+	-	-	+	+	*Proteus* species
78	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
79	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
80	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
81	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
82	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
83	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
84	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
85	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
86	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*

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

Table 3.2.1: Biochemical test results of the isolates from cancer patients and their probable identity (cont.)

Isolate number	Selective media used for isolation	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
87	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
88	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
89	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
90	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
91	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
92	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
93	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Escherichia coli*
94	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species
95	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella* species

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

The table 3.2.1 includes outcome of the biochemical tests done on the isolates from cancer patients. This also includes the most probable identity of the isolates. The interpretation is based on biochemical characteristics of the isolates.

Table 3.2.2: Biochemical test results of the isolates from control and their probable identity.

Isolate number	Selective media used for isolation	MRVP		Catalase	Oxidase	Citrate	Appearance on Hicrhome agar	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	
C1	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C2	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
C3	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C4	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C5	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C6	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C7	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C8	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C9	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C10	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
C11	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C12	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C13	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C14	EMB	-	+	+	-	+	Purple	-	+	-	Pink	Rod	Y/Y	+	+	+	-	+	*Klebsiella*species
C15	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C16	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species
C17	EMB	+	-	+	-	-	Blue	+	-	+	Pink	Rod	Y/Y	+	+	+	-	+	*Esherechia coli*
C18	Cetrimide	-	-	+	+	+	Colorless	+	-	-	Pink	Rod	R/R	-	-	-	-	+	*Pseudomonas* species

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

The table 4 includes outcome of the biochemical tests done on the isolates from control. This also includes the most probable identity of the isolates. The interpretation is based on biochemical characteristics of the isolates.

3.3 Identification of the isolates

After the selection from selective media and biochemical tests the probable organisms were *Pseudomonas* species, *Klebsiella*species, *Escherichia coli* and *Proteus* species.

The number and ratio of isolated organisms are given below in table 6.

Table 3.3: Percentage identity of the isolates

Organism	Total in cancer patients (%)	Infection (Post-op) (%)	Infection (Pre-op) (%)	No infection (%)	Control (%)
Pseudomonas	46 (46)	73 (16)	27 (59)	3 (9)	9 (7)
Klebsiella	20 (20)	41 (9)	7 (15)	4 (20)	4 (3)
Proteus	33 (33)	0	33	0	0
E. coli	14 (14)	14 (3)	9 (20)	2 (6)	9 (8)

This table shows the highest number of isolates were *Pseudomonas* species followed by *Klebsiella*species and *E. coli.* The least number of isolates were *Proteus*.

The graphical representation of the isolates in terms of their probable identity and source are given below.

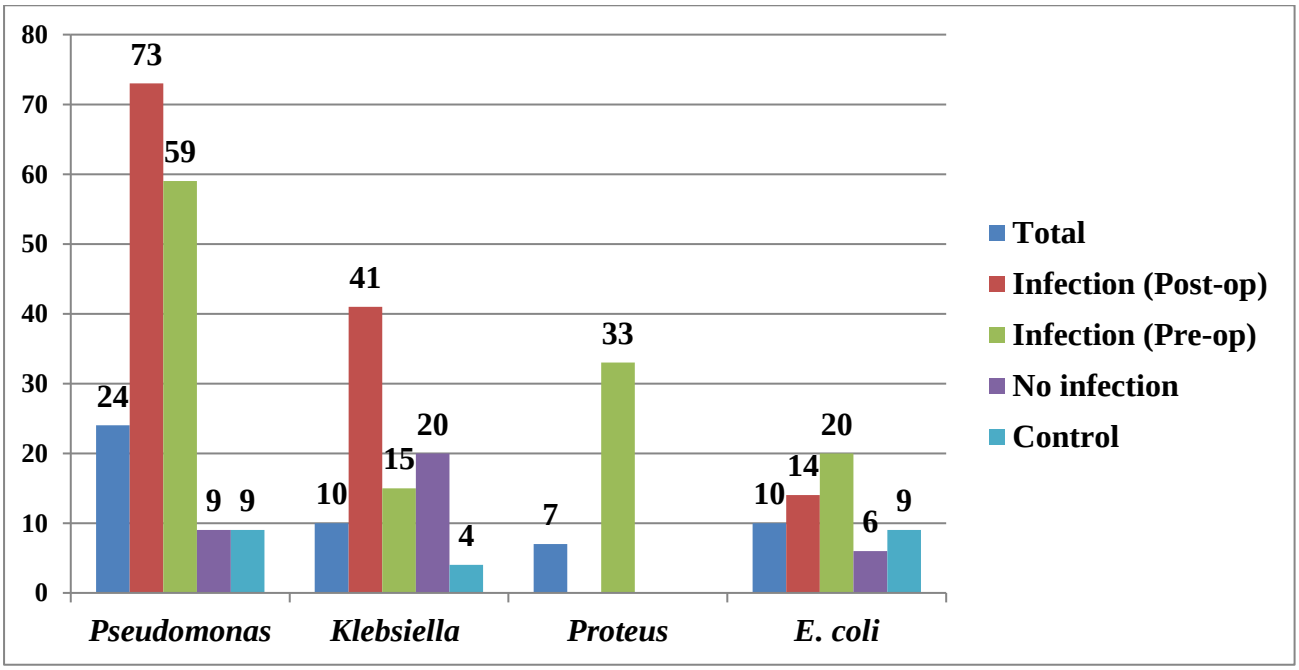


Figure 3.3: Graphical representation of the percentage of isolates

3.4 Results from antibiotic susceptibility test

The 95 isolates from cancer patients and 18 isolates from the control group were tested for antibiotic susceptibility with 17 antibiotics. The result from the AST is given below.

Table 3.4.1: Table of antibiotic sensitivity testing interpretation on the isolates from cancer patients

	AK	GEN	CIP	ND	LEV	CFM	CTR	CAZ	CF	CXM	IMI	AMP	AMX	P	CL	LD	MT
	ZS (I)	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**
1	21 **S**	30**S**	21**S**	- **R**	25**S**	- **R**	- **R**	19 **S**	22**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
2	25**S**	22**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	30**S**	- **R**	- **R**	- **R**	22 **S**	20**S**	- **R**
3	29**S**	21**S**	24**S**	- **R**	21**S**	24 **S**	21 **S**	23**S**	18**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	17**S**	- **R**
4	20**S**	26**S**	22**S**	- **R**	30**S**	- **R**	- **R**	26**S**	19**S**	- **R**	34**S**	- **R**	- **R**	- **R**	- **R**	19**S**	- **R**
5	21**S**	25**S**	31**S**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	- **R**	26**S**	- **R**	- **R**	- **R**	- **R**	22**S**	- **R**
6	22**S**	- **R**	34**S**	- **R**	24**S**	- **R**	- **R**	22**S**	21**S**	- **R**	29**S**	- **R**	- **R**	- **R**	9 **R**	18**S**	- **R**
7	25**S**	21**S**	29**S**	- **R**	- **R**	- **R**	23**S**	- **R**	24**S**	- **R**	21 **S**	- **R**	- **R**	- **R**	24 **S**	26**S**	- **R**
8	29**S**	29**S**	26**S**	- **R**	20**S**	- **R**	- **R**	25**S**	22**S**	- **R**	25**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
9	22**S**	21**S**	30**S**	- **R**	21**S**	- **R**	- **R**	21**S**	19**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	8 **R**	- **R**
10	- **R**	25**S**	22**S**	- **R**	26**S**	- **R**	- **R**	20**S**	26 **S**	- **R**	32**S**	- **R**	- **R**	- **R**	- **R**	23 **S**	- **R**
11	23**S**	- **R**	26**S**	- **R**	- **R**	27 **S**	- **R**	24**S**	23**S**	21**S**	22**S**	- **R**	- **R**	- **R**	- **R**	19 **S**	- **R**
12	28**S**	22**S**	25**S**	- **R**	29**S**	- **R**	18**S**	- **R**	- **R**	- **R**	19**S**	- **R**	- **R**	- **R**	- **R**	20**S**	- **R**
13	26**S**	29**S**	23**S**	- **R**	21**S**	- **R**	- **R**	22 **S**	21**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
14	- **R**	- **R**	21 **S**	- **R**	24**S**	22 **S**	- **R**	25**S**	18**S**	- **R**	21**S**	- **R**	- **R**	- **R**	12 **R**	- **R**	- **R**
15	24**S**	26**S**	- **R**	- **R**	27**S**	- **R**	- **R**	19**S**	23**S**	- **R**	24**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
16	27**S**	21**S**	19**S**	- **R**	22**S**	- **R**	24 **S**	28**S**	25**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	17**S**	- **R**
17	22**S**	25**S**	28**S**	- **R**	27**S**	- **R**	- **R**	21**S**	24**S**	24**S**	28**S**	- **R**	- **R**	- **R**	19 **S**	29**S**	- **R**
18	29**S**	27**S**	26**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
19	19 **S**	23**S**	29**S**	- **R**	29**S**	19 **S**	- **R**	24**S**	29**S**	- **R**	32**S**	- **R**	- **R**	- **R**	- **R**	22**S**	- **R**
20	- **R**	31**S**	21**S**	- **R**	21**S**	- **R**	21 **S**	- **R**	25**S**	- **R**	21**S**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
21	25**S**	24 **S**	- **R**	- **R**	20**S**	- **R**	- **R**	- **R**	28**S**	- **R**	23**S**	- **R**	- **R**	- **R**	- **R**	20**S**	- **R**
22	31**S**	28**S**	30 **S**	- **R**	18 **S**	- **R**	19 **S**	26**S**	- **R**	- **R**	27**S**	- **R**	- **R**	- **R**	21 **S**	11 **R**	- **R**

ZS-Zone size (cm) **I**-Interpretation **R**- Resistant **S**- Sensitive

Table 3.4.1: Table of antibiotic sensitivity testing interpretation on the isolates from cancer patients (cont.)

	AK	GEN	CIP	ND	LEV	CFM	CTR	CAZ	CF	CXM	IMI	AMP	AMX	P	CL	LD	MT
	ZS (I)	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**
23	19**S**	21**S**	24**S**	- **R**	28**S**	- **R**	**S**	25**S**	21 **S**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**
24	24**S**	25**S**	29**S**	- **R**	21**S**	- **R**	- **R**	23**S**	19**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	19**S**	- **R**
25	- **R**	22**S**	30**S**	- **R**	- **R**	21 **S**	23 **S**	- **R**	- **R**	- **R**	32**S**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
26	22 **S**	26**S**	32**S**	- **R**	25 **S**	- **R**	- **R**	21**S**	24**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	27**S**	- **R**
27	25**S**	22**S**	- **R**	- **R**	29**S**	- **R**	- **R**	18**S**	19**S**	- **R**	29**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
28	- **R**	- **R**	- **R**	- **R**	31**S**	- **R**	- **R**	20**S**	22**S**	- **R**	- **R**	- **R**	- **R**	- **R**	28 **S**	- **R**	- **R**
29	28**S**	25**S**	31**S**	- **R**	- **R**	19 **S**	- **R**	21**S**	25**S**	- **R**	33**S**	- **R**	- **R**	- **R**	- **R**	31**S**	- **R**
30	21**S**	23**S**	28 **S**	- **R**	28**S**	- **R**	- **R**	- **R**	21**S**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
31	25**S**	26**S**	27**S**	- **R**	26**S**	- **R**	20 **S**	19**S**	- **R**	- **R**	29**S**	- **R**	- **R**	- **R**	12 **R**	18**S**	- **R**
32	- **R**	- **R**	30**S**	- **R**	29**S**	- **R**	- **R**	21**S**	18**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	22**S**	- **R**
33	22**S**	27**S**	29**S**	- **R**	- **R**	19 **S**	- **R**	- **R**	- **R**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
34	21**S**	24**S**	29**S**	- **R**	27**S**	- **R**	21 **S**	20**S**	22**S**	23**S**	39**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
35	19**S**	22**S**	27**S**	- **R**	25**S**	- **R**	- **R**	20**S**	21**S**	- **R**	27**S**	- **R**	- **R**	- **R**	18 **S**	- **R**	- **R**
36	18**S**	- **R**	- **R**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**	- **R**	- **R**	- **R**	32**S**	- **R**
37	20**S**	21**S**	24**S**	- **R**	26**S**	- **R**	- **R**	22**S**	21**S**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
38	18**S**	23**S**	28**S**	- **R**	24**S**	- **R**	- **R**	- **R**	19**S**	- **R**	30**S**	- **R**	- **R**	- **R**	- **R**	19**S**	- **R**
39	- **R**	- **R**	31 **S**	- **R**	21**S**	- **R**	- **R**	19**S**	- **R**	- **R**	18**S**	- **R**	- **R**	- **R**	- **R**	9 **R**	- **R**
40	20**S**	19**S**	29**S**	- **R**	23**S**	- **R**	- **R**	21 **S**	24**S**	- **R**	- **R**	- **R**	- **R**	- **R**	13 **R**	18**S**	- **R**
41	22**S**	23**S**	26**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	21**S**	24**S**	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**
42	24**S**	19**S**	30**S**	- **R**	26**S**	- **R**	- **R**	25**S**	22**S**	- **R**	29**S**	- **R**	- **R**	- **R**	- **R**	23**S**	- **R**
43	21**S**	22**S**	28**S**	- **R**	23**S**	- **R**	- **R**	22**S**	18**S**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
44	22**S**	25**S**	33**S**	- **R**	21**S**	- **R**	- **R**	20**S**	21**S**	- **R**	30**S**	- **R**	- **R**	- **R**	29 **S**	23 **S**	- **R**
45	17**S**	23**S**	29**S**	- **R**	19**S**	22 **S**	- **R**	18**S**	22**S**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
46	- **R**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	- **R**	17**S**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
47	18**S**	19**S**	27**S**	- **R**	22**S**	- **R**	- **R**	25**S**	- **R**	- **R**	18**S**	- **R**	- **R**	- **R**	- **R**	30**S**	- **R**
48	20**S**	21**S**	29**S**	- **R**	24**S**	- **R**	17 **S**	22**S**	19**S**	- **R**	21**S**	- **R**	- **R**	- **R**	17 **S**	19**S**	- **R**
49	21**S**	24**S**	22**S**	- **R**	25**S**	- **R**	- **R**	19**S**	- **R**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	33**S**	- **R**
50	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**	**S**	17**S**	- **R**	- **R**	22**S**	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**

ZS-Zone size (cm) **I**-Interpretation **R**- Resistant **S**- Sensitive

Table 3.4.1: Table of antibiotic sensitivity testing interpretation on the isolates from cancer patients (cont.)

	AK	GEN	CIP	ND	LEV	CFM	CTR	CAZ	CF	CXM	IMI	AMP	AMX	P	CL	LD	MT
	ZS (I)	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**
51	18**S**	22**S**	29**S**	- **R**	23**S**	- **R**	- **R**	19**S**	17**S**	- **R**	29**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
52	21**S**	20**S**	21**S**	- **R**	21**S**	- **R**	- **R**	- **R**	20**S**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	27**S**	- **R**
53	25**S**	23**S**	28**S**	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	31**S**	- **R**
54	19**S**	22**S**	25**S**	- **R**	18**S**	- **R**	- **R**	20**S**	- **R**	- **R**	18**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
55	23**S**	18**S**	31**S**	- **R**	- **R**	- **R**	- **R**	23**S**	21**S**	- **R**	21**S**	- **R**	- **R**	- **R**	- **R**	18**S**	- **R**
56	21**S**	20**S**	30**S**	- **R**	24**S**	- **R**	- **R**	22**S**	23**S**	- **R**	31**S**	- **R**	- **R**	- **R**	24 **S**	22**S**	- **R**
57	- **R**	- **R**	27	- **R**	26	22 **S**	- **R**	- **R**	21	- **R**	28	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
58	23**S**	18**S**	- **R**	- **R**	21**S**	- **R**	- **R**	17**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
59	19**S**	22**S**	22**S**	- **R**	25**S**	- **R**	19 **S**	21**S**	20**S**	- **R**	30**S**	- **R**	- **R**	- **R**	- **R**	26 **S**	- **R**
60	21**S**	24**S**	33**S**	- **R**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**	33**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
61	18**S**	26**S**	21**S**	- **R**	22**S**	18 **S**	- **R**	23**S**	18**S**	- **R**	25**S**	- **R**	- **R**	- **R**	19 **S**	27**S**	- **R**
62	20**S**	24**S**	28**S**	- **R**	19**S**	- **R**	- **R**	20**S**	- **R**	- **R**	21**S**	- **R**	- **R**	- **R**	- **R**	18**S**	- **R**
63	23**S**	- **R**	- **R**	- **R**	21**S**	- **R**	21 **S**	18**S**	17**S**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
64	19**S**	17**S**	26**S**	- **R**	18**S**	- **R**	- **R**	19**S**	20**S**	- **R**	20**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
65	17**S**	19**S**	30**S**	- **R**	19**S**	21 **S**	- **R**	- **R**	18**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
66	21**S**	20**S**	- **R**	- **R**	21**S**	- **R**	- **R**	21**S**	- **R**	18 **S**	29**S**	- **R**	- **R**	- **R**	27 **S**	23**S**	- **R**
67	22**S**	18 **S**	21**S**	- **R**	22**S**	- **R**	23 **S**	23**S**	- **R**	- **R**	32**S**	- **R**	- **R**	- **R**	- **R**	30**S**	- **R**
68	- **R**	- **R**	30**S**	- **R**	25**S**	- **R**	- **R**	19**S**	25**S**	- **R**	35**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
69	19**S**	20**S**	32 **S**	- **R**	- **R**	- **R**	- **R**	24**S**	22**S**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
70	21**S**	21**S**	22**S**	- **R**	22**S**	19 **S**	20 **S**	20**S**	- **R**	- **R**	22**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
71	24**S**	23**S**	25**S**	- **R**	20**S**	- **R**	- **R**	17**S**	20**S**	- **R**	28**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
72	- **R**	22**S**	21**S**	- **R**	24**S**	- **R**	- **R**	19**S**	23**S**	- **R**	25**S**	- **R**	- **R**	- **R**	- **R**	26**S**	- **R**
73	23 **S**	20**S**	26**S**	- **R**	26**S**	- **R**	- **R**	- **R**	21**S**	- **R**	35**S**	- **R**	- **R**	- **R**	- **R**	10 **R**	- **R**
74	25**S**	21**S**	28**S**	- **R**	21**S**	- **R**	- **R**	21 **S**	16**S**	- **R**	33 **S**	- **R**	- **R**	- **R**	- **R**	23**S**	- **R**
75	22**S**	24**S**	31**S**	- **R**	- **R**	17 **S**	- **R**	- **R**	- **R**	20**S**	31**S**	- **R**	- **R**	- **R**	- **R**	19**S**	- **R**
76	21**S**	19**S**	- **R**	- **R**	28**S**	- **R**	- **R**	19**S**	18**S**	- **R**	29**S**	- **R**	- **R**	- **R**	- **R**	20**S**	- **R**
77	- **R**	- **R**	29**S**	- **R**	20**S**	- **R**	24 **S**	22**S**	- **R**	- **R**	26**S**	- **R**	- **R**	- **R**	29 **S**	24**S**	- **R**

ZS-Zone size (cm) **I**-Interpretation **R-** Resistant **S**- Sensitive

Table 3.4.1: Table of antibiotic sensitivity testing interpretation on the isolates from cancer patients (cont.)

	AK	GEN	CIP	ND	LEV	CFM	CTR	CAZ	CF	CXM	IMI	AMP	AMX	P	CL	LD	MT
	ZS (I)	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**
78	20**S**	17**S**	28**S**	- **R**	- **R**	- **R**	- **R**	18**S**	21**S**	- **R**	33**S**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**
79	18**S**	- **R**	30**S**	- **R**	29**S**	21 **S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	9 **R**	- **R**	- **R**
80	21**S**	21**S**	- **R**	- **R**	27**S**	- **R**	- **R**	22**S**	23**S**	- **R**	39**S**	- **R**	- **R**	- **R**	- **R**	28**S**	- **R**
81	22**S**	23**S**	25 **S**	- **R**	33**S**	- **R**	17 **S**	19**S**	19**S**	22 **S**	28**S**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
82	- **R**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	23**S**	19**S**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	9 **R**	- **R**
83	24**S**	21**S**	22**S**	- **R**	31**S**	- **R**	- **R**	- **R**	21**S**	- **R**	28**S**	- **R**	- **R**	- **R**	25 **S**	18**S**	- **R**
84	19**S**	20**S**	29**S**	- **R**	29**S**	- **R**	19 **S**	21**S**	- **R**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	30**S**	- **R**
85	19**S**	17**S**	26**S**	- **R**	21**S**	- **R**	- **R**	25**S**	20**S**	- **R**	29**S**	- **R**	- **R**	- **R**	11 **R**	25**S**	- **R**
86	21 **S**	24**S**	- **R**	- **R**	- **R**	19 **S**	- **R**	- **R**	17**S**	- **R**	32**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**
87	17**S**	21**S**	30**S**	- **R**	27**S**	- **R**	- **R**	22**S**	22**S**	19 **S**	18**S**	- **R**	- **R**	- **R**	19 **S**	22**S**	- **R**
88	- **R**	- **R**	33**S**	- **R**	30**S**	22 **S**	- **R**	18**S**	21**S**	- **R**	22**S**	- **R**	- **R**	- **R**	- **R**	24**S**	- **R**
89	18**S**	18**S**	27**S**	- **R**	26**S**	- **R**	20 **S**	17**S**	24**S**	- **R**	27**S**	- **R**	- **R**	- **R**	- **R**	27**S**	- **R**
90	23**S**	22**S**	25**S**	- **R**	29**S**	- **R**	- **R**	20**S**	22**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	21**S**	- **R**
91	- **R**	19**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	25**S**	- **R**	- **R**	- **R**	26 **S**	- **R**	- **R**
92	25**S**	20**S**	29**S**	- **R**	22**S**	- **R**	- **R**	21**S**	20**S**	- **R**	29**S**	- **R**	- **R**	- **R**	12 **R**	8 **R**	- **R**
93	22**S**	17**S**	26**S**	- **R**	25**S**	- **R**	21 **S**	23**S**	21**S**	- **R**	31**S**	- **R**	- **R**	- **R**	- **R**	29**S**	- **R**
94	20**S**	21**S**	22**S**	- **R**	27**S**	- **R**	- **R**	- **R**	19**S**	- **R**	33**S**	- **R**	- **R**	- **R**	8 **R**	18**S**	- **R**
95	- **R**	- **R**	28**S**	- **R**	- **R**	18 **S**	- **R**	19**S**	- **R**	- **R**	- **R**	- **R**	- **R**	- **R**	29 **S**	- **R**	- **R**

ZS-Zone size (cm) **I**-Interpretation **R**- Resistant **S**- Sensitive

The table includes the zone sizes of the different isolates from cancer patients 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.

The antibiotic susceptibility results from the control group are as follows.

Table 3.4.2: Table of antibiotic sensitivity testing interpretation on the isolates from control group

	AK	GEN	CIP	ND	LEV	CFM	CTR	CAZ	CF	CXM	IMI	AMP	AMX	P	CL	LD	MT
	ZS (I)	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**	**ZS (I)**
C1	28 **S**	21 **S**	34 **S**	22 **S**	29 **S**	25**S**	21**S**	17**S**	- **R**	22**S**	21**S**	18**S**	- **R**	- **R**	23**S**	25**S**	-**R**
C2	25**S**	20**S**	31**S**	20**S**	25**S**	20**S**	23**S**	19**S**	22**S**	25**S**	24**S**	- **R**	- **R**	- **R**	27**S**	28**S**	23 **S**
C3	21**S**	27**S**	29**S**	19**S**	30**S**	21**S**	19**S**	- **R**	25**S**	17**S**	22**S**	- **R**	17**S**	- **R**	30**S**	21**S**	- **R**
C4	- **R**	21**S**	- **R**	22**S**	28**S**	24**S**	24**S**	21**S**	- **R**	- **R**	19**S**	- **R**	21**S**	- **R**	21**S**	26**S**	- **R**
C5	28**S**	25**S**	28**S**	20**S**	21**S**	- **R**	22**S**	25**S**	21**S**	21**S**	24**S**	- **R**	- **R**	- **R**	25**S**	29**S**	- **R**
C6	22**S**	- **R**	35**S**	- **R**	27**S**	22**S**	18**S**	23**S**	26**S**	25**S**	21**S**	22**S**	- **R**	- **R**	27**S**	22**S**	26 **S**
C7	26**S**	22**S**	31**S**	23**S**	25**S**	23**S**	21**S**	- **R**	19**S**	18**S**	25**S**	- **R**	- **R**	- **R**	24**S**	24**S**	- **R**
C8	21**S**	24**S**	37**S**	21**S**	31**S**	19**S**	- **R**	19**S**	22**S**	23**S**	19**S**	- **R**	- **R**	19 **S**	22**S**	21**S**	- **R**
C9	19**S**	23**S**	22**S**	25**S**	23**S**	25**S**	23**S**	21**S**	25**S**	- **R**	19**S**	- **R**	- **R**	- **R**	- **R**	19**S**	28 **S**
C10	24**S**	- **R**	29**S**	- **R**	30**S**	21**S**	25**S**	25**S**	21**S**	21**S**	21**S**	20 **S**	- **R**	- **R**	19**S**	29**S**	-**R**
C11	27**S**	25**S**	- **R**	24**S**	- **R**	24**S**	19**S**	22**S**	24**S**	24**S**	25**S**	- **R**	- **R**	17**S**	21**S**	25**S**	19 **S**
C12	- **R**	19**S**	29**S**	21**S**	26**S**	20**S**	21**S**	26**S**	- **R**	22**S**	24**S**	- **R**	- **R**	- **R**	25**S**	- **R**	-**R**
C13	25**S**	- **R**	31**S**	17**S**	28**S**	- **R**	26**S**	21**S**	20**S**	19**S**	22**S**	- **R**	- **R**	- **R**	22**S**	19**S**	24 **S**
C14	22**S**	21**S**	33**S**	20**S**	21**S**	22**S**	22**S**	17**S**	22**S**	25**S**	- **R**	- **R**	- **R**	- **R**	27**S**	23**S**	23 **S**
C15	28**S**	25**S**	35**S**	18**S**	29**S**	17**S**	21**S**	19**S**	17**S**	21**S**	25**S**	23 **S**	- **R**	- **R**	21**S**	25**S**	- **R**
C16	19**S**	22**S**	27**S**	21**S**	31**S**	19**S**	27**S**	22**S**	21**S**	24**S**	23**S**	- **R**	- **R**	- **R**	24**S**	22**S**	- **R**
C17	21**S**	19**S**	29**S**	22**S**	33**S**	21**S**	23**S**	21**S**	19**S**	- **R**	19**S**	- **R**	- **R**	- **R**	28**S**	26**S**	- **R**
C18	- **R**	22**S**	- **R**	19**S**	26**S**	20**S**	19**S**	23**S**	- **R**	21**S**	21**S**	- **R**	- **R**	- **R**	31**S**	28**S**	- **R**

ZS-Zone size (cm) **I**-Interpretation **R-** Resistant **S-** Sensitive

The table includes the zone sizes of the different isolates from 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.

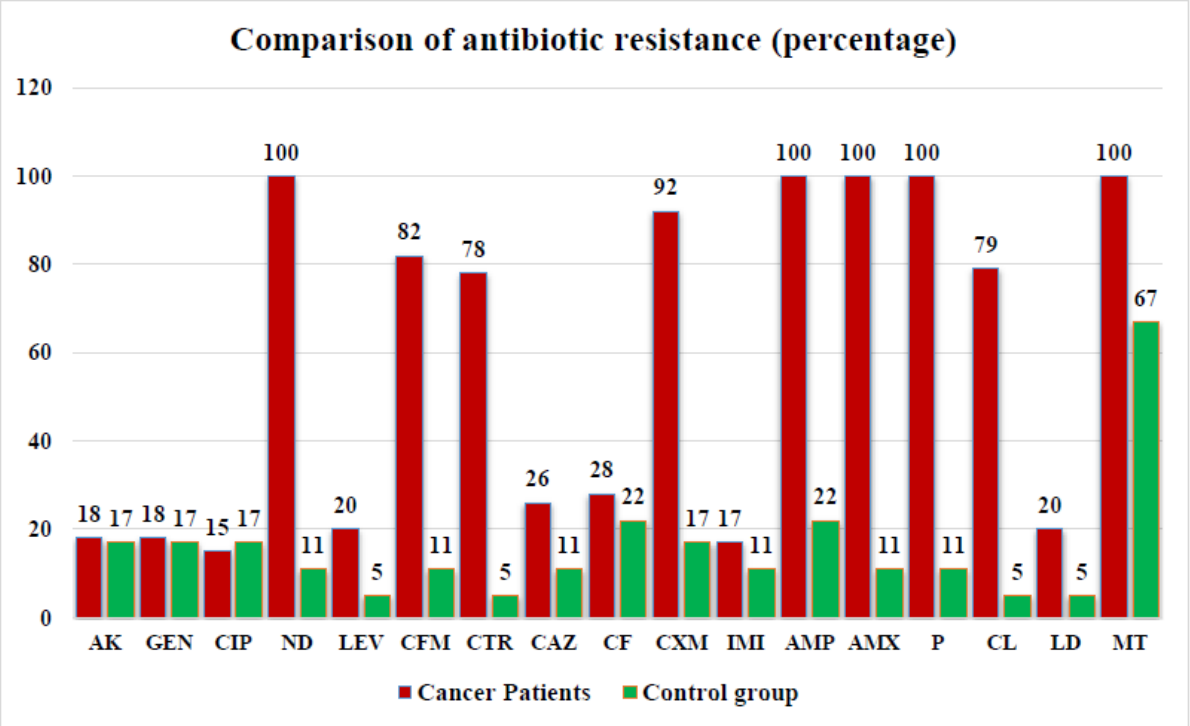


Figure 3.4: Comparison of antibiotic resistance for different antibiotics

All the isolates from cancer patients were resistant to nalidixic acid, metronidazole, and amoxicillin and penicillin-G. It was followed by cefuroxime, where 92% of the isolates were resistant to it. The least resistance was seen against ciprofloxacin (15%), then amikacin and gentamicin both having a percentage of 18%.

In the control group, the highest level of resistance was seen against metronidazole (67%). The least level of resistance was seen against levofloxacin, cefepime, colistin and linezolid (5%).

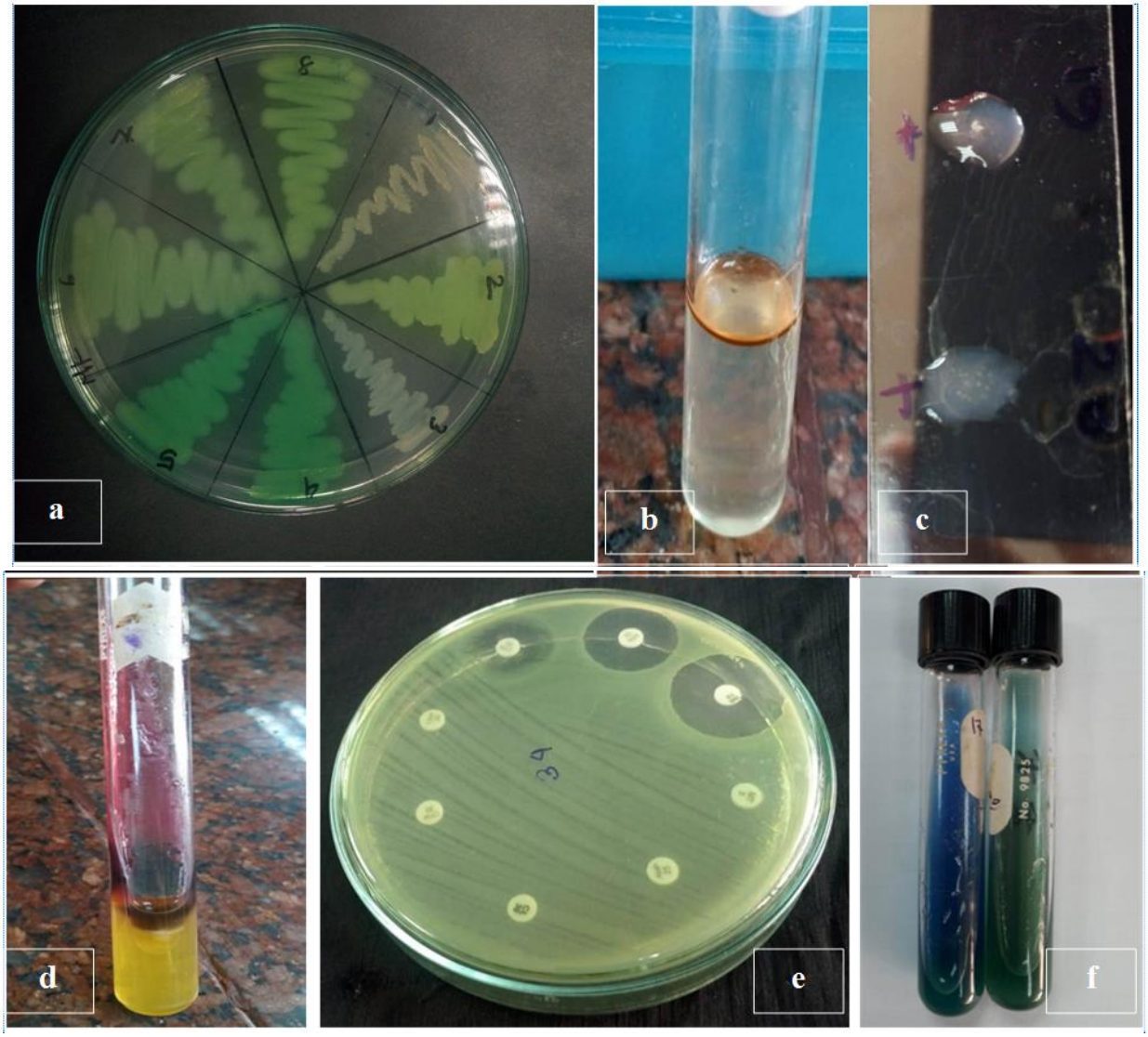


Figure: a.*Pseudomonas* species grown on cetrimide agar. b. Voges-Proskauer negative result. c. Catalase positive result. d. TSI slant. e. Antibiotic susceptibility test with antibiotic discs. f. Citrate positive (left) citrate negative (right)

3.5 Results from detection of antibiotic resistant gene

The antibiotic resistant gene *vim*-2 was detected using PCR procedure. From the samples which were resistant to the antibiotic imipenem, 8 were selected for PCR amplification with *vim*-2 primers. The expected band size of the primers is 865. After gel electrophoresis, the agarose gel was taken under the UV transilluminator to see the presence of the expected band.

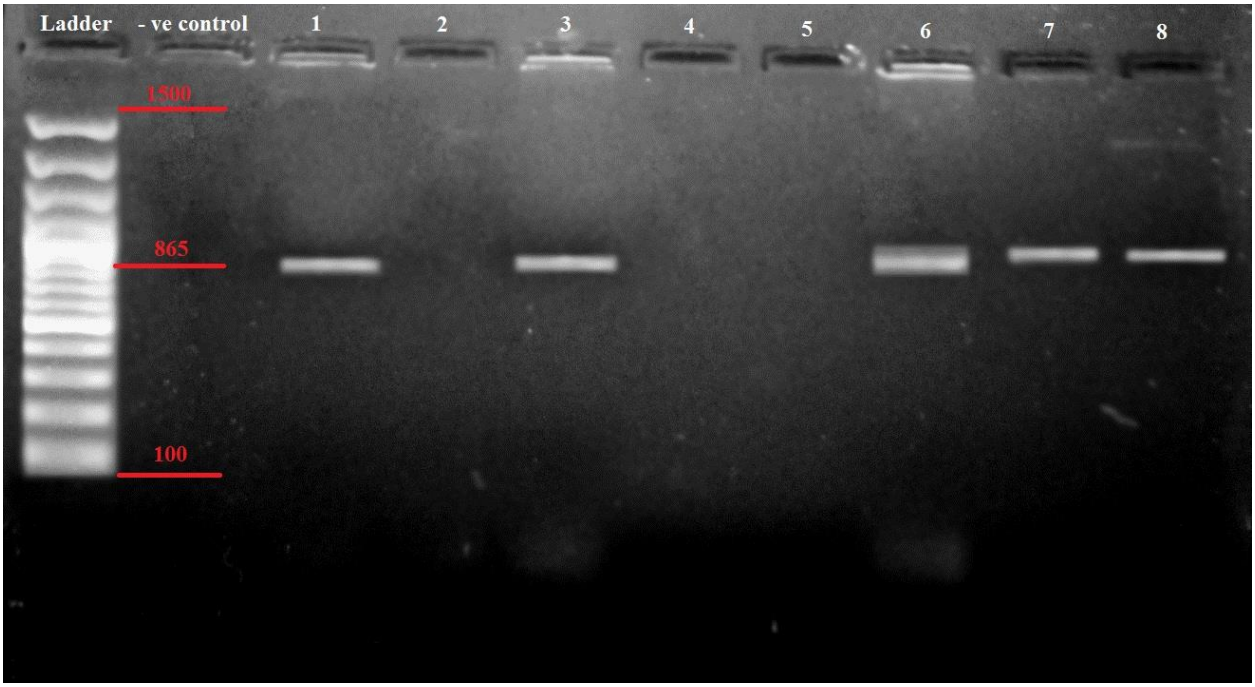


Figure 3.5: Result of gel electrophoresis for the presence of *vim*-2 gene

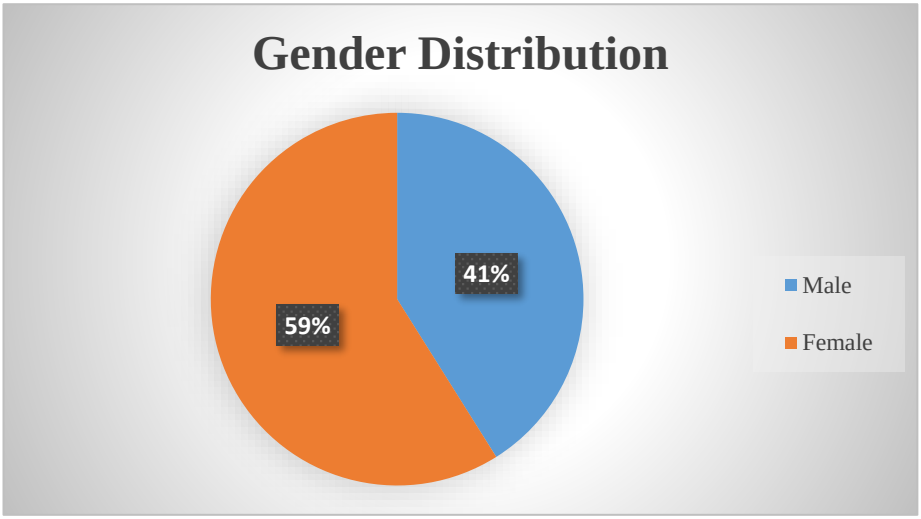
Above is the image of the agarose gel where PCR products were placed under the UV transilluminator. The ladder is placed at the first well and the negative control is placed beside that well. The 8 samples that were used were labeled from 1-8. Except sample 2, 4 and 5 the other 5 gave positive results with corresponding band size of 865 bp.

3.6 Analysis of survey according to the Questionnaire

The statistical analysis was done with the data collected from the survey of the oral cancer patients. It was done to check the gender, age and demographic distribution of the patients that went to the treatment facility in the time being. Along with that, estimation and illustration of the predisposing factors were also analyzed. Apart from that, connection between treatment, progression and duration time were also checked.

3.6.1 Distribution of gender in the study population

Graph 3.6.1: Pie Chart for male and female patients



This graph shows how the gender is distributed among the cancer patient in the study. Majority of the patients were female with a percentage of 59. The percentage of male patient is 41. The difference between two genders is 18%. If analyzed deeply, The Dhaka region had significantly more female patients than other ones.

3.6.2 Distribution of Age among the patients

Graph 3.6.2: Percentage distribution of Age groups of patients

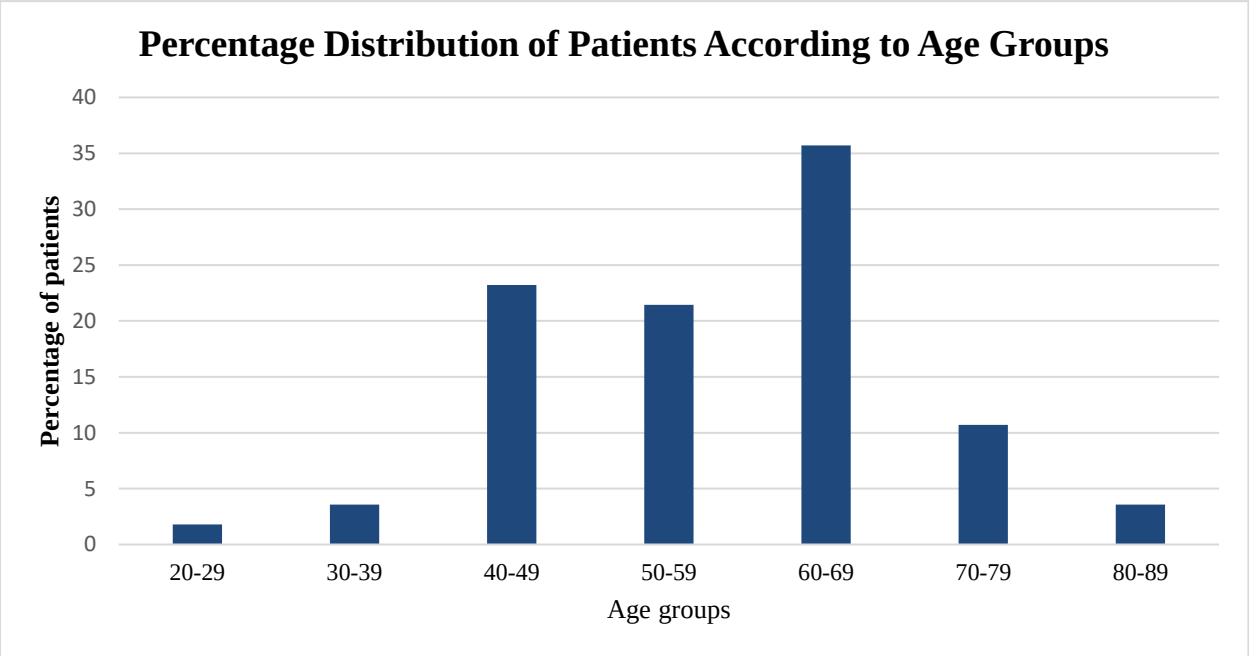


Table3.6.2: Percentage distribution of Age groups of patients

Age group	Percentage of Patients
20-29	1.78
30-39	3.57
40-49	23.21
50-59	21.42
60-69	35.71
70-79	10.71
80-89	3.57

The distribution of oral cancer patients in terms of age is given above. While the graph shows the illustration, the table depicts the values of age distribution. The group with the highest value is for 60-69. The second highest value is for 40-49 years old. On the other hand, the group with the lowest percentage is for 20-29. It can be seen that oral cancer is more prevalent in the group of 40-70. The average age was calculated to be 55.89 years and mode was 60.

3.6.3 Geographical distribution

Graph 3.6.3: Percentage distribution based on location

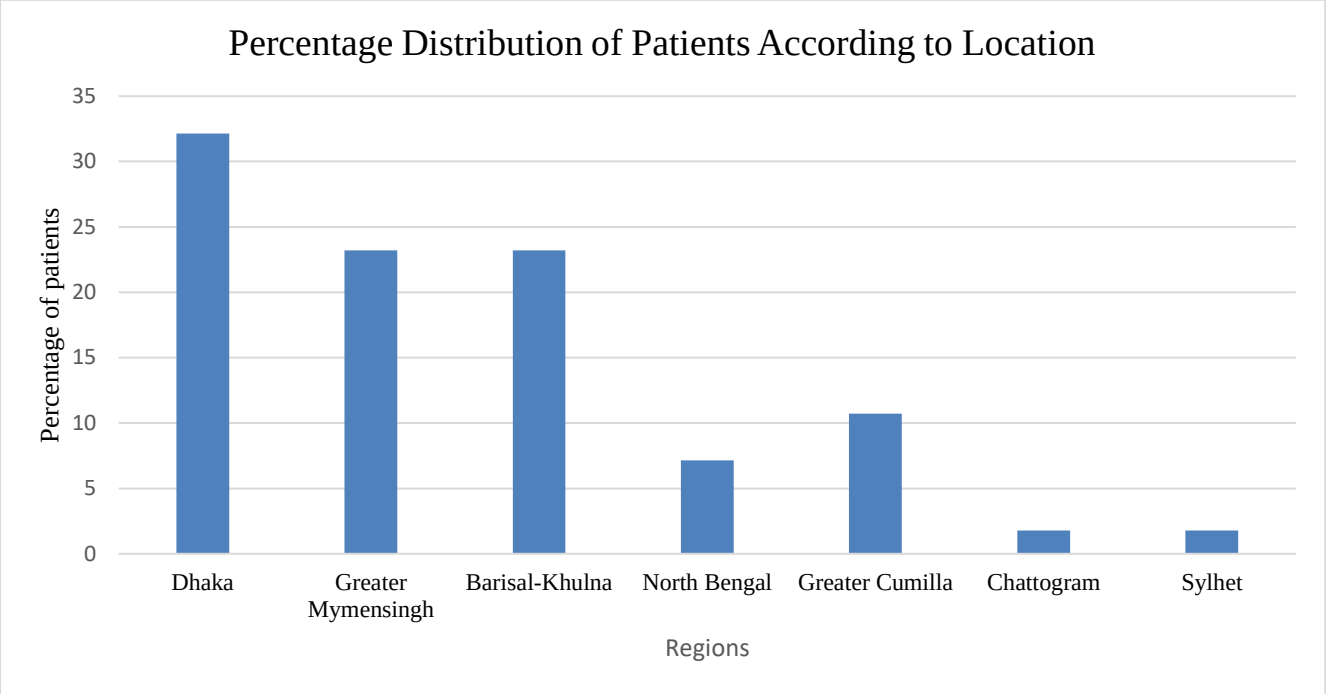


Table3.6.2: Percentage distribution based on location

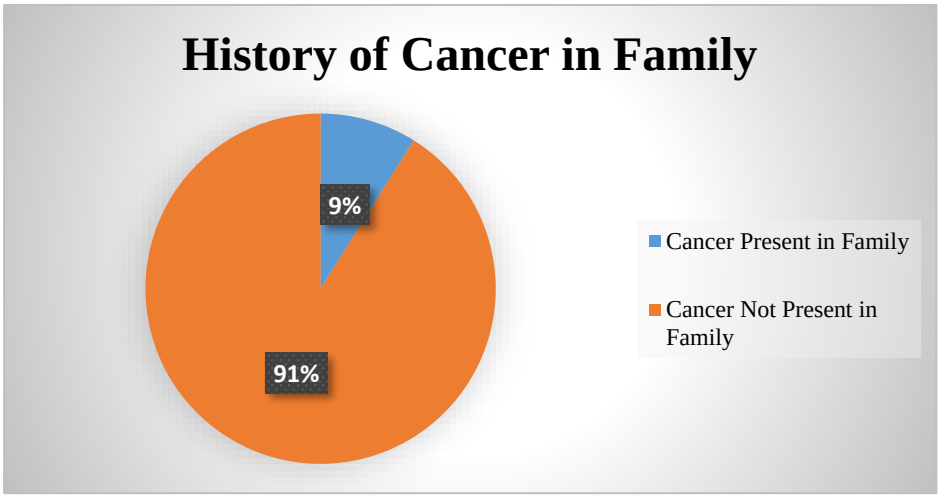
Region	Percentage of Patients
Dhaka	32.14
Greater Mymensingh	23.21
Barisal-Khulna	23.21
North Bengal	7.14
Greater Cumilla	10.71
Chattogram	1.78
Sylhet	1.78

The highest number of the cancer patient from this survey belonged to Dhaka zone. Its nearby Mymensingh zone also has high number. Barisal-Khulna region also had equal percentage of patients. The lowest numbers of patients were from Sylhet and Chattogram equally with a percentage of 1.78.

3.6.5 Presence of cancer in family members

The graph below illustrates how many percent of the patients already had someone in their family with cancer. Only 9% patients had cancer present in the family.

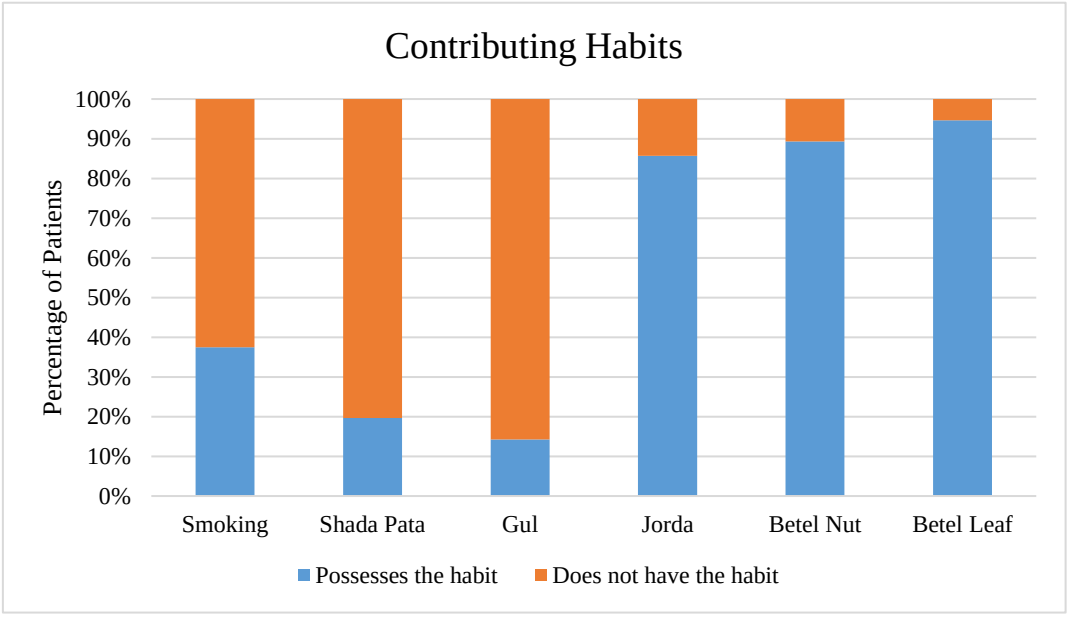
Graph 3.6.4: Pie chart of presence of cancer in the family



3.6.5 Distribution of predisposing factors of oral cancer

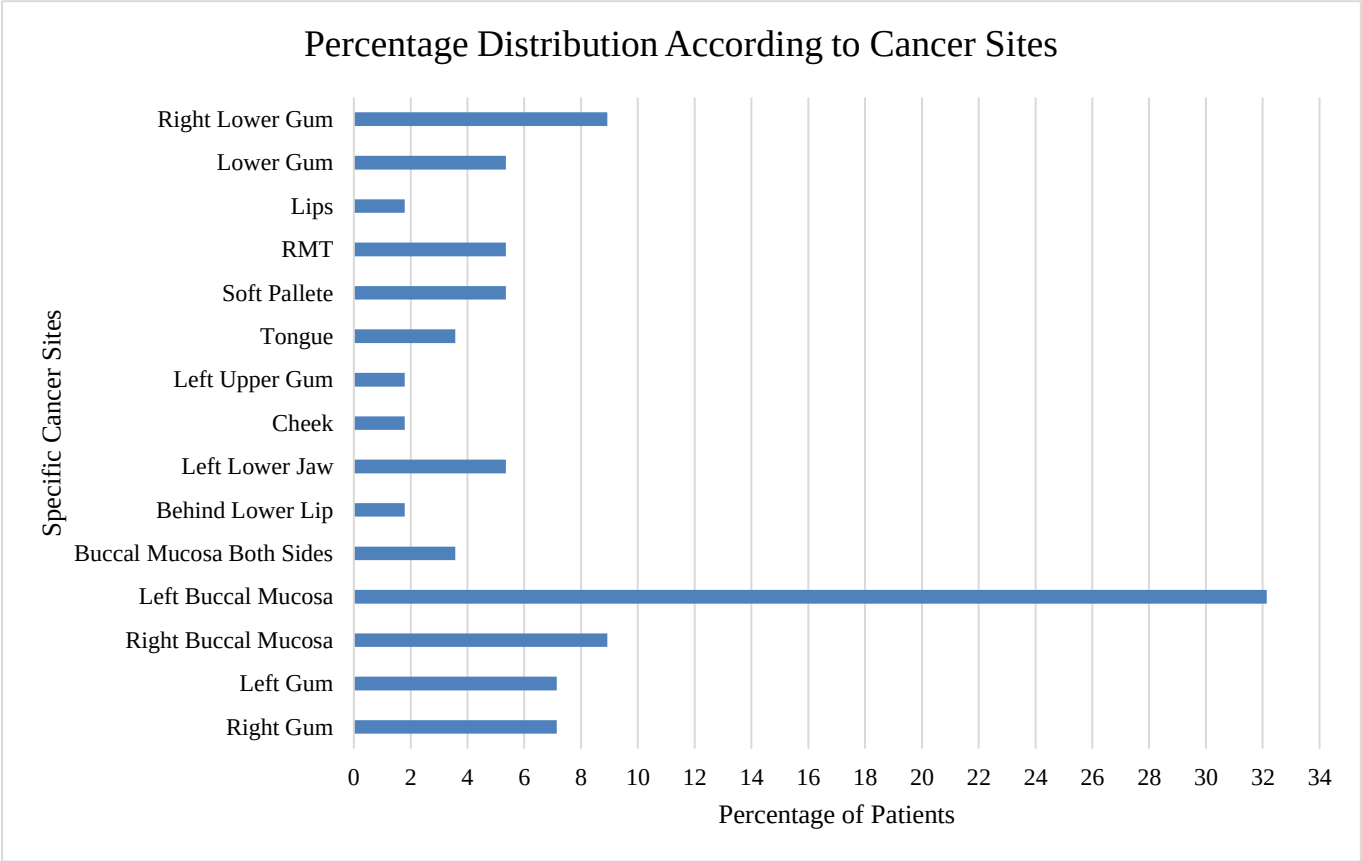
Consumption of nicotine, betel leaf, betel nut and other intoxicant leaves are considered predisposing factors of oral cancer. Here the distribution is given. The most number of patients had regular uptake of betel nut and betel leaf (90%).

Graph 3.6.5: Stacked Column for percentage of user of contributing habits



3.6.6 Distribution of cancer sites

Graph 3.6.6: Percentage distribution based on Cancer sites

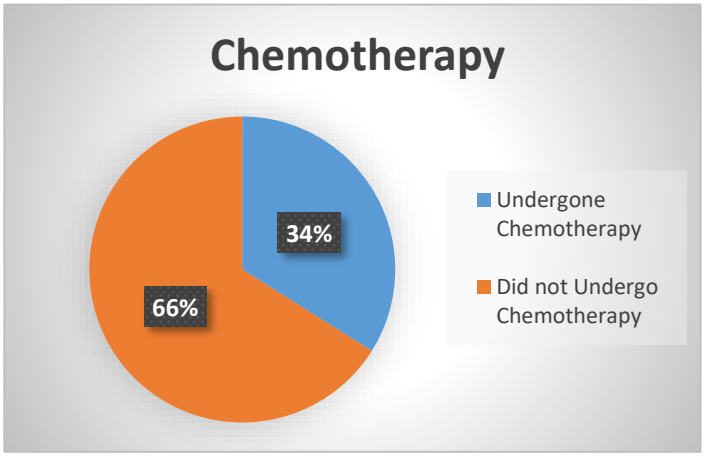


The oral cancer can take place in any part of the oral cavity. It can be even extended to the lymphatic glands in the throat. Here, the distribution is given on the graph. It can be seen that the left buccal mucosa is the most prevalent site with a percentage of more than 32. On the other hand, lips have the lowest percentage of less than 2.

3.6.7 Distribution of treatment procedure

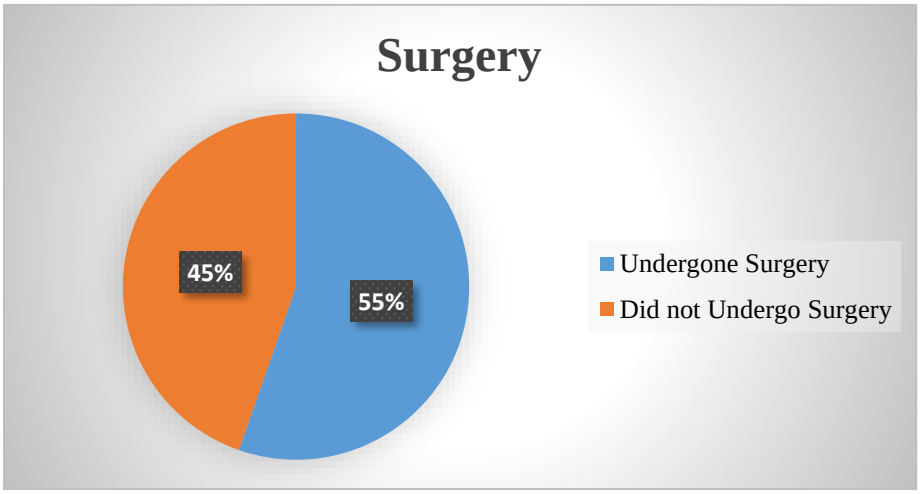
Cancer treatment can have multiple options. It can be treated with surgery or chemotherapy or ever radio therapy. The following consists of the distribution of treatment found in the patients.

Graph 3.6.7.1: Pie diagram of chemotherapy treatment among patients



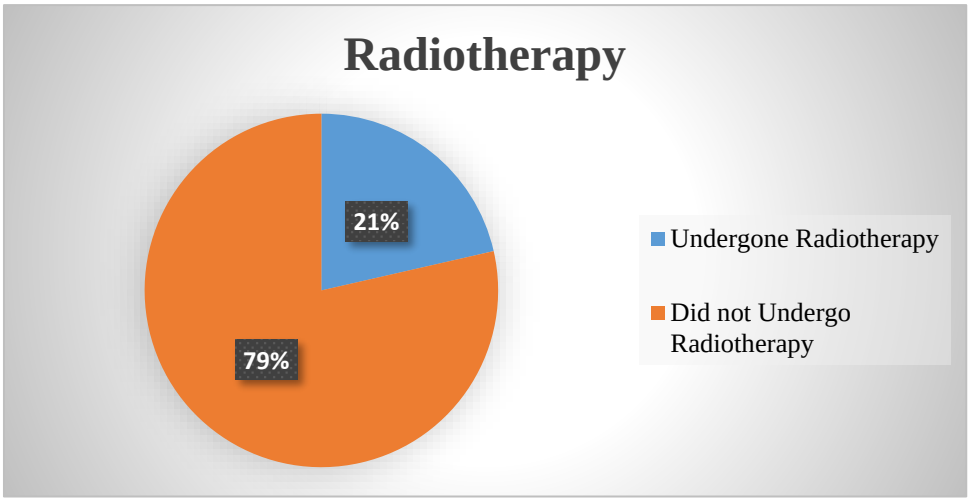
Of all the patients, 34% had to undergo chemotherapy and the rest 66% did not have to.

Graph 3.6.7.2: Pie diagram of surgery treatment among patients



In terms of surgery, 55% of the patients had to undergo it. This is higher than the percentage of chemotherapy treatment done there. It should also be noted that some of the patients have taken all three methods: surgery, chemo and radiotherapies.

Graph 3.6.7.3: Pie diagram of chemotherapy treatment among patient

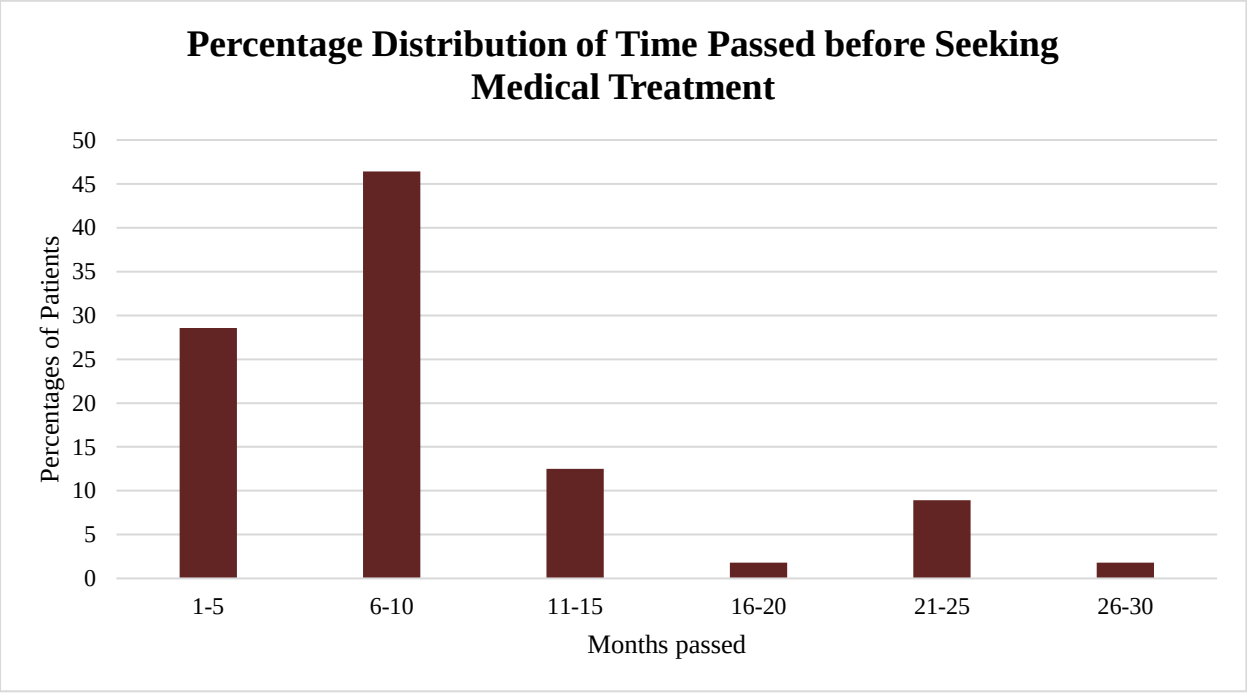


Patients going through radio therapy had the least percentage which was 21. The rest 79% did not have to take radiotherapy at the time being.

3.6.8. Distribution of time duration between the time duration taken to seek treatment

The graph below shows the time duration patients waited before they sought treatment at the study institute. The highest percentage of people had the time gap of 6-10 months. The average was 8.75 months and mode was 6 months.

Graph 3.6.8: Percentage of patients in different time groups showing the frequency distribution of patients



Chapter 4

Discussion

4. Discussion

This study was aimed at isolating gram negative opportunistic bacilli from the ulcerative lesions of oral cancer patients. Oral cancer patients often develop severe ulcer in their cancer sites. These sites then become colonized by many opportunistic bacteria. The purpose of this study was to identify the opportunistic pathogens along with finding the most prevalent one residing on the wound site.

The organisms that were screened in this study were *Pseudomonas* species, *Klebsiella*species, *Escherichia coli* and *Proteus* species. These organisms have been notorious for their opportunistic tendencies. Due to having multiple resistance mechanism against antibiotics, these microbes can become very hard to treat (Peleg *et al*., 2010). Among the isolates,*Pseudomonas* genus was the most prevalent. *P. aeruginosa* have been responsible for many nosocomial infections and a major cause of pneumonia (Gaynes& Edwards, 2005). In this study out of 100 swab samples from cancer patients, *Pseudomonas* had the prevalence of 46%. This is close to the value from another study with *P. aeruginosa* residing in hospital where the percent was found to be 38 (Hidron*et al*., 2008). However, in regards of isolates from postoperative patients, the percentage of *Pseudomonas* was higher. Out of 21 samples, 28 isolates were found, of which 73 percent was *Pseudomonas.* This indicates heavy presence of the bacteria in the hospital vicinity. However, to comment 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.

The next most prevalent organism was *Proteus* species with a percentage of 33. Similar result was seen in a study by Mordi&Momoh (2009), where 26.8% isolates from various wounds throughout the body were *Proteus* species. Although, in this current study, *Proteus* isolates were found only in patients who already had visible infections before treatment in the cancer hospital.

The prevalence of *Proteus* was followed by *Klebsiella*with 20%. In several studies, it was found out that 3%-7% nosocomial infections were due to this species (Horan *et al*., 1988). Moreover, it is one of the main causes of pneumonia. Especially, among immunosuppressed patients such as cancer patients, the rate of pneumonia infection increases (Carpenter, 1990). In the samples from post-operative patients, the percentage of *Klebsiella*isolates was higher than in pre-operative patients. Again, this indicates presence of this microorganism in the

hospital facility. Similar situation was seen in another study done at a public hospital where the prevalence of high antibiotic resistance was seen (Poudyal*et al*, 2011).

In terms of *E. coli*, 14% of the samples belonged to this bacteria. *E. coli* is deemed as one of the most major causes of hospital acquired infections. However, from the cancer patients, a higher percentage was found in pre-operative patients than post-operative patients. Although, presence of this organism in both cases pose serious health risks to the patients since the infections tend to be chronic and the bacteria are resistant to most antibiotics. Moreover, they have the capability to produce extended spectrum beta lactamases that can degrade beta-lactam antibiotics (Livermore &Hawkey, 2005). The presence of *E. coli* and *Proteus* can pose great risk as they are capable of producing extended spectrum beta lactamases which can degrade many major antibiotics (Pitout&Laupland, 2008)

Of the 100 patients that were included in the study, 32 had no visible signs of infection on their cancer site. However, upon inoculation on bacterial isolation media, 9 samples provided positive culture. *E. coli*, *Pseudomonas* species and *Klebsiella*species were found there. *Klebsiella* (9%) had the highest percentage followed by *Pseudomonas* (20%) and the least percentage was *E. coli* (6%). In comparison, 120 swab samples were taken from control group. Over there, the highest percentage was seen in *E. coli* (7%), the second highest being *Pseudomonas* (6%) and the least percentage was *Klebsiella* (3%). The presence of these microbes in control group can be due to any other 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 sourced 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. However, due to several factors that need to be investigated properly, the control group could show the positive results. Nevertheless, the major difference in percentage between prevalence of the bacteria from cancer patients to the bacteria in the control group shows role of immune-suppression being a catalyst in allowing pathogenic microbes to grow (Minah*et al*., 1985). Additionally, radiation therapy can increase the susceptibility to infections in the cancer sites as it increase neutropenia (Bohl, 1991). However, the degree of neutropenia in the cancer patients under this study were not undertaken, therefore, a link between succession of pathogenic microbes in the wound site and the level of neutropenia could not be made.

This study focused on assessing the antibiotic susceptibility pattern of the opportunistic pathogens along with their identification. The antibiotic sensitivity testing was done with 17 antibiotics that are used in the hospitals for isolates from cancer patients and isolates from control group. Patients who are already under power chemotherapy and low immunity need antibiotics with fewer side effects. After the AST, the results showed that all the isolates from cancer patients were resistant to Penicillin group antibiotics, which included ampicillin, amoxicillin and penicillin-G. Similar result was seen for metronidazole and nalidixic acid. The rise of resistance is already evident worldwide (Bud, 2007). The finding from this study corroborated with that.

However, the incident of penicillin resistance is not a new case. Since, 1950s there have been reports of penicillin resistance (Spellberg& Gilbert, 2015). In other reports, resistance to quinolones such as nalidixic acid was also mentioned. There has been a rise in resistance to nalidixic acid in *E. coli* (Gellert, 1977) due to *gyr*mutation. However, not only *E. coli* but also the other species were resistant to nalidixic acid. This could be due to horizontal gene transfer between the microbes residing in the infections. In contrast, in this study the other quinolones such as ciprofloxacin and levofloxacin were highly effective against the isolates found. Similar result was seen in another study where all the isolates were resistant to nalidixic acid and 74.83% were sensitive to ciprofloxacin (Ruiz *et al*., 2002).

In terms of imipenem, 17% isolates were resistant. This is lower in compared to many studies where the resistance level was found up to 57% (Teo*et* al., 2016). However, colistin resistance was seen in 79%. This resistance can occur due to mobile elements like *mcr*genes that are plasmid mediated (Sun *et al*., 2018). Colistin resistance imposes a lethal threat on the patients. In Slovakia, 80% of the infected patients who had colistin resistant gram negative bacilli were dead (Beno*et al*., 2006). This indicates a great threat to the patients.

In contrast, the effective antibiotic group was aminoglycosides, where both amikacin and gentamicin were effective against the isolates. It is to be mentioned that the patients in this study are from poor rural community. Therefore, it can be expected they had lower exposure to antibiotics than urban society. Lack of exposure to antibiotics could result in lesser extent of resistance. According to the patient history majority of patients had no prior incidents of major infections which require antibiotic treatment. They did not have history of typhoid, urinary tract infection or other bacterial infections. This can be considered as them having

lesser antibiotic usage, which in return gave a higher level of susceptibility compared to other tertiary hospital facilities (Arlet*et al*, 1990).

Regarding the antibiotic resistance, 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. Here, the presence of gene *vim*-2 was investigated with the aid of PCR techniques. The forward and reverse primers of this gene were used to amplify template DNA. Positive results showed a band of 865 bp. Among the 8 used isolates, 5 gave positive results. In another study done in immunocompromised patients, metalobeta lactamase gene was prevalent (Chakraborty, 2010). This gene can breakdown many antibiotic groups ranging from cephalosporin, carbapenems and penicillin (Poirel *et al*., 2000). These groups of antibiotics were used in this study to check the susceptibility of the isolates against these.

In terms of the statistical analysis, all the major qualitative factors were subjected to chi square test. Medical history related factors were tested for association with treatment procedures and gender was tested against location, site of cancer, differentiation and time gap before seeking medical help. Area was tested for association with nicotine or tobacco intake habit. Differentiation of cancer cells was tested against treatment methods and site of cancer.

Among the statistical analyses done, only a few were significant. No statistically significant association was observed through chi square test between location and gender, or nicotine intake habit. Only shadapata use was attributed to a P value of .013 which showed that, people of Barisal-Khulna and greater Cumilla used more of it. No significant association was seen between location and site of cancer.

On the other hand, independent samples T test showed significant differences in treatment procedures on the basis of time gap before seeking medical treatment. On average, those who a gap of 10.9 months from showing first sights of oral cancer symptoms had to take surgery as a treatment method but those who came within 6 months did not have to go surgical procedure. Similarly those who took chemotherapy had a delay of 12 months and the others had only 7 months on average. For radio therapy, the test yielded same result that it was statistically significant that, late medical attention is associated with radio therapy. The average time delays for both groups were: 13.4 and 7.4 months.

P values for the three tests are: .033, .029 and .003. Thus the null hypotheses were rejected and the means are statistically different.

Time delay was also different for well, poor and moderately differentiated cancer cells. It was calculated that people with well differentiated cells came to hospital 12 months later on average. For moderate and poor the means were 5.6 and 7.1 month. ANOVA and Post Hoc analyses revealed that the delay of treatment for differentiated cancer cell rendering group was statistically higher (P value .004 and .01). But there were no statistically significant difference of mean between poor and moderately differentiated cell groups.

However, no statistical significance was found in the association of treatment method to differentiation stage. Only radio therapy was associated to well differentiated cell group (p value .041 in fisher's test). No association was found between site of cancer and differentiation stage.

The most common cancer site in the patients was the buccal mucosa. It had a combined percentage of more than 41. Similar outcomes were seen in other studies. In a study with survey, the most common cancer site was buccal mucosa (Rutvij*et al*, 2018). However, in another study, it was seen the most prevalent site was the tongue (Gupta *et al*,2016).

On top of that, no association was found between pain and infection at cancer site. Income only was higher for those who opted for surgery. Other than that no statistically significant associations were seen with chemo or radio therapies. Age did not associate with differentiation of cancer cells or site as well.

Cancer is a mortal disease that takes millions of lives every year. As there is no permanent cure yet, management to reduce the damages is the best solution. Microbes play an intricate role in the progression of a disease. Gram negative bacilli can prolong the infection and complicate the healing process. Antibiotic resistance aggravates the scenario. To mitigate the damage, it is important to assess the degree of resistance. In future, the molecular characteristics should be evaluated. Moreover, plasmid profiling can be done to check presence of acquired plasmids.

Chapter 5

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5. References

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