Identification of the Multi-Drug Resistant Genes in the Bacteria Isolated from the Saliva of Smoker Oral Cancer Patients and Comparative Analysis with Smoker Normal Individuals

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing the degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Abstract

Cancer poses a severe threat to world health in the modern period. Cancer is abnormal cell proliferation that can affect different bodily components. Due to cultural behaviors like tobacco consumption and betel nut chewing, in south Asian countries like Bangladesh, oral cancer is one of the most common types of cancer. Moreover, oral cancer is the 13th most prevalent cancer globally among different types of cancers, and the number of affected people is increasing yearly. In Bangladesh, oral cancer is the second most prevalent cancer in males and the third most prevalent in females. Patients with oral cancer often experience reduced immune systems, further weakened by cancer treatments. As a result, these individuals are at a heightened risk of multi-drug-resistant bacterial colonization and subsequent infection. In this study, microbes were identified and isolated from preoperative oral cancer patients associated with smoking tobacco consumption. Their resistance profile with the standard antibiotics used was determined, and ESBL genes' presence among these microbes was evaluated. To identify the presence of opportunistic organisms, oral swab samples are taken from 40 preoperative oral cancer patients with a significant history of smoking and 40 healthy individuals who were regular smokers. After screening from the patient group, 68 (57.14%) of the organism were gram-negative bacteria, and 51(45.86%) were gram-positive bacteria, where the most prevalent organisms were *Staphylococcus* spp. 27 (22.69%), followed by *Klebsiella* spp. 26 (21.85%), *Pseudomonas* spp. 23 (19.32%), *Streptococcus* spp. 17(14.29%), *Proteus* spp. 13 (10.92%), *Enterococcus* spp. 7(5.88%). The least prevalent was *Escherichia* spp. 6 (5.04%). In the control group, the most prevalent organism was *Staphylococcus* spp 19 (27.94%), and the least prevalent was *Proteus* spp, as no isolates of proteus were found. In the control group, the second highest organisms were

Streptococcus spp 16(23.53%), followed by *Klebsiella* spp 15(22.06%), *Pseudomonas* spp 9 (13.23%), *Escherichia* spp 7 (10.29%) and *Enterococcus* spp 2 (2.94%).

Subsequently, the isolates were all taken for antibiotic sensitivity testing (AST) against antibiotics from 11 different groups used in hospitals. It was observed that gram-positive isolates of the patient group exhibited 100% resistance to antibiotic amoxicillin, cloxacillin, and oxacillin. The gram-negative isolates exhibited 100% resistance to vancomycin, amoxicillin, and penicillin. Even though all the isolates from the patient group showed some percentage of resistance toward the antibiotics, the gram-positive isolates did not show any resistance towards the antibiotic imipenem. Moreover, both gram-positive and gram-negative isolates of the patient group exhibited high resistance to amoxicillin, and the least resistance was seen against amikacin, gentamicin, imipenem, and ciprofloxacin.

In contrast, the microbes of the control group showed less resistance to these antibiotics and showed comparatively higher sensitivity to them. Furthermore, to determine whether or not these organisms have any genes for antibiotic resistance, PCR was done to find six distinct ESBL genes NDM, bla-NDM, SHV, bla-CTX-M, bla-TEM, and bla-IMP. Of the total of these six ESBL genes examined, three genes (NDM, bla-NDM-1, and bla-IMP) were detected in different organisms in different numbers in this study.

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1. Introduction

1.1.Oral Cancer

Oral cancer is referred to as cancer that affects the mouth, tongue, lips, salivary glands, hard palate, and tonsil glands (Sultana and Malik, 2014). In the past few decades, oral cancer has become a major global threat and is considered a significant health concern by the World Health Organization (WHO). Among different types of cancers, oral cancer is the 13th most prevalent cancer in the world. Every year, the number of people affected by oral cancer is staggering. By 2040, the rate of oral cancer is predicted to rise to around 40%, with a subsequent increase in mortality.

Most oral cancers develop on the tissue lining of the mouth and gums, tongue, the base of the tongue, around the throat area, and at the back of the mouth. According to the National Institute of Dental and Craniofacial Research (2023), the prevalence of oral cancer is mainly seen in people over 50, and the prevalence is most common in patients over 65 with an average age of 60. Chewing or smoking tobacco and the consumption of alcohol are linked to around 70-80% of all oral cancers (Chocolatewala, 2010).

Several significant factors encompass the risks of oral cancer, a tumor that affects the mouth and throat tissues. It is worth mentioning that tobacco intake, whether by smoking or using smokeless goods, introduces carcinogenic substances into the body. These compounds can cause genetic changes and cellular damage, ultimately contributing to cancer development. Simultaneously, the excessive and continuous consumption of alcohol irritates oral cells,

increasing their vulnerability to the development of malignancies. (Hooper et al., 2009; Irani, 2020).

1.2. Types of Oral Cancers

Oral cancer comprises a range of diverse forms, each displaying specific characteristics and anatomical sites within the oral cavity and surrounding structures. The primary categories of oral cancer encompass oral squamous cell carcinoma (OSCC), the most predominant form, and develops from the stratified squamous epithelial cells that line the oral mucosa. More than 90% of all oral cancer cases are found to be OSCC (Bagan et al., 2010; Peng et al., 2016). Oral Verrucous carcinoma (OVC), a variation with lower aggressiveness, presents as growths resembling warts and is commonly found in the oral cavity. It constitutes 2-12% of all oral carcinomas, and its 5-year survival rate is estimated at around 50% Adenocarcinoma originates from the salivary glands. In contrast, mucoepidermoid carcinoma is characterized by both glandular and squamous cell elements (Peng et al., 2016).

Moreover, oral cancer can encompass the lips, commonly known as lip cancer, which is frequently linked to extended periods of sun exposure. Additionally, malignancies originating from the tongue, floor of the mouth, palate, gums, and inner lining of the cheeks contributes to the diverse manifestations of oral cancer. The presence of several kinds of oral cancer highlights the intricate nature of this disease, requiring accurate diagnostic evaluation and customized treatment strategies to ensure the most effective patient care.

1.3. Global Situation of Oral Cancer

The prevalence of oral cancer on a global scale is a matter of great concern within the field of public health since it is associated with a substantial impact on morbidity and death rates. Based on recent statistical data, oral cancer is identified as one of the top ten most prevalent types of cancer globally, constituting around 3% of all reported cancer cases. The prevalence of oral cancer exhibits geographical disparities, with elevated rates documented in specific areas, notably Southeast Asia and Central and Eastern Europe. The annual incidence of oral cancer is expected to be over 350,000 cases, with a corresponding mortality rate of around 150,000 deaths (Ghantous & Abu Elnaaj, 2017). Moreover, there are inequalities in gender distribution, with men exhibiting a higher susceptibility compared to women. Men show a 2.6 percent higher chance of developing oral cancer than women (Silverman, 2001). Significantly, risk factors such as the use of tobacco, consumption of alcohol, and infection with human papillomavirus (HPV) remain crucial in the causation of oral cancer. The numbers mentioned above underscore the pressing need for comprehensive prevention measures, timely identification, and efficacious treatment options to alleviate the worldwide ramifications of oral cancer and enhance patient outcomes.

Oral cancer is considered Southeast Asia's fourth most prevalent cancer, with 149,102 newly reported cases in 2018. Oral cancer has the highest prevalence among males in Southeast Asia, with 110,710 newly reported cases, accounting for 11.3% of all cancer cases in the region. While oral cancer is commonly associated with the elderly population, typically affecting individuals between 50 and 70, it is essential to note that children as young as ten can also be diagnosed with this disease (Sarode et al., 2020).

In 2012, a total of 73,860 newly diagnosed cases of oral cancer were recorded among males in Europe, while 25,770 newly diagnosed cases were reported among females. In the United States of America (USA), it has been estimated that there are approximately 47,010 new cases of oral squamous cell carcinoma (OSCC), accounting for approximately 1.2% of the total number of reported malignancies. Oral squamous cell carcinoma (OSCC) generally comprises 3% of all malignant tumors in males and 2% in females (Neville & Day, 2002). On the other hand, the incidence of oral cancer was lowest among the African population, with little over 17,000 patients recorded as confirmed cases of oral cancer in 2012 (Sarode et al., 2020).

1.4. The situation of Oral Cancer in Bangladesh

Oral squamous cell carcinoma (OSCC) is the predominant form of cancer diagnosed in Southeast Asian nations such as Sri Lanka, Pakistan, India, and Bangladesh. It accounts for more than 25% of all newly reported cases of OSCC in these regions (Sarode et al., 2020). Cancer is anticipated to significantly contribute to morbidity and mortality rates in Bangladesh throughout the forthcoming decades. According to projections, the anticipated number of newly diagnosed cancer cases, estimated at 12.7 million, is expected to increase to 21.4 million by 2030. Based on data from the Bangladesh Bureau of Statistics, cancer ranks as the sixth most prevalent cause of mortality. According to the International Agency for Research on Cancer, Bangladesh's predicted cancer-related mortality rates were 7.5% in 2005 and are projected to increase to 13% by 2030 (Hussain & Sullivan, 2013). There are significant discrepancies between male and female patients when it comes to the occurrence of oral cancer

in Bangladesh, which is skewed toward one gender. According to statistical data, men account for roughly 70% of all oral cancer diagnoses, with women making up the remaining 30% (Hussain & Sullivan, 2013). This striking discrepancy highlights a significant gender bias in the nation's incidence of mouth cancer.

Moreover, a report published in 2020 found that the total number of patients suffering from cancer in Bangladesh is about 13 – 15 lakhs, and about 11.9% of all males who have cancer are suffering from oral cancers. In comparison, the percentage of females suffering from oral cancer is only 6.5% of the country's total cancer-affected population (Sah & Akhter, 2020). Even then, oral cancer is the second and third most prevalent cancer in Bangladesh in males and females, respectively (Hussain, 2013; Sah & Akhter, 2020).

1.5. The Risk of Infection in Oral Cancer Patients

The occurrence of oral cancer in individuals presents a variety of heightened infection vulnerabilities, hence exacerbating their overall health condition. Patients with oral cancer, whether due to the disease itself or the treatments administered, experience impaired immune systems, rendering them vulnerable to opportunistic infections. Surgical operations and invasive therapies can compromise mucosal barrier integrity, facilitating microbial entry and colonization (Yusuf et al., 2023). Furthermore, the impairment of salivary gland function and the decline in oral hygiene resulting from pain and functional restrictions might contribute to the proliferation of bacteria, hence heightening the vulnerability to infections (Khajuria & Metgud, 2015). In addition, utilizing immunosuppressive drugs has the potential to further

aggravate these susceptibilities. The complex relationship between oral cancer and infection requires careful surveillance, preventive strategies, and customized interventions to minimize the possible adverse effects of infections on the well-being and treatment results of individuals with oral cancer.

The potential for bacterial infection is a significant issue that warrants attention in the population of individuals with oral cancer, as it has considerable consequences for their general well-being and the effectiveness of their therapy. Patients with oral cancer often experience reduced immune systems, further weakened by cancer treatments. As a result, these individuals are at a heightened risk of bacterial colonization and subsequent infection. It is worth noting that opportunistic microorganisms, such as *Staphylococcus aureu*s and *Streptococcus* species, have been recognized as prevalent causative agents in cases of infection (Khajuria & Metgud, 2015). The complex relationship between the primary disease, its therapies, and bacterial infections highlights the importance of thorough infection monitoring, timely intervention, and customized antimicrobial approaches to minimize the adverse effects of bacterial infections on the health and treatment outcomes of individuals with oral cancer.

Bacterial infections can potentially worsen the patient's general health condition, which may result in a deterioration of their performance status and decreased ability to tolerate intensive treatments. In addition, administering antibiotics to address bacterial infections can intersect with cancer treatments, potentially leading to changes in drug metabolism or disruptions in the effectiveness of therapeutic drugs. In addition, bacterial infections can induce inflammation

and cause tissue damage, hindering the process of wound healing and potentially impacting the results of surgical procedures.

1.6. Opportunistic Microbes in Oral Cancer Patients

The complex interaction between oral cancer and opportunistic microorganisms has been recognized as a crucial component of cancer-associated problems. Individuals diagnosed with oral cancer exhibit a heightened vulnerability to the colonization and subsequent infections caused by opportunistic bacteria. These bacteria exploit the impaired immune system and change the oral environment intrinsic to the disease and its therapeutic interventions. According to Khajuria and Metgud (2015), various species of opportunistic microbes can be found in the oral microbiome of oral cancer patients who are suffering from OSCC, which includes but are not limited to *Streptococcus spp, pseudomonas spp, enterococcus spp, klebsiella spp.* Anaerobic bacteria exhibiting pathogenic characteristics, including *Actinomyces, Clostridium, Fusobacterium, Prevotella, and Porphyromonas*, were observed to be responsible for the formation of biofilms on the surface of the tumor.

Conversely, these bacteria were only sporadically detected on the healthy mucosal surface of the same patient. Several of these bacteria were found to be present in the microbiome of both cancer and healthy patients. However, *klebsiella pneumoniae* among gram-negative bacteria and *Enterococcus faecalis* were more commonly found in tumor sites and almost rare in the case of the healthy population.

1.6.1. *Klebsiella* spp:

Klebsiella spp are a notable category of opportunistic bacteria that have received substantial recognition in infectious illnesses. *Klebsiella spp*., members of the *Enterobacteriaceae* family, exhibit a wide array of bacterial species, with *Klebsiella pneumoniae* being the most significant and intensively researched member in clinical settings (Podschun & Ullmann, 1998). These gram-negative bacilli are widespread in several environmental sources, such as soil, water, and vegetation. Additionally, they constitute a component of the typical human microbiota, primarily inhabiting the gastrointestinal system. Nevertheless, *Klebsiella* species can transition into highly virulent pathogens, taking advantage of compromised immune systems and weakened protective barriers to initiate infections of varying severity. It is worth mentioning that they are widely recognized for their ability to induce a range of diseases, both within healthcare settings (nosocomial) and in the community. These infections include but are not limited to urinary tract infections, pneumonia, bloodstream infections, and surgical site infections. Furthermore, the presence of innate and acquired resistance mechanisms in these organisms, namely against specific categories of antibiotics such as beta-lactams, has given rise to apprehensions regarding the limited choices available for efficacious therapeutic interventions. In addition, it should be noted that some variants of *Klebsiella pneumoniae* have gained notoriety due to their capacity to generate extended-spectrum beta-lactamases (ESBLs) and carbapenemases, hence exacerbating the escalating worldwide predicament of antibiotic resistance (Pitout et al., 2004).

1.6.2. *Pseudomonas spp:*

Pseudomonas spp are a varied and medically important category of gram-negative bacteria. It is commonly found in several ecological habitats. *Pseudomonas aeruginosa* is a highly significant and adaptable opportunistic pathogen that distinguishes itself from other species. The pathogen is widely acknowledged for its notable propensity to induce a diverse range of illnesses, varying in severity, with a specific predilection for immunocompromised persons. *Pseudomonas aeruginosa* is a prominent etiological agent responsible for nosocomial infections, encompassing surgical site infections, urinary tract infections, and bloodstream infections (Tortora, 1982).

1.6.3. *Streptococcus spp*:

The *Streptococcus* species encompasses a wide range of gram-positive bacteria with significant clinical importance. The bacteria exhibit a distinct morphology, with either spherical or ovoid cells. They are widely distributed across several habitats, including the human body (Tortora, 1982). *Streptococcus pneumoniae, Streptococcus pyogenes,* and *Streptococcus agalactiae* are noteworthy representatives of the *Streptococcus* genus, as they have been extensively researched owing to their unique pathogenic characteristics. *Streptococcus pneumoniae*, commonly known as pneumococcus, is a prominent etiological agent causing bacterial pneumonia, meningitis, and otitis media, exhibiting localized and invasive presentations. *Streptococcus pyogenes*, commonly referred to as Group A Streptococcus (GAS), is well-known for its ability to induce many infections, including

strep throat and impetigo, as well as more invasive severe conditions, including necrotizing fasciitis and streptococcal toxic shock syndrome. *Streptococcus agalactiae*, commonly referred to as Group B *Streptococcus* (GBS), is a significant etiological agent of newborn sepsis and meningitis, in addition to infections occurring in pregnant women and those with impaired immune systems (Krzyściak et al., 2013).

1.6.4. *Staphylococcus spp:*

Staphylococcus spp are a diverse group of gram-positive bacteria. Among these species, *Staphylococcus aureus*, which has been intensively researched, is particularly notable as a versatile pathogen with a wide range of virulence (Tortora, 1982). This pathogen significantly contributes to community-acquired and hospital-acquired infections, encompassing a broad spectrum of manifestations, ranging from bare skin and soft tissue infections to more invasive severe conditions like bacteremia, endocarditis, and osteomyelitis. *Staphylococcus epidermidis*, despite being commonly considered a commensal organism, has gained recognition as a notable opportunistic pathogen linked to infections related to medical devices, particularly in those with impaired immune systems or those who have undergone surgical procedures (Vuong & Otto, 2002).

1.6.5. *Enterococcus spp:*

The group of gram-positive bacteria known as *Enterococcus* spp. is of great therapeutic significance. These bacteria exhibit a wide array of species, showcasing distinct attributes

that differentiate them as symbiotic inhabitants of the human gastrointestinal system and potent pathogens that take advantage of favorable conditions. *Enterococcus faecalis* and *Enterococcus faecium* are considered the most clinically relevant species within the *Enterococcus* genus. *Enterococcus* species have traditionally been regarded as commensal organisms, although their ability to cause infections has become more apparent, particularly in healthcare-associated environments. The bacteria in question demonstrate a remarkable ability to flourish in hostile surroundings and withstand antimicrobial substances. Certain strains have even developed resistance mechanisms that pose challenges to therapeutic interventions, such as the emergence of vancomycin-resistant Enterococcus (VRE) (Vu & Carvalho, 2011).

1.6.6. *Proteus spp:*

Proteus spp. refers to a collection of gram-negative bacteria. These bacteria are distinguished by their capacity for movement and ability to establish colonies exhibiting swarming behavior (Tortora, 1982). They comprise multiple species, with *Proteus mirabilis* and *Proteus vulgaris* being the most notable among them. *Proteus* species have been associated with wound infections, infections of the respiratory system, and cases of bacteremia. The difficulty in controlling proteus infections is underscored by their notable ability to develop resistance mechanisms, such as extended-spectrum beta-lactamases (ESBLs) and carbapenemases (Ojdana et al., 2014).

1.6.7. *Escherichia coli:*

Escherichia coli, a prominent and thoroughly researched member of the *Enterobacteriaceae* family, possesses significant importance in the realm of infectious diseases. The bacterium is a gram-negative, rod-shaped microorganism widely distributed throughout the gastrointestinal system of humans (Tortora, 1982). It is notable for its ability to exist as both a harmless commensal and a highly virulent pathogen. *Escherichia coli* consists of a wide range of strains, each exhibiting unique virulence profiles and capabilities. While numerous strains play crucial roles in nutrient metabolism and maintaining gut equilibrium, specific pathogenic variants can induce a broad range of illnesses. One example of a pathogenic strain of *Escherichia coli* (*E. coli*) is *enterohemorrhagic E. coli* (EHEC), which is well-known for its connection to outbreaks of foodborne illnesses and the development of hemolytic uremic syndrome. Another strain, *uropathogenic E. coli* (UPEC), primarily contributes to urinary tract infections. Moreover, it should be noted that *extraintestinal pathogenic Escherichia coli* (ExPEC) strains can potentially cause infections that extend beyond the confines of the gastrointestinal system, giving rise to various medical disorders, including sepsis and meningitis. In addition, the occurrence of *E. coli* bacteria that are resistant to antibiotics, frequently carrying extended-spectrum beta-lactamases (ESBLs) and carbapenemases, has presented significant difficulties in clinical treatment (Kaper et al., 2004).

1.7. Oral Microflora in Healthy Population

The composition of microorganisms in the oral cavity of a healthy population is a multifaceted and ever-changing ecosystem. It consists of various microorganisms that preserve dental health and general physical and mental state. The complex microbial community predominantly occupies diverse oral surfaces, such as the teeth, gingiva, tongue, and mucosal epithelium. The oral microflora mainly comprises bacterial phyla such as *Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria*. Commensal bacteria, including *Streptococcus mitis, Streptococcus oralis,* and *Streptococcus sanguinis*, play a role in the production of biofilms on dental surfaces and facilitate the earliest phases of colonization (Sharma et al., 2018).

Prominent members encompass *Neisseria spp., Haemophilus spp., and Veillonella spp*., collectively contributing to the intricate and balanced microbial ecosystem. These bacteria engage in several tasks, including food metabolism, acid buffering, and avoiding colonization by potentially harmful species (Yamashita & Takeshita, 2017).

The presence of opportunistic microflora within the mouth cavity of individuals who are in good health is a complex and intricate component of oral microbial ecology. Although the oral microbiota often coexists in a commensal relationship, specific individuals within this microbial community have the inherent ability to transition into opportunistic pathogens under appropriate circumstances. *Fusobacterium nucleatum*, which is typically regarded as a commensal organism, has the ability to engage in polymicrobial interactions that contribute to the production of biofilms and the development of oral illnesses. In addition, *Enterococcus*

faecalis, commonly found in the gastrointestinal tract, can establish colonies in oral sites and impact oral health outcomes. *Candida* species are known for their capacity to induce oral candidiasis under conditions of compromised host immunity. The oral microbiome's dynamic and diverse nature is highlighted by opportunistic microflora, which necessitates more investigation to understand the processes that regulate the shift from commensalism to pathogenicity (Sharma et al., 2018).

1.8. Common Antibiotics Used for Treating Oral Cancer

Cancer patients frequently demonstrate heightened vulnerability to infections due to many causes, encompassing reduced immune functionality, invasive medical interventions, and extended periods of hospitalization. Antibiotics are significant in this framework since they effectively accomplish multiple crucial objectives. Firstly, preventative strategies are employed to avoid infections, particularly in surgical operations and neutropenic phases generated by chemotherapy. Prophylactic antibiotics reduce the likelihood of bacterial colonization and infections that follow, preventing potential interruptions in treatment and enhancing patient outcomes. Furthermore, antibiotics are utilized in a therapeutic manner to address existing infections, encompassing localized wound infections and systemic sepsis. The prudent utilization of antibiotics, guided by the identification and susceptibility testing of microorganisms, also tackles the increasing issue of antibiotic resistance, guaranteeing suitable drugs to effectively combat the infecting pathogens.

The management of oral cancer frequently requires the utilization of antibiotics in order to address infections, mitigate potential consequences, and uphold the overall health and well-being of patients. Multiple categories of antibiotics are often utilized, each exhibiting unique modes of action and range of effectiveness.

Beta-lactam antibiotics

Beta-lactam antibiotics are a class of antibiotics that contain a beta-lactam ring in their chemical structure. This class of antibiotics includes penicillins, cephalosporins, and carbapenems. The bactericidal effect of these substances is achieved through the inhibition of bacterial cell wall production, which occurs through binding to penicillin-binding proteins. Penicillins, such as ampicillin and amoxicillin, are frequently employed in the medical field to selectively combat a broad spectrum of gram-positive and certain gram-negative bacteria. Cephalosporins, such as ceftriaxone, exhibit a broad spectrum of antimicrobial activity and demonstrate efficacy against numerous gram-negative microorganisms. Carbapenems, exemplified by imipenem, provide a wide range of effectiveness against gram-positive and gram-negative bacteria, encompassing strains that have developed resistance to alternative antibiotics (Oates et al., 1988).

Macrolide

Macrolide antibiotics are a class of antimicrobial agents widely used in clinical practice. Macrolides, such as erythromycin and clarithromycin, inhibit bacterial protein synthesis by

their binding affinity to the 50S ribosomal subunit. Narrow-spectrum antibiotics exhibit notable efficacy against gram-positive bacteria and are frequently employed in treating infections attributed to *Streptococcus spp.* and *Staphylococcus aureus* (Gaynor & Mankin, 2003).

Fluoroquinolone

Fluoroquinolone antibiotics are a class of antimicrobial agents. Fluoroquinolones, such as ciprofloxacin and levofloxacin, inhibit bacterial DNA synthesis by selectively targeting DNA gyrase and topoisomerase IV enzymes (Paton & Reeves, 1988). These compounds demonstrate a wide range of effectiveness against gram-negative and gram-positive bacteria, making them a commonly employed treatment option for infections that can be managed with oral antibiotic therapy.

Glycopeptide

Glycopeptide antibiotics are a class of antimicrobial agents. Vancomycin, a glycopeptide compound, is an inhibitor of bacterial cell wall formation through its specific binding to the D-alanyl-D-alanine segment of the cell wall precursor (Reynolds, 1989). Frequently employed for managing infections resulting from methicillin-resistant *Staphylococcus aureus* (MRSA) and other bacteria belonging to the gram-positive category (Appelbaum, 2007).

Aminoglycoside

Aminoglycoside antibiotics are a class of antimicrobial agents. Aminoglycosides, such as gentamicin and tobramycin, exert their antibacterial effects by interfering with bacterial protein synthesis through their binding affinity to the 30S ribosomal subunit. Antibiotics with a mostly gram-negative spectrum of activity are frequently employed in treating severe infections, often in conjunction with other antimicrobial agents (Forge & Schacht, 2000).

The determination of appropriate antibiotics for individuals with oral cancer should be informed by various considerations, including the particular bacteria causing the infection, susceptibility patterns, any allergies the patient may have, and the possibility of drug interactions. To optimize treatment outcomes and minimize the burden of infections in this patient population, it is imperative to employ antibiotics judiciously, in conjunction with extensive infection surveillance and multidisciplinary care.

1.9. Objectives

1. This investigation aimed to discover and screen microorganisms in individuals with oral cancer infections and significant smoking history. Subsequently, the obtained data were compared with isolates from a cohort of smokers who exhibited no signs of illness.

2. This study it was investigated the antibiotic resistance profile and identified multi-drug resistant bacteria in light of the increasing prevalence of multi-drug resistant organisms.

3. Identifying the genes responsible for conferring resistance to a diverse range of antibiotic drugs. The discovery of the most efficient antibiotics was conducted to reduce patient suffering.

4. Aimed to conduct a comprehensive survey to estimate the epidemiological, etiological, and socio-economic characteristics of oral cancer patients in Bangladesh. The survey focused on various factors, including the duration of smoking, daily cigarette consumption, cancer duration, and family history of cancer.

2. Materials and Methods

2.1.Study Place:

The laboratory work for research was done in the Biotechnology and Microbiology laboratory of the Mathematics and Natural Sciences Department at BRAC University. The research was done in collaboration with the National Institute of Cancer Research and Hospital, Bangladesh. Data and clinical swab samples were collected from 40 oral cancer patients treated at this hospital.

2.2. Study Duration:

The duration of this research work was from October 2022 to August 2023.

2.3. Study Population:

Swab samples and data were collected from the oral cavity of oral cancer patients treated at the National Institute of Cancer Research and Hospital, Bangladesh. Forty swab samples were thus taken from patients from their cancer sites. However, those who did not have cancer and had a significant smoking history were included in the control group.

2.4. Sample Collection

2.4.1. Bacterial Collection:

The saliva originating from the tumor site within the oral cavity of individuals diagnosed with oral cancer was obtained using sterilized cotton swabs. Subsequently, this sample was

added to a sterilized 0.9% sodium chloride solution and inoculated to nutrient agar (NA) and selective agars using a streaking technique. The agar was placed within the laboratory setting and then incubated at 37 degrees Celsius to facilitate bacterial growth. Subsequent investigations were conducted following the proliferation of microorganisms on nutrient and selective agar media. The control group, which did not exhibit any signs of cancer, underwent a comparable procedure. Once more, in the case of another control group, the sterilized cotton swabs were utilized to gently apply pressure on the gingival region, sublingual area, and buccal mucosa of the individuals who exhibited no signs of cancer.

Figure 2.1: Sterilized cotton swabs used for sample collection.

2.4.2. Data Collection

A survey was also done to check the etiological, demographic, and socio-economic conditions of oral cancer patients in Bangladesh. The consent of the patient and the volunteers were received before the collection of data. Afterward, the participant's signature or thumbprint was taken to ensure their legality of participation in this research. The questionnaire for the survey is given in the Table below:

Table 2.1: Questionnaire for the survey.

Survey Topics	Questions	Answer
1. Particulars of the Participant	i. Name:	
	ii. Age:	
	iii. Sex:	
	iv. Marital Status:	
	v. Occupation:	
	vi. Education:	
	vii. Address:	
	viii. Telephone Number	
2. History		
a. Smoker Oral Cancer Patient	i. Type of cancer	
	ii. Duration of cancer	
	iii. Smoking history	
	iv. Duration of Smoking	
	v. Number of cigarettes per day	
b. Smoker Normal Individual	i. Duration of Smoking	
	ii. Number of cigarettes per day	
3. Medical History		
	i. Prevalence of cancer in the family	

	ii.	Prevalence of any other diseases	
	iii.	Prevalence of mental health disorders	
4. Drug History			
5. Clinical Examination	i.	Site of Cancer	
	ii.	Size of Tumor	
	iii.	Tumor Location	
	iv.	Recommendation	

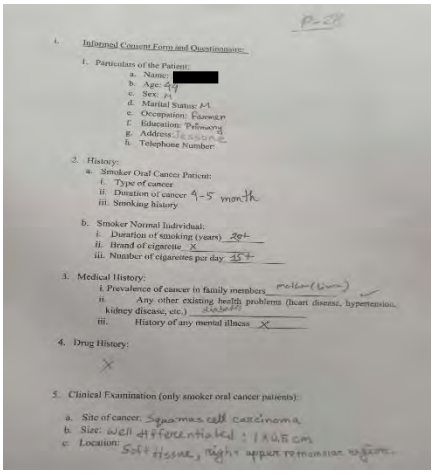
I, _____ (name of patient/subject) _____, voluntarily give my consent to participate in this study and my consent to collect the necessary saliva sample for this purpose.

_____

Signature of the subject/Thumb Print Date of collection: _____

_____

Signature of Sample Collector



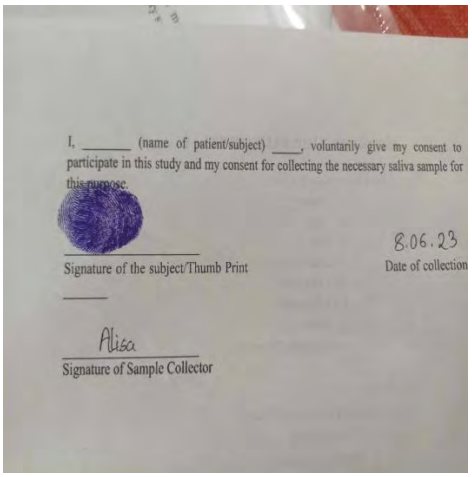
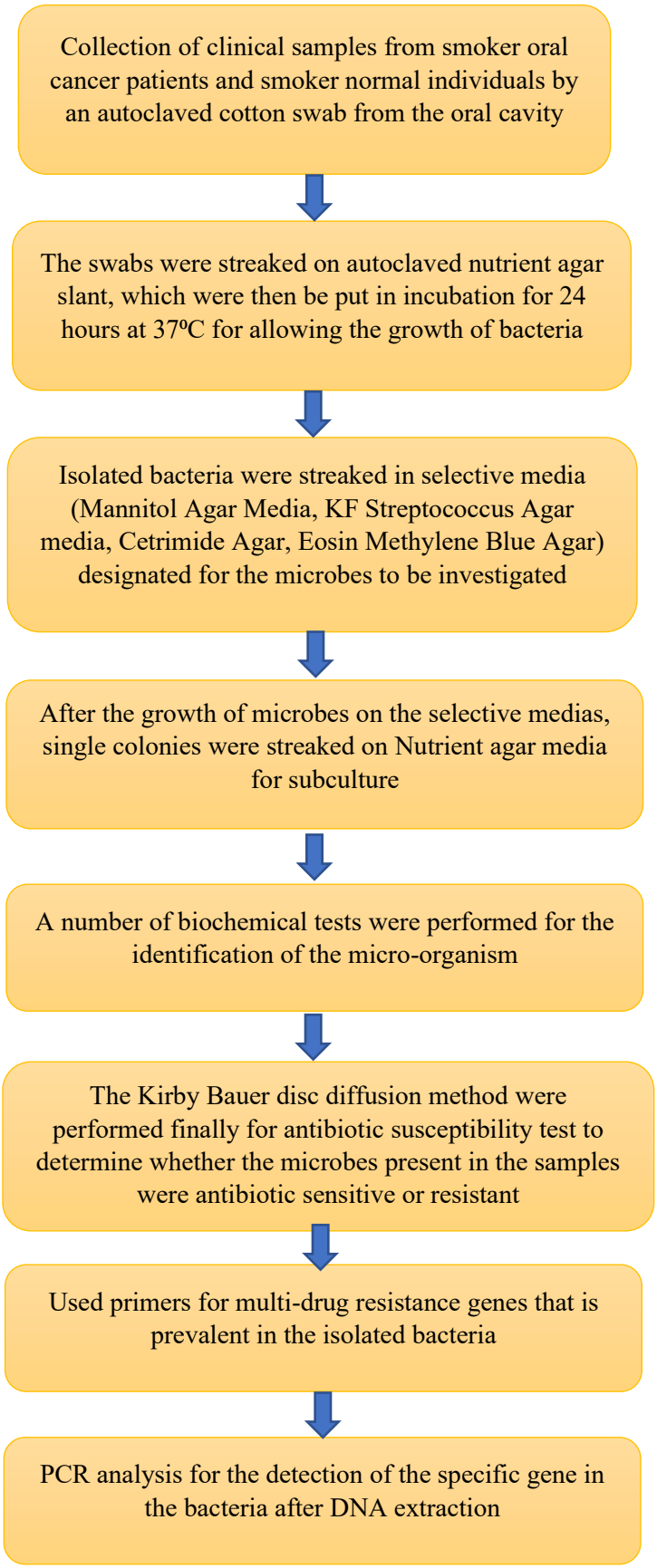


Fig 2.2: Survey Questionaire for Collecting Data

2.5. Experimental Workflow



2.6.Types of equipment:

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

2.7. Culture Media Used for Bacterial Isolation

The strategic implementation of various culture media in bacterial isolation and identification is based on the intricate demands of microbiological analysis. Culture media selection depends on various criteria, such as the particular bacteria under investigation, their specific nutritional requirements, and the intended diagnostic or research goals. Various media formulations are designed to meet the metabolic requirements of different bacterial species, hence supporting their growth and aiding in their isolation.

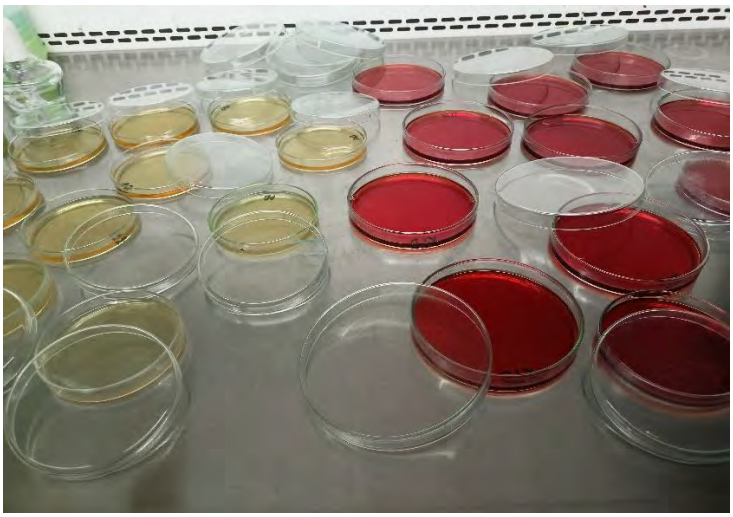


Figure 2.3: Different types of media were prepared.

2.7.1. Nutrient Agar:

Nutrient agar is a culture medium that cultivates various non-fastidious microorganisms. This medium is popular because it supports the growth of diverse bacteria and fungi. The preparation involves the measurement of 28 grams of nutritional agar powder, which was subsequently dissolved in 1 liter of distilled water within a conical flask. Subsequently, the substance underwent the process of boiling. The conical flask was covered with aluminum foil and then placed in the autoclave for sterilization. After sterilization, the lukewarm liquid media was carefully transferred onto a petri dish.

2.7.2. Mannitol Salt Agar (MSA):

Mannitol Salt Agar (MSA) is a selective and differential medium commonly used in microbiology laboratories. It is primarily employed for isolating and identifying

pathogenic *Staphylococcus* species, particularly *Staphylococcus aureus*. The Mannitol Salt Agar is a type of selective media employed to identify and isolate gram-positive bacteria, particularly *Staphylococcus* species, while concurrently inhibiting the growth of other bacterial groups. The substance is comprised of sugar mannitol and the pH indicator phenol red. The organism exhibits the ability to undergo mannitol fermentation, resulting in the production of an acidic byproduct. This byproduct causes a color change in the agar medium containing phenol red, turning it from its original color to yellow. *Staphylococcus aureus* can undergo mannitol fermentation.

During the study, 111.02 grams of MSA powder was solubilized in 1 liter of distilled water within a conical flask. Subsequently, the combination underwent the process of boiling. The conical flask was covered with aluminum foil and then placed in the autoclave for sterilization. After sterilization, the warm liquid media was carefully transferred onto a petri dish. The number provided by the user is 17.

2.7.3. Kenner Fecal (KF) Streptococcus agar media

The KF Streptococcus agar media, specifically KF (Kenner Fecal) Streptococcus Agar, is a selective medium for isolating and identifying fecal streptococci. This medium's nitrogen and carbon source is derived from the enzymatic hydrolysis of animal tissue. Yeast extract is a source of essential vitamins and trace components within the medium. Most fecal streptococci can metabolize maltose and lactose. The presence of Sodium Azide inhibits

the growth of gram-negative bacteria. The presence of acid is identified through Bromocresol blue, shown by a discernible alteration in hue from purple to yellow. The 1% Triphenyltetrazolium Chloride (TTC) supplement leads to colonies exhibiting pink-to-red coloration.

During the research study, KF Streptococcus Agar Media production involved the initial preparation of 0.2N NaOH. The concentration was achieved by dissolving 0.08 g of NaOH in 10 ml of deionized water (dH_2O) within a test tube. The resulting solution was subsequently subjected to sterilization using autoclaving. Following the sterilizing process, a solution of 0.03g of Bromocresol purple was dissolved in 10 ml of NaOH that had been autoclaved. Subsequently, a quantity of 76.4 grams of potassium fluoride powder was obtained. The Streptococcus Agar Media was dissolved in one liter of distilled water. A solution containing 5 ml of Bromocresol purple dissolved in NaOH was introduced to the powder media and afterward subjected to boiling. After heating 10 mL of TTC, it was thoroughly mixed into the heated medium. Subsequently, the tepid liquid was carefully transferred onto the surface of a sterile petri dish.

2.7.4. Eosin Methylene Blue

Eosin methylene blue agar (EMB agar) is a selective media commonly used in laboratory settings to isolate and identify gram-negative bacteria. This particular agar effectively inhibits the growth of gram-positive bacteria, which can be attributed to eosin and

methylene dyes within the medium. In the realm of media, it is seen that bacteria that engage in lactose fermentation exhibit colonies of distinct colors. In contrast, bacteria that do not partake in lactose fermentation display colonies that lack coloration. The presence of *Escherichia coli* in Eosin Methylene Blue (EMB) media results in the development of a green sheen due to its ability to metabolize lactose and subsequently decrease the pH of the media. The pink coloration is observed in other gram-negative bacteria that do not ferment lactose, while colonies of *Aerobacter aerogenes* exhibit a distinctive brown core.

The process of preparing eosin methylene blue agar entails the dissolution of 35.96 grams of powder in 1 liter of distilled water, followed by boiling. After boiling, the flask was hermetically sealed using aluminum foil and subsequently subjected to autoclaving. Subsequently, the substance was transferred onto Petri dishes and employed after solidification.

2.7.5. Cetrimide Agar

Cetrimide Agar is employed in the process of isolating gram-negative *Pseudomonas* bacteria. Pyocyanin production is seen, manifesting a characteristic greenish tint (Leoboffe and Pierce, 2011).

The solution was formulated by combining 46.7 grams of powder with 1 liter of distilled water. Following the dissolution of the powder in water via boiling, the resulting solution

was subsequently covered using aluminum foil and subjected to autoclaving. Subsequently, the liquid medium was transferred into sterile Petri plates previously dried and sterilized.

2.7.6. HiChrome Agar

Hi-Chrome is a selective agar medium commonly used for preliminary identification of bacteria frequently associated with urinary tract infections. The agar medium employed in this study exhibits selectivity towards microorganisms commonly associated with urinary tract infections, *including Klebsiella pneumonia, Enterococcus fecalis, Staphylococcus aureus, Proteus spp, E. coli,* and *Pseudomonas aeruginosa.* These microbes show significant and discernible color variations when grown on this medium. *Escherichia coli* exhibits pink-purple colonies; *Staphylococcus aureus* displays golden-yellow colonies, *Proteus* spp. manifests brown colonies, *Enterococcus faecalis* creates blue colonies; *Klebsiella pneumonia* generates blue mucoid colonies, and *Pseudomonas spp.* yields colorless colonies when cultivated on Hi-Chrome agar.

2.8.Biochemical Test:

A set of biochemical tests were performed to confirm the identification of the bacteria formed in the media. The methods were done according to the microbiology laboratory manual (Cappuccino & Sherman, 2014).

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

2.8.1. Gram staining:

The Gram staining method is employed to differentiate between bacteria that are classified as gram-positive and gram-negative. A small amount of bacteria from a previously cultured sample was applied onto a clean glass slide, followed by gram staining.

2.8.2. Methyl Red (MR) test:

The methyl red test aimed to assess the bacteria's capacity to oxidize glucose, resulting in the generation and stability of a significant concentration of acid end products. A solution of MR-VP broth was made by dissolving 7g of peptone, 5g of dextrose, and 5g of di-potassium hydrogen phosphate in 1 liter of distilled water. The resulting solution was then autoclaved at a pressure of 15 psi and a temperature of 121◦C. Each test tube was filled with 7 ml of the prepared MR-VP broth. Utilizing aseptic methodology, a minute quantity of the experimental bacterium derived from a pure culture cultured for 24 hours was introduced into the tube using an inoculating loop. Subsequently, the tubes were incubated for 24 hours at a temperature of 37◦C. Following a 24-hour incubation period, a volume of 3.5 ml was extracted from the culture tubes and transferred to sterile test tubes for the Voges-Proskauer test. The leftover broth was then subjected to an additional 24-hour incubation period. Following a 48-hour incubation period, the remaining aliquot of the culture tubes was subjected to adding five drops of methyl red indicator. This was done in order to facilitate the prompt observation of a red color development, which serves as an indicative of a positive outcome. (Cappuccino & Sherman, 2014)

2.8.3. Voges Proskauer:

The Voges-Proskauer test enhanced the differentiation between enteric organisms, aiming to identify their ability to produce non-acidic or neutral end products, such as acetyl-methyl-carbinol. After incubating the aliquot of MR-VP broth for 24 hours, a volume of

0.6 ml (equivalent to 12 drops) of a 5% solution of alpha naphthol (Baritte A) was introduced. This was followed by adding 0.2 ml (equivalent to 4 drops) of a 40% potassium hydroxide solution (Baritte B). The tube was subjected to gentle agitation to facilitate the exposure of the medium to atmospheric oxygen for 30 seconds. Subsequently, the medium was left undisturbed for 10 to 15 minutes. The test was conducted within one hour of reagent addition, as McDevitt (2009) stated. The hue pink indicated a positive outcome, whereas the absence of any color change suggested a negative outcome.

2.8.4. Citrate utilization test:

A citrate utilization test was conducted to distinguish between enteric organisms by assessing their capacity to ferment citrate as the sole carbon source through the enzyme citrate permease. Simmons citrate agar slants containing 2 ml were made by subjecting them to autoclaving at a pressure of 15 psi at a temperature of 121◦C. A fraction of the experimental bacteria derived from the 24-hour-old uncontaminated culture was introduced into the vials using a streak inoculation technique employing an inoculating needle. The vials were then incubated for 48 hours at a temperature of 37◦C (Cappuccino & Sherman, 2014). The color blue indicated a positive outcome, whereas the color green indicated a poor outcome.

2.8.5. Catalase test:

The experiment involved conducting a catalase test to assess the bacteria's capacity to break down hydrogen peroxide by producing the enzyme catalase. A tiny slide was inserted into a petri dish. Aseptic techniques were employed to transfer a limited quantity of germs from a 24-hour uncontaminated culture onto a microscope slide. A droplet of hydrogen peroxide (H_2O_2) with a concentration of 3% was carefully applied onto the organism on the microscopic slide using a dropper. The sample was after that examined for the prompt appearance of bubbles, which would indicate beneficial outcomes (Reiner, 2010).

2.8.6. Oxidase test:

The Oxidase test was conducted to ascertain the existence of the enzyme cytochrome oxidase within the bacterial sample. A filter paper was immersed in the Gaby and Hadley oxidase test reagent and allowed to desiccate. A well-isolated colony obtained from a pure 24-hour culture was selected using an inoculating loop. The colony was then transferred onto filter paper and examined for any observable alterations in color. (Shields & Cathcart, 2010).

2.8.7. Triple sugar iron test (TSI):

A triple sugar iron test was performed to distinguish between several families or genera of the Enterobacteriaceae by assessing their capacity to reduce sulfur, ferment carbohydrates,

and generate gas. The Triple sugar iron's base powder was introduced into distilled water, subsequently subjected to boiling, and then transferred into test tubes. Subsequently, the sample underwent autoclaving under conditions of 15 pounds per square inch (psi) at a temperature of 121 degrees Celsius. During sustained warmth, the object was deliberately positioned at an inclined angle, solidifying into oblique formations. A minute quantity of the experimental bacteria derived from the 24-hour-old uncontaminated culture was introduced into the tubes using a stab and streak inoculation technique utilizing an inoculating needle. The screw caps were seen to be inadequately secured, and subsequently, the tubes were subjected to incubation for 24 hours at a temperature of 37◦C. (Cappuccino & Sherman, 2014).

2.8.8. Motility Indole Urease test (MIU):

The MIU test assessed the bacteria's capacity to generate indole, evaluate motility, and digest urea through the enzyme urease. The MIU medium was sterilized using autoclaving at a pressure of 15 pounds per square inch (psi) at 121 degrees Celsius. The media was subjected to a cooling process, reducing its temperature to around 50-55◦C. Following this, aseptically, 100ml of the urea glucose solution was introduced into the base medium, which had a volume of 900 ml. Subsequently, a 6 ml solution was aliquoted into individual sterile test tubes, forming a semi-solid medium. Aseptically, a minute quantity of the experimental bacteria derived from a pure culture cultured for 24 hours was introduced into the tubes by a stab inoculation technique employing an inoculating needle. Subsequently, the tubes were incubated at a temperature of 37◦C for 24 hours. The visual characteristics and

pigmentation of the media were assessed after the incubation period. (Cappuccino and Sherman, 2014).

2.8.9. Indole test:

The experiment involved conducting an indole synthesis test to assess the bacteria's capacity to metabolize the amino acid tryptophan through the enzyme tryptophanase. A 5 ml of Tryptophan broth was made in each test tube using autoclaving at a pressure of 15 psi and a temperature of 121◦C. The experimental bacteria, obtained from a 24-hour-old pure culture, were aseptically implanted into tubes using a loop inoculation method. The tubes were then incubated at a temperature of 37◦C for 48 hours. To assess the presence of indole, Kovac's reagent was introduced into the tubes by adding five drops (MacWilliams, 2009). The color red was indicative of a positive outcome, whereas the color yellow was indicative of a negative outcome.

2.9. Antibiotic resistance and susceptibility analysis

Evaluating the efficacy of antibiotic susceptibility testing for notable bacterial isolates is paramount. This test aims to identify potential medication resistance in prevalent pathogens and confirm their sensitivity to preferred medicines for certain diseases. The Kirby-Bauer disk diffusion method was employed to conduct antimicrobial susceptibility testing on Muller-Hinton agar following the guidelines set out by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial susceptibility pattern was assessed using thirteen specifically

chosen antibiotics sourced from commercial antimicrobial disks. These antibiotics encompass diverse modes of action, such as targeting cell walls, nucleic acids, and proteins. The bacterial suspension was introduced into Mueller Hinton agar plates, and afterward, antibiotic discs were positioned on the culture. Following the incubation period, the antimicrobial effectiveness was assessed by quantifying the diameter of the inhibition zones. Based on the observed diameter of the inhibition zone, bacterial strains were categorized as susceptible (S) or resistant (R).

2.9.1. Preparation of Muller Hinton Agar (MHA)

Muller Hinton agar is widely recognized as a useful medium for antibiotic susceptibility testing. All bacteria that are inoculated into this medium will exhibit growth, as it is a medium that lacks selectivity and differentiation. A solution was prepared by dissolving 38g of Mueller Hinton agar powder in 1 liter of distilled water through boiling and stirring. The conical flask's aperture was covered with aluminum foil and subjected to autoclaving to ensure sterility. After sterilization, the liquid was transferred into Petri plates that had been rendered sterile.

2.9.2. Bacterial Suspension preparation:

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

2.9.3. List of antibiotics:

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

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

Class of antibiotics	Group of Antibiotic(s)	Name of Antibiotic(s)	Disc Code	Disc Potency (µg)	Inhibition Zone Measurements		
					Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Aminoglycosides	**Aminoglycosides**	Gentamicin	GEN	10	12	13-14	15
		Amikacin	AK	30	14	15-16	17
Beta-lactam	**Carbapenems**	Imipenem	IMI	10	13	14-15	16
	Cephalosporins	Ceftazidime	CAZ	30	14	15-17	18
		Ceftriaxone	CTR	30	13	14-20	21
	Penicillin	Amoxicillin	AMX	10	13	14-17	20
		Penicillin-G	P	10	14/28	12/21-21/28	15/19
		Oxacillin	OX	1	10	11-12	13
		Cloxacillin	COX	5	15	16-19	20
	Penicillin combination	Amoxyclav	AMC	10	13	14-17	20
Glycopeptides	**Glycopeptides**	Vancomycin	VA	30	14	15-16	17
Macrolides	**Macrolides**	Erythromycin	E	15	13	14-22	23
		Azithromycin	AZM	15	13	14-17	18
Fluroquinolone	**Quinolones**	Ciprofloxacin	CIP	5	15	16-20	21
Tetracycline	**Tetracycline**	Tetracycline	TE	30	14	15-18	19
Oxazolidinone	**Others**	Linezolid	LZ	30	20	21-22	23

2.9.4. Inoculation and disc diffusion:

A Muller Hinton agar plate was inoculated with an autoclaved cotton swab. The autoclaved cotton swab was immersed in the bacterial suspension mixture. Subsequently, the swab was evenly distributed throughout the Mueller-Hinton agar (MHA) plate's surface to establish a uniform grass culture. Following the streaking procedure, the plate was subjected to a drying period of 5 minutes. Subsequently, the antibiotic discs were carefully positioned on the plate using sterile forceps. The placement of discs was arranged in a manner that ensured the absence of overlap and maintained an evenly distributed area. Following the placement of the discs, the plates were inverted and subjected to incubation at a temperature of 37ºC for 16-18 hours. Following the incubation time, the zones were quantified and then analyzed.

2.10. Molecular Detection

The primary aim of molecular detection in the context of gene identification is to employ sophisticated molecular methodologies to accurately and selectively amplify, visualize, and validate the existence of the target genes of interest within intricate biological materials. The technology described in this study leverages the fundamental concepts of nucleic acid hybridization and polymerase chain reaction (PCR), allowing for the retrieval of genetic information with excellent sensitivity and specificity.

2.10.1. DNA isolation:

Genomic DNA of the selected isolates was extracted via the boiling method. The isolates were streaked in Nutrient agar media for the boiling method and incubated overnight at 37°C. Next, in Eppendorf tubes, 150 microliter TE buffer was taken, and one loopful of the selected Isolate was mixed in the buffer evenly with the help of a vortex machine. Then a thermocycler was used as a heat block where the sample was heated at 95°C for 20 minutes. Then the cell suspension was centrifuged at four °C, 10000 rpm for 10 minutes, leading to the precipitation of the cell debris. After that, the final supernatant was collected, which contained the DNA.

2.10.2. PCR Amplification:

PCR amplification was carried out to verify the genus and species of some isolates*: Streptococcus* spp, *Staphylococcus* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa, and Klebsiella pneumoniae*. Also, the presence of particular resistant genes was determined by performing the polymerase chain reaction. PCR amplification was carried out for the beta-lactamase gene of the family NDM, bla-NDM, bla-CTXm, bla-TEM, bla-IMP, and SHV. The following pairs of primers are used for this PCR amplification.

Table 2.3: The oligonucleotide primers set as forward and reverse for bacterial identification

Name of bacteria	Primer sequence	PCR conditions	No. of cycles	Product size
Streptococcus spp.	Str1-F: 5'-GTACAGTTGCTTCAGGACGTATC-3' Str2- R: 5'-ACGTTCGATTTCATCACGTTG-3'	94°C for 10 min 95°C for 15 sec 60°C for 1min 65°C for 1sec 72°C for 3 min.	40	137bp
Staphylococcus spp.	TStaG422 (F): 5′-GGCCGTGTTGAACGTGGTCAAATCA-3′ TStag765 (R): 5′-TIACCATTTCAGTACCTTCTGGTAA-3′	94°C for 5 min 94°C for 30 sec 55°C for 30sec 72°C for 30sec 72°C for 7 min.	30	370bp
Staphylococcus aureus	Nuc – F: 5'-GCGATTGATGGTGATACGGT-3' Nuc -R: 5'-AGCCAAGCC TTGACGAACTAAAGC-3'	95°C for 5 min 95°C for 1 min 55°C for 45 sec 72°C for 1 min. 72°C for 10 min.	30	279
Pseudomonas aeruginosa.	PA-SS (F): 5'-GGGGGATCTTCGGACCTCA-3' PA-SS (R): 5'-TCCTTAGAGTGCCCACCCG-3'	95°C for 2 min 94°C for 20sec 58°C for 20sec 72°C for 40sec 72°C for 1min	30	956
Klebsiella pneumoniae	KP Pf-F: 5′-ATTTGAAGAGGTTGCAAACGAT-3′ KP Pr1-R: 5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′	94°C for 10 min 94°C for 30sec 60°C for 45 sec 72°C for 45sec 72°C for 10 sec.	30	133

Table 2.4: The oligonucleotide primers set as forward and reverse for antibiotic resistant genes

Gene	Primer sequence	PCR Condition	Number of Cycle	Amplicon Size
NDM	NDM-F: 5'-GGTTTGGCGATCTGGTTTTC-3' NDM-R: 5'-CGGAATGGCTCATCACGATC-3'	95°C for 5 min 94°C for 30 sec 58°C for 30 sec 72°C for 30 sec 72°C for 7 min	36	264bp
bla-NDM	bla-NDM-1- F: 5'ACCGCCTGGACCGATGACCA-3' bla-NDM-1- R: 5'-GCCAAAGTTGGGCGCGGTTG-3'	95°C for 7 min 94°C for 30 sec 58°C for 30 sec 72°C for 30 sec 72°C for 7 min	36	264bp
bla-IMP	bla-IMP-F: 5′-GAAGGCGTTTATGTTCATAC-3′ bla-IMP-R: 5′-GTATGTTTCAAGAGTGATGC-3′	95°C for 5 min 95°C for 45 sec 60°C for 45 sec 72°C for 1 min 72°C for 8 min	35	587bp
bla-CTX-M	bla-CTX-M F: 5'-ACGCTGTTGTTAGGAAGTG-3' bla-CTX-M R: 5'-TTGAGGCTGGGTGAAGT-3'	94°C for 3 min 94°C for 60 sec 58°C for 30 sec 72°C for 60 sec 72°C for 10 min	30	857bp
SHV	SHV(F): 5'-TACCATGAGCGATAACAGCG-3' SHV(R) 5'-GATTTGCTGATTTCGCTCGG-3'	94°C for 3 min 94°C for 60 sec 58°C for 30 sec 72°C for 60 sec 72°C for 10 min	30	450bp
bla-TEM	bla-TEM F: 5' AAAATTCTTGAAGACG-3' bla-TEM R: 5' TTACCAATGCTTAATCA-3'	94°C for 3 min 94°C for 30 sec 50°C for 30 sec 72°C for 2 min 72°C for 10 min	35	980bp

The PCR was carried out on the sample under suitable and different conditions for each gene primer, as given in the Table. Here, 2 µL of DNA template, 7.5 microliters of PCR master mix (Thermofisher), 2.5 microliters of nuclease-free water, 0.5 microliter of forward and 0.5 microliters of reverse primer were used in a total of 13 microliter reaction mixture. The PCR master mix contained an equal amount of dNTP, $MgCl_2$, and Taq polymerase. The PCR products were analyzed by electrophoresis with 1.2 to 1.5% agarose gel concentration. The gel was stained with ethidium-bromide and visualized under a UV transilluminator.

3. Results

The present study examined antimicrobial resistance patterns of 40 bacterial samples from the mouth of oral cancer patients and 40 bacterial samples from the mouth of normal healthy individuals.

3.1. Colony characteristics of bacterial isolates on different agar plates:

Oral swabs were collected from the patients' and control groups and inoculated in the 0.9% sodium chloride. Next, the inoculated saline solution spread plate method was performed on the selective media. Mannitol sugar agar media and KF Streptococcus agar media were used for isolating Gram-positive bacteria, and Eosin methylene blue agar media, Xylose Lysine Deoxycholate (XLD) Agar, MacConkey agar, and Cetrimide agar media were used for isolating Gram-negative bacteria. All 40 samples from oral cancer patients showed growth in the selective media. While among the 40 control groups, 35 showed growth on the selective media selected for the growth of gram-positive and gram-negative opportunistic microbes. The patient groups were labeled P1 to P-40, and the control groups were labeled C1 to C40, respectively. The Table below shows the isolates' appearance and type of growth in the selective media.

Table 3.1: Isolates' appearance and type of growth in the selective media

Selective Media	Colony characteristics	Observed Isolate in the patient group	Observed Isolate in the control group	Total Isolates
MSA	White non-fermenting	16	12	28
	Yellow fermenting	11	7	18
KF Streptococcus Agar media	pink colony with yellow zone	17	16	33
	Pink/red colony	7	2	9
Cetrimide	White	7	4	11
	Green	16	5	21
EMB	Pink/purple mucoid	26	15	41
	Colorless/orangish lush	15	_	13
	Black/ green sheen	6	7	13
XLD Agar	Large, Flat, Yellow colonies	4	5	9
	Yellow colonies	12	_	12
	Mucoid yellow colonies	23	14	37
	Pink, Flat, Rough colonies	20	9	29
MacConkey Agar	Non mucoid red/pink	4	6	10
	Red round	5	1	6
	Pale pink	26	12	38
	Green-brown, fluorescent growth	15	9	24

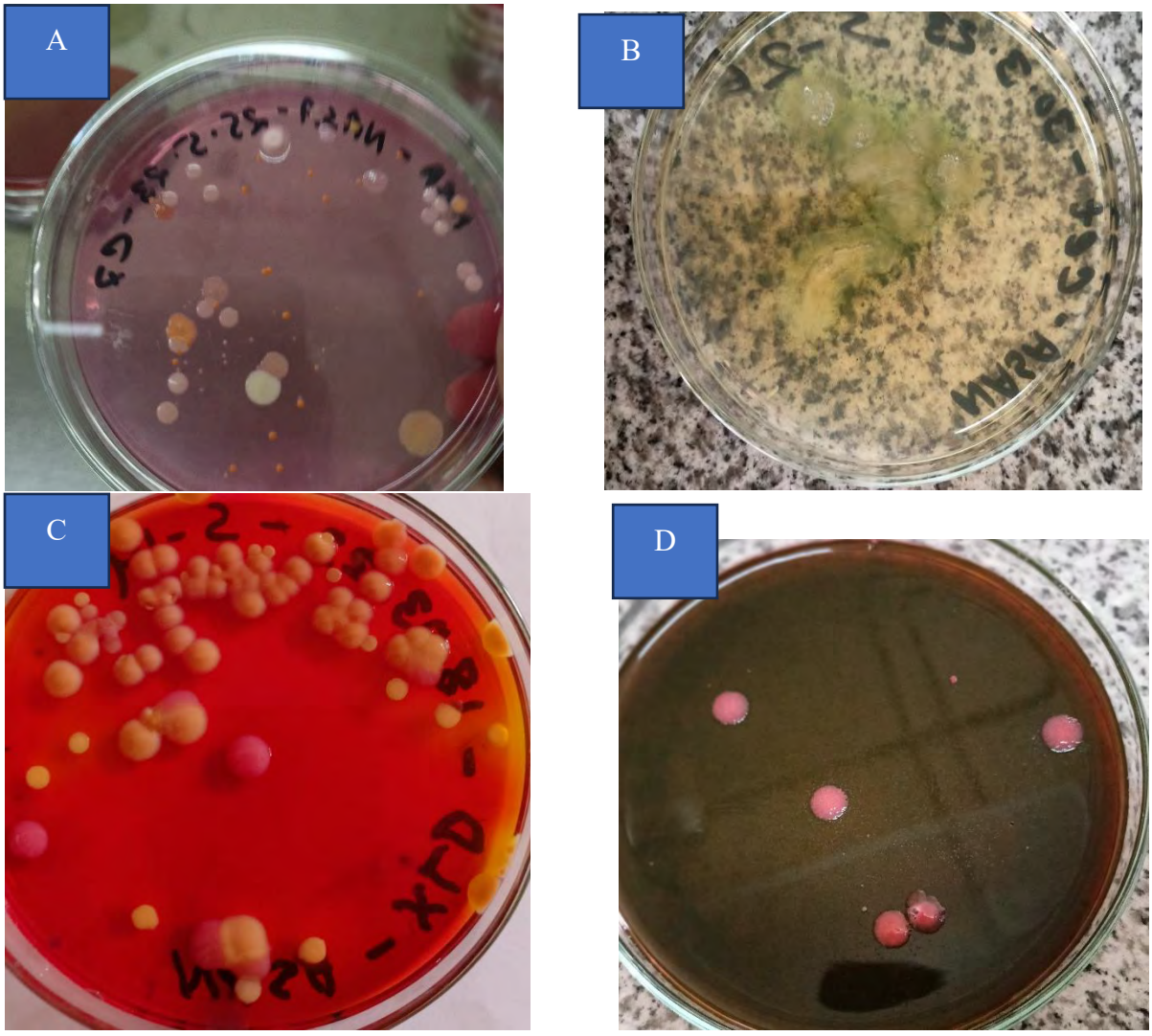


Figure 3.1: Figure A-D shows the colony morphology in selective media MSA, Cetrimide, XLD, And EMB respectively.

3.2. Identification of isolates based on biochemical tests results:

In Tables 3.2 and 3.3, the biochemical test of the found gram-positive and gram-negative isolates of the patient group and control group are shown

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon's citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
1	P1	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
2	P2	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
3	P2	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
4	P4	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	R/Y	+	+	+	+	+	*Streptococcus spp*
5	P5	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
6	P5	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
7	P6	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
8	P7	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	R/Y	+	+	+	-	-	*Staphylococcus spp*
9	P8	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
10	P8	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
11	P9	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
12	P10	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
13	P10	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
14	P11	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
15	P11	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
16	P12	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
17	P13	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
18	P14	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon's citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
19	P15	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
20	P15	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
21	P17	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
22	P18	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
23	P19	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
24	P20	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
25	P20	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
26	P21	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
27	P21	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
28	P22	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
29	P22	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
30	P23	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
31	P23	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
32	P24	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
33	P24	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
34	P25	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
35	P26	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
36	P27	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
37	P29	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
38	P29	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	Streptococcus *spp*
39	P30	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
40	P30	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
41	P31	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
42	P32	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
43	P32	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
44	P34	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
45	P35	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	R/Y	+	+	+	-	-	*Staphylococcus spp*
46	P36	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
47	P37	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
48	P38	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
49	P38	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
50	P39	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
51	P40	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon's citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
1	P1	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
2	P2	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
3	P2	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
4	P3	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
5	P3	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
6	P3	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
7	P3	Cet, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
8	P4	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
9	P4	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
10	P5	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
11	P5	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
12	P6	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
13	P6	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
14	P7	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
15	P8	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
16	P11	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
17	P11	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
18	P11	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
19	P12	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
20	P12	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
21	P12	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
22	P13	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
23	P13	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
24	P12	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
25	P14	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
26	P15	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
27	P15	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
28	P15	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
29	P16	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
30	P17	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon's citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
31	P17	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
32	P17	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
33	P18	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
34	P19	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
35	P19	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
36	P20	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
37	P21	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
38	P21	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
39	P22	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
40	P22	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
41	P22	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
42	P23	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
43	P23	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon's citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
44	P24	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
45	P26	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
46	P26	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
47	P28	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
48	P29	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
49	P29	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
50	P29	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
51	P31	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
52	P32	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
53	P32	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
54	P33	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
55	P33	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
56	P34	Cet, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*

'P'=Patient, '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
57	P35	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
58	P36	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
59	P36	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
60	P37	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
61	P37	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
62	P38	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
63	P38	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
64	P38	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
65	P38	EMB, XLD	Light Brown	Pink	Rod	-	+	+	-	+	+	+	+	R/Y	+	-	-	+	+	*Proteus spp*
66	P39	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
67	P40	Cet, Mac, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
68	P40	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*

'P'=Patient, '+'= positive, '-' = negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
1	C1	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
2	C2	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
3	C2	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
4	C3	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
5	C4	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
6	C5	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
7	C5	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
8	C6	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
9	C7	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
10	C7	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
11	C8	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
12	C8	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
13	C9	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
14	C10	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
15	C10	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*

'C'= Control, '+'= positive, '-' = negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
16	C11	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
17	C12	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
18	C12	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
19	C14	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
20	C14	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
21	C15	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
22	C16	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
23	C17	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
24	C18	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
25	C19	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
26	C19	KF	Blue	Purple	Cocci	-	-	-	-	-	+	+	-	Y/Y	+	+	+	-	-	*Enterococcus spp*
27	C21	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
28	C21	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
29	C23	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*

'C'= Control, '+'= positive, '-' = negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
30	C26	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
31	C28	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
32	C30	KF	No Color	Purple	Cocci	-	-	-	-	-	+	-	+	Y/Y	+	+	+	-	-	*Streptococcus spp*
33	C31	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
34	C33	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
35	C34	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
36	C36	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*
37	C39	MSA	Golden Yellow	Purple	Cocci	-	+	-	-	+	+	+	+	Y/Y	+	+	+	-	-	*Staphylococcus spp*

'C'= Control, '+'= positive, '-' = negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
1	C5	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
2	C5	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
3	C6	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
4	C7	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
5	C8	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
6	C9	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
7	C12	Cet, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
8	C13	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
9	C15	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
10	C16	EMB, XLD, Mac	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
11	C17	EMB, Mac	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
12	C18	EMB, XLD, Mac	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
13	C19	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
14	C19	EMB, XLD, Mac	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*

'C'='Control', '+'= positive, '- '= negative; Y= Yellow, R= Red

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

Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
15	C22	EMB, XLD, Mac	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
16	C22	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
17	C23	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
18	C25	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
19	C27	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
20	C29	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
21	C32	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
22	C32	EMB, Mac	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
23	C34	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
24	C35	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
25	C35	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
26	C37	Cet, XLD	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*

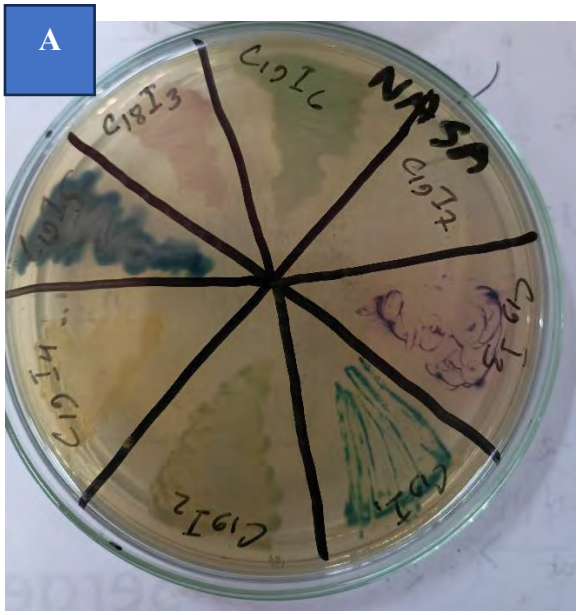
'C'='Control', '+'= positive, '- '= negative; Y= Yellow, R= Red

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

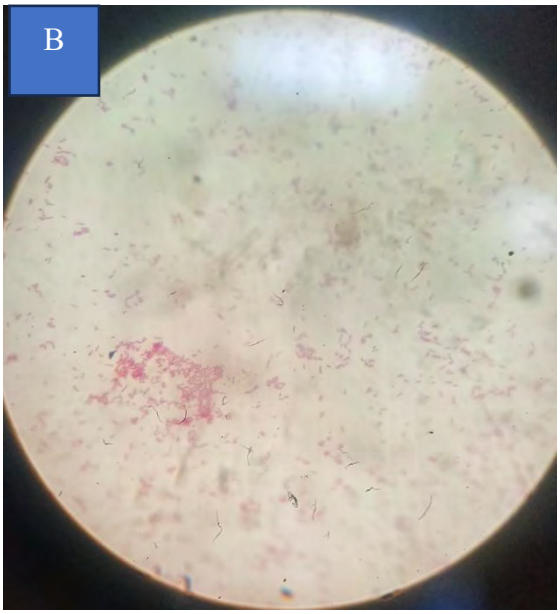
Serial no:	Isolate no:	Media used for isolation	Appearance in HiChrome media	Gram Staining		Oxidase test	Catalase test	MIU			MR VP		Simmon' s citrate	TSI						Probable Organism
				Color	Shape			motility	indole	urease	Methyl Red	Voges Proskauer		slant/ Butt	glucose	lactose	sucrose	H2s production	Gas production	
27	C38	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
28	C38	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*
29	C40	Cet, XLD, Mac	Green	Pink	Rod	+	+	+	-	-	-	-	+	R/R	-	-	-	-	+	*Pseudomonas spp*
30	C40	EMB, XLD	Dark blue	Pink	Rod	-	+	-	-	+	-	+	+	Y/Y	+	+	+	-	+	*Klebsiella spp*
31	C40	EMB, Mac, XLD	Purple	Pink	Rod	-	+	+	+	-	+	-	-	Y/Y	+	+	+	-	+	*Escherichia spp*

'C'='Control', '+'= positive, '- '= negative; Y= Yellow, R= Red

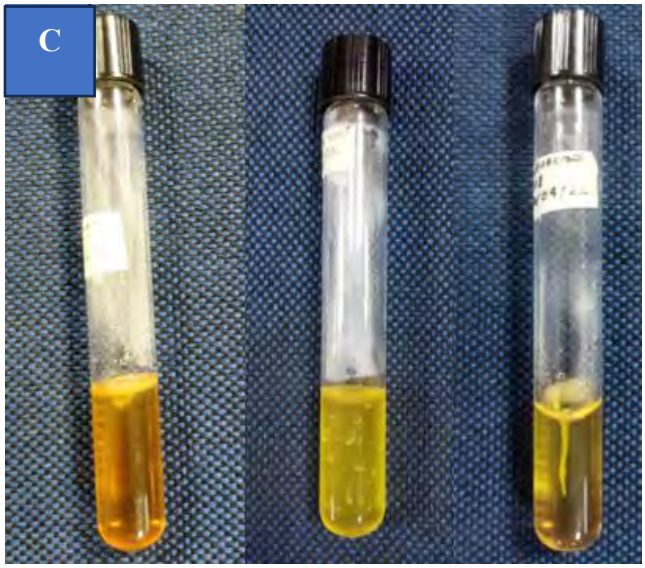
A



B



C



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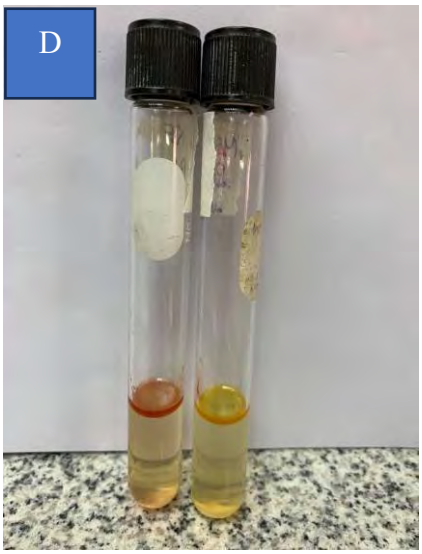

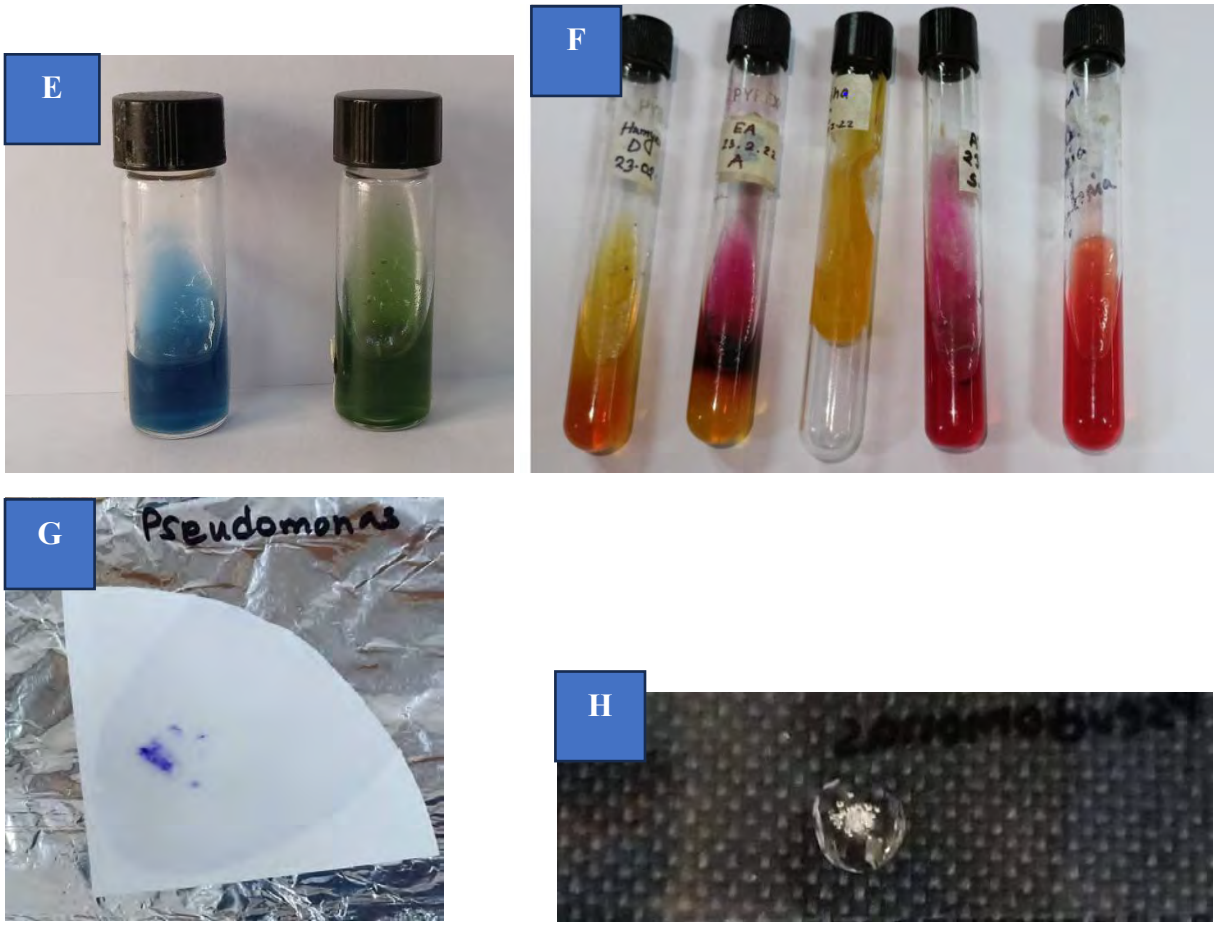

Figure 3.2: [A] isolates in HiChrome media. [B] Microscopic observation of gram-negative bacteria. [C] Motility test.[D] Indole test positive (left) negative (right). [E] Citrate test negative (green) positive (blue). [F] TSI slant. [G] oxidase test (positive). [H] Catalase test (positive).

3.3. Percentage identity of the identified isolates

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

The ratio of selected isolates found is given below.

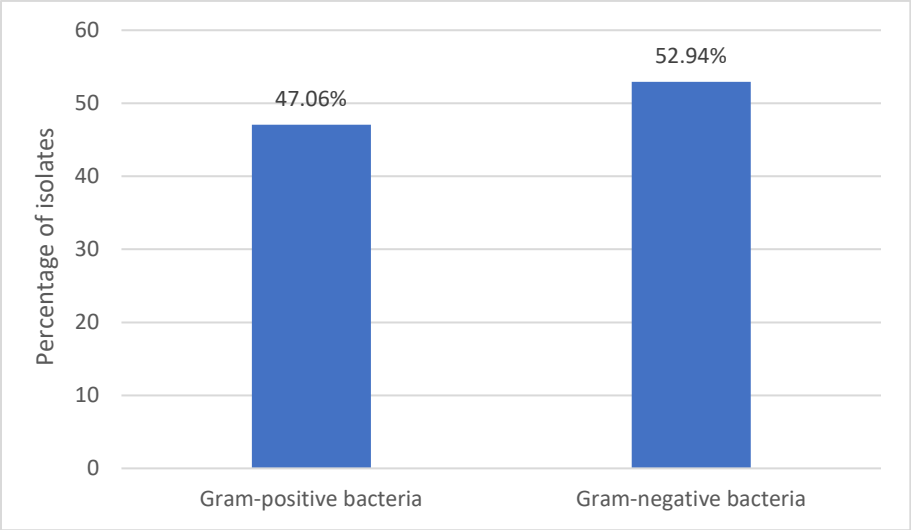

Figure 3.2: Overall Percentage of the Gram-positive and Gram-negative bacteria.

Figure 3.2 represents that the higher percentage of isolates found were gram-negative bacteria which is 52.94% and 47.06% percentage of isolates found were gram-positive bacteria.

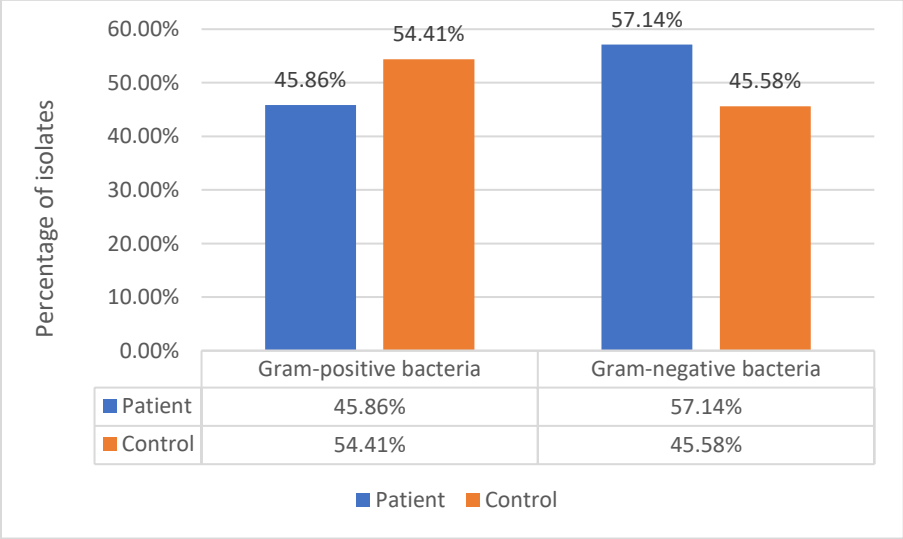

Figure 3.3: Percentage of the Gram-positive and Gram-negative bacteria in the patient and control group.

Figure 3.3 shows the Percentage of Gram-positive and Gram-negative bacteria in the patient and control groups. Here, it is seen that in the patient group, a higher percentage of bacteria are gram-negative bacteria, whereas in the control group, the percentage of gram-positive bacteria is higher.

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

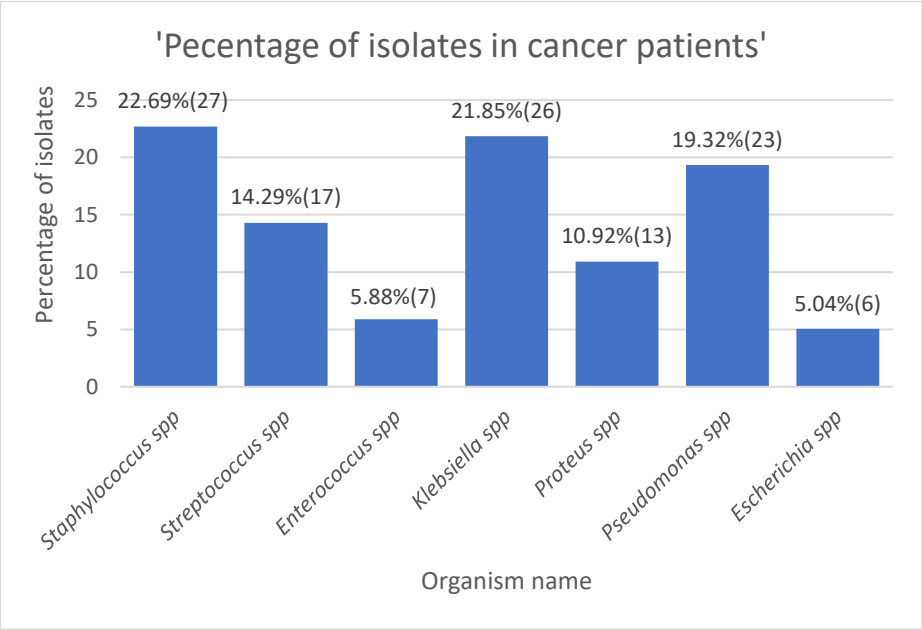

Figure 3.4: Percentage of isolates in cancer patients.

Figure 3.4 shows amongst the cancer patients the most prevalent organisms were *Staphylococcus* spp 27 (22.69%) followed by *Klebsiella spp* 26(21.85%), *Pseudomonas* spp 23 (19.32%), *Streptococcus* spp 17(14.29%), *Proteus* spp 13(10.92%), *Enterococcus* spp 7(5.88%). Here, the least prevalent organism was *Escherichia* spp 6 (5.04%).

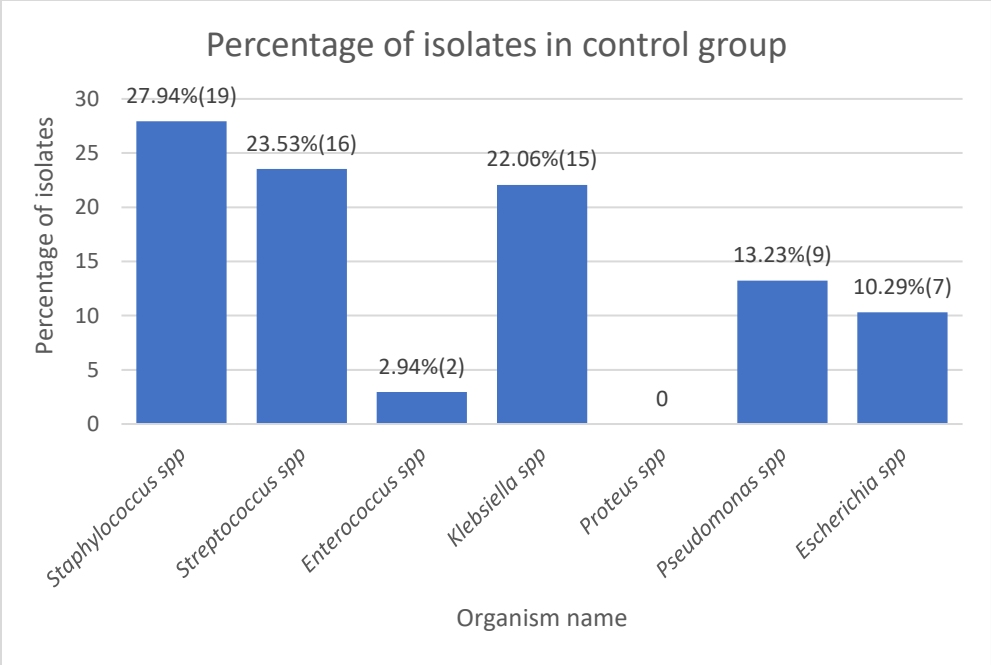

Figure 3.5: Percentage of isolates in control.

Again, figure 3.5 shows in the case of the control group the most prevalent organism was *Staphylococcus* spp 19 (27.94%) and the least prevalent was *Proteus* spp as no isolates of proteus were found. The second highest organism were *Streptococcus* spp 16(23.53%) followed by *Klebsiella* spp 15(22.06%), *Pseudomonas* spp 9(13.23%), *Escherichia* spp 7(10.29%) and *Enterococcus* spp 2(2.94%).

3.4. Antibiotic susceptibility test results:

The antibiotic Sensitivity Test (AST) gives us an idea about an organism's resistivity or sensitivity pattern. This test allows us to choose an effective antibiotic against that organism during an infection. To find out the resistivity pattern, AST was done. One hundred nineteen isolates from the cancer patients and 68 isolates from the control group were tested for antibiotic susceptibility

with antibiotics from 11 different groups. For gram-positive isolates, ten antibiotics used that were Imipenem, Gentamicin, Amikacin, Ceftazidime, Erythromycin, Amoxicillin, Cloxacillin, Oxacillin, Ciprofloxacin, and Linezolid. Next, for gram-negative isolates, 11 different antibiotics- Imipenem, Gentamicin, Amikacin, Ceftriaxone, Vancomycin, Amoxicillin, Penicillin-G, Azithromycin, Ciprofloxacin, Tetracycline, and Amoxyclav were used.

Due to the significant number of strains and antibiotics examined, instead of presenting the raw data in the form of tables, these results of AST have been illustratively presented. The results are summarized in percentages of resistant populations against specific medications.

3.4.1. Antibiotic resistance pattern for isolated Gram-positive bacteria:

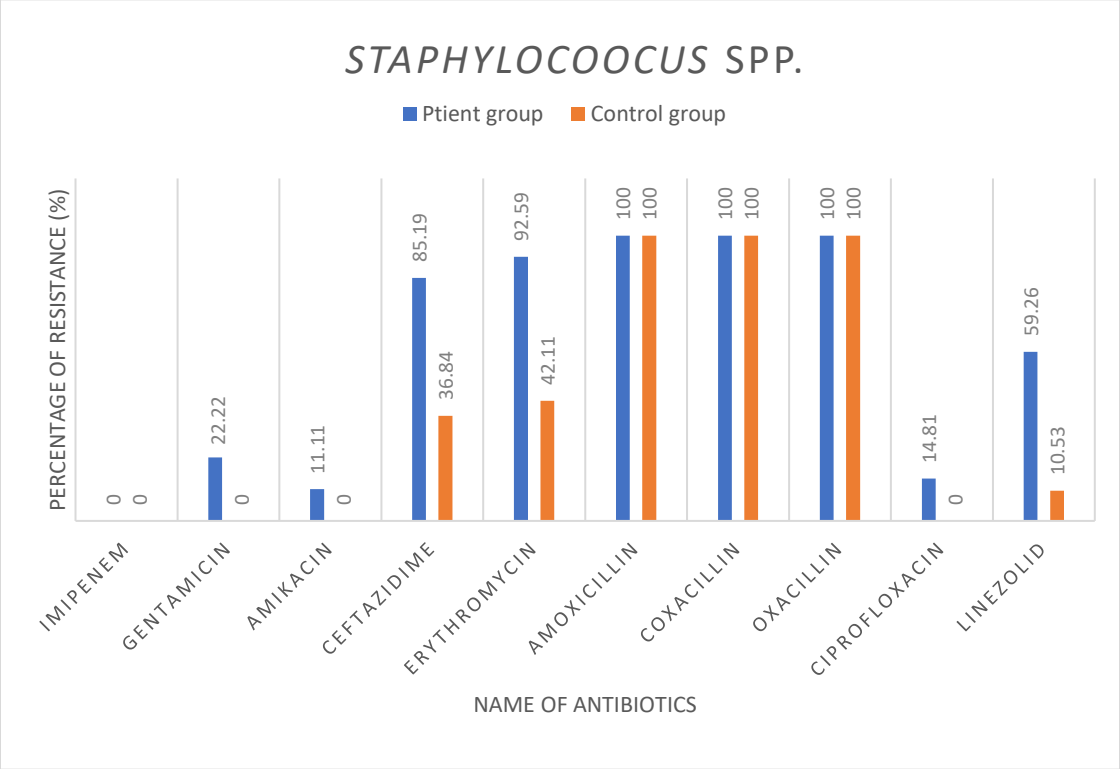

Figure 3.6: Antibiotics resistance pattern for *Staphylococcus* spp.

Figure 3.6 illustrates the antibiotics resistance pattern for *Staphylococcus* spp. Here, both the patient and control group showed 100% resistance to antibiotic amoxicillin, cloxacillin, and oxacillin and 0% resistance towards imipenem. In the case of other antibiotics patient groups isolates showed much higher resistance than the control group. Moreover, in the case of gentamicin, the Amikacin ciprofloxacin control group showed 100% sensitivity.

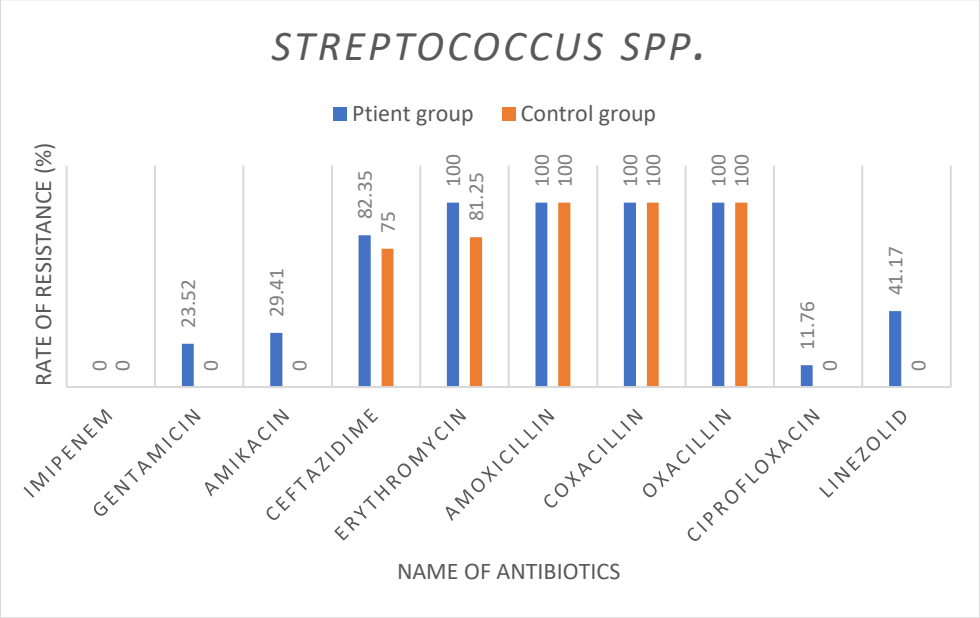

Figure 3.7: Antibiotics resistance pattern for *Streptococcus* spp.

Similarly, the bar graph in Figure 3.7 shows the antibiotics resistance pattern for *Streptococcus* spp. Here both the patient and control group showed 100% resistance to antibiotic amoxicillin, cloxacillin, and oxacillin and 0% resistance towards imipenem. Also, isolates from the patient group shows 100% resistance towards erythromycin which was 81.25% for the control group. Next, in the case of gentamicin, amikacin, ciprofloxacin, linezolid control group showed 0% resistance.

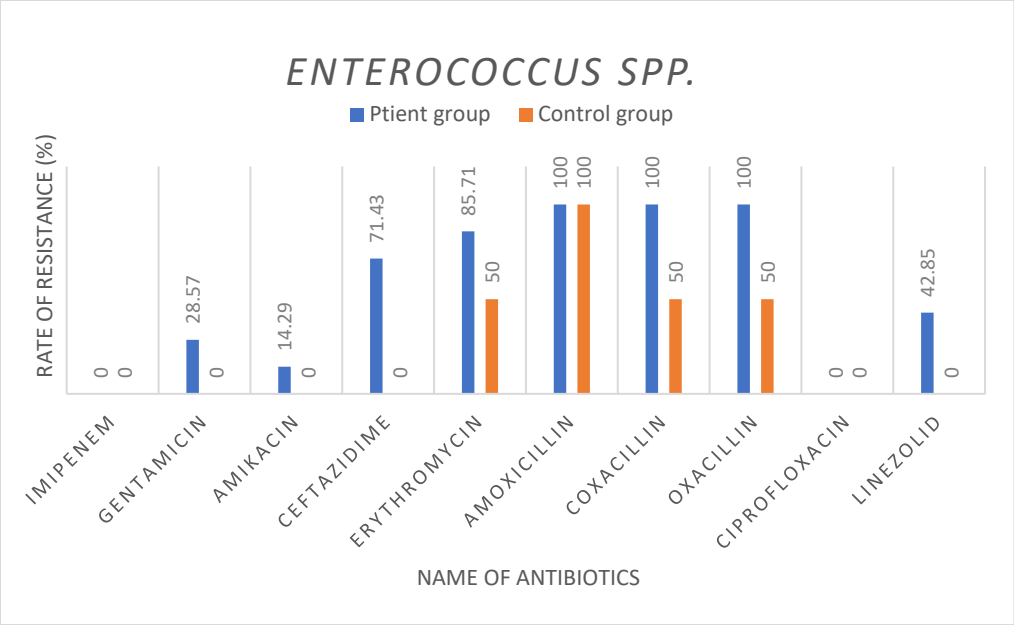

Figure 3.8: Antibiotics resistance pattern for *Enterococcus* spp.

Again, figure 3.8 displays the antibiotics resistance pattern for *Enterococcus* spp. Here, only for amoxicillin the isolates from both groups showed 100% resistance but for both the antibiotics imipenem and ciprofloxacin all isolates showed 100% sensitivity. Next, in patient isolates cloxacillin and oxacillin showed 100% resistance, which was 50% in the case of the control group. Apart from, imipenem and ciprofloxacin, control group isolates also showed 100% sensitivity towards gentamicin, amikacin, linezolid, and ceftazidime.

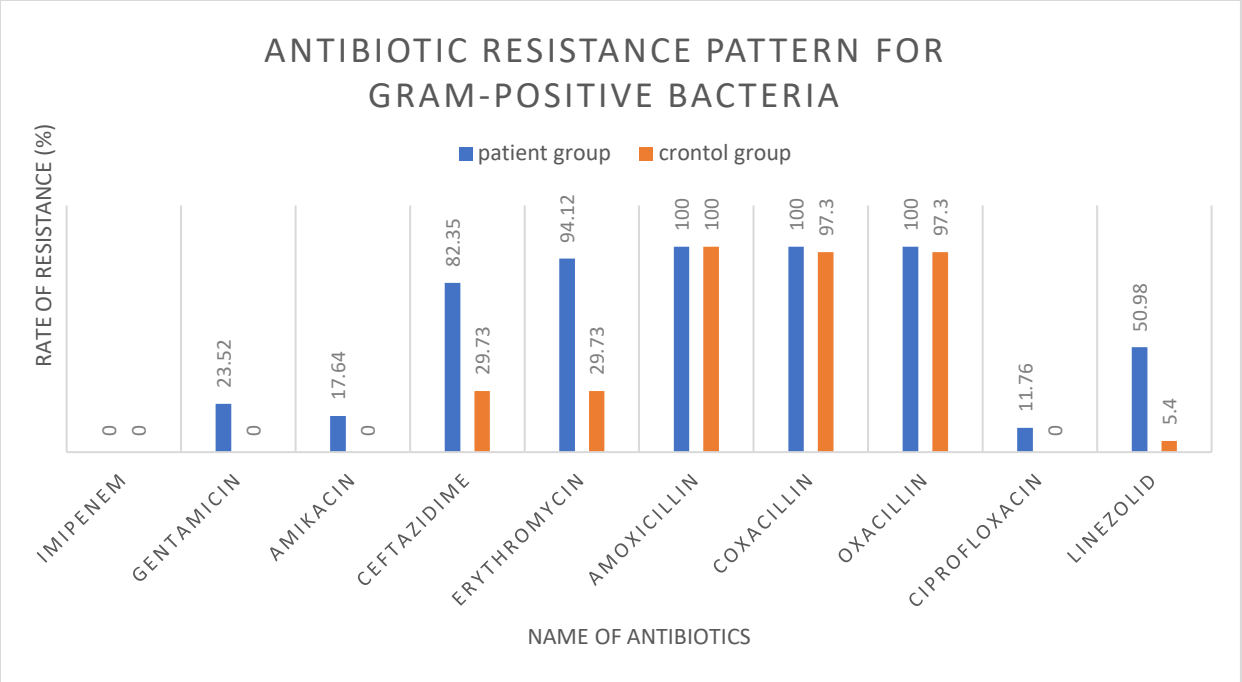

Figure 3.9 Resistance pattern of all three isolated Gram-positive bacteria.

Figure 3.9 represents the antibiotic resistance pattern for all gram-positive isolates in this study. Here it is seen that most of the isolates from cancer patients were 100% resistant to amoxicillin, cloxacillin, and oxacillin. The resistance of isolates was followed by erythromycin with a percentage of 94.12% resistance and ceftazidime with 82.2% resistance. Imipenem showed no resistance against the isolated Gram-positive isolates from both oral cancer patients and control groups. Next, for the control group, all the isolates showed 100% resistance to amoxicillin and 97.3% resistance to cloxacillin and oxacillin antibiotics. Including imipenem, no resistance was found in the case of amikacin, gentamicin, ciprofloxacin, and linezolid antibiotics.

3.4.2. Antibiotic resistance pattern for isolated Gram-negative bacteria:

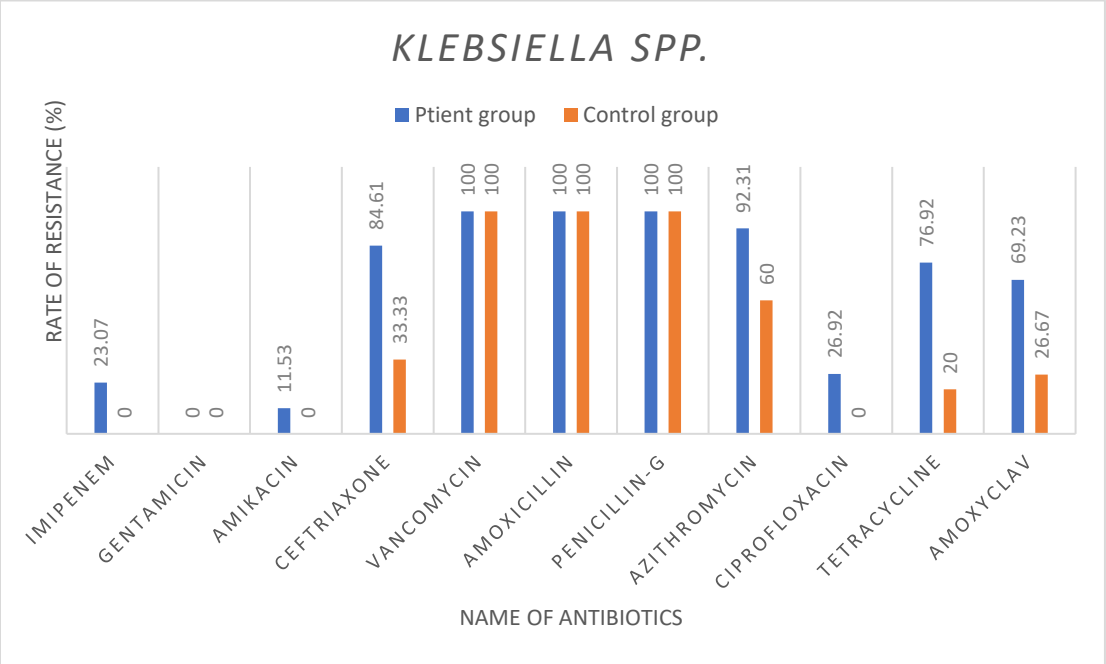

Figure 3.10: Antibiotics resistance pattern for *Klebsiella* spp.

Figure 3.10 represents the antibiotics resistance pattern for *Klebsiella* spp. From the bar graph it is seen that for vancomycin, amoxicillin, and penicillin *Klebsiella* spp isolate from both the patient and control group were 100% resistant, and for gentamicin both the groups are 100% sensitive. Also, the control group showed 100% sensitivity towards imipenem, amikacin, and ciprofloxacin. While the patient group showed some percentage of resistance toward all other antibiotics except gentamicin.

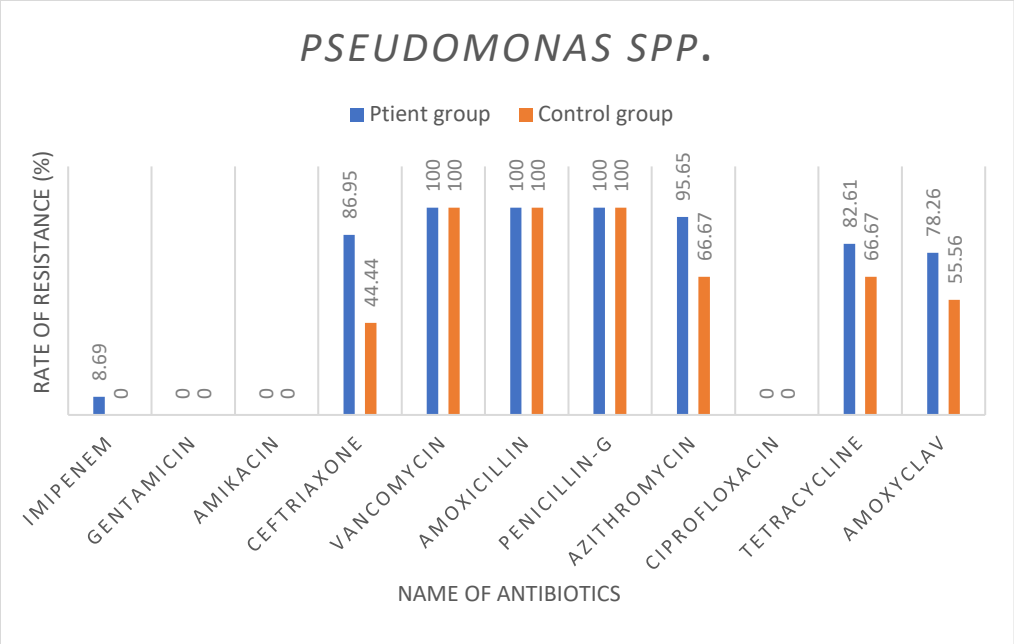

Figure 3.11: Antibiotics resistance pattern for *Pseudomonas* spp.

Figure 3.11 shows the antibiotics resistance pattern for *Pseudomonas* spp. All the isolates of *Pseudomonas* spp from both groups showed 100% resistance towards vancomycin, amoxicillin, and penicillin antibiotics and showed 100% sensitivity towards gentamicin, amikacin, and ciprofloxacin. Apart from these control groups isolates also showed 0% resistance towards imipenem which was 8.69% for the patient grouip.

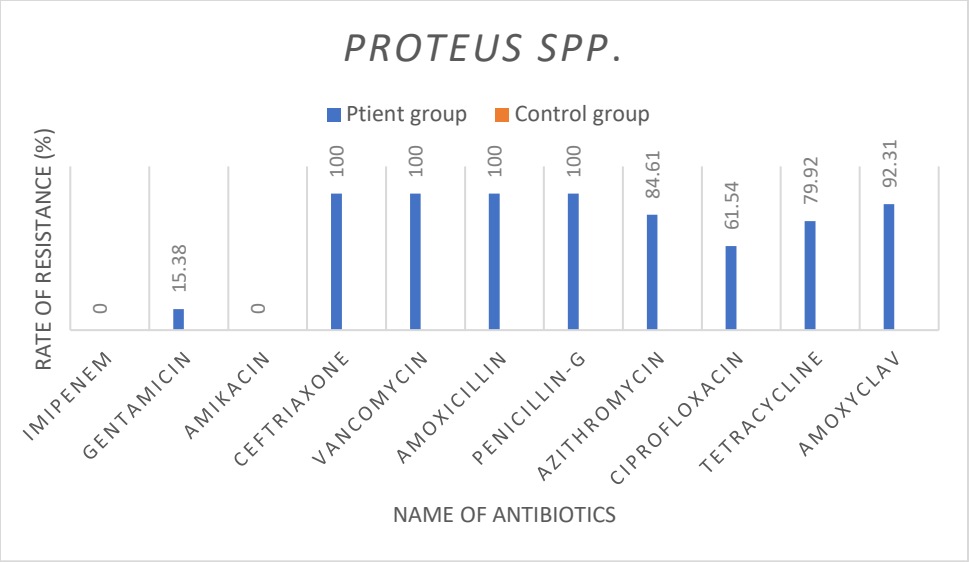

Figure 3.12: Antibiotics resistance pattern for *Proteus* spp.

Figure 3.12 shows the Antibiotic resistance pattern for *Proteus* spp in the patient group only as no *Proteus* spp isolates were found for the control group. Here, it is seen all the isolates showed 100% resistance towards ceftriaxone, vancomycin, amoxicillin, and penicillin antibiotics and showed 0% resistance towards imipenem and amikacin.

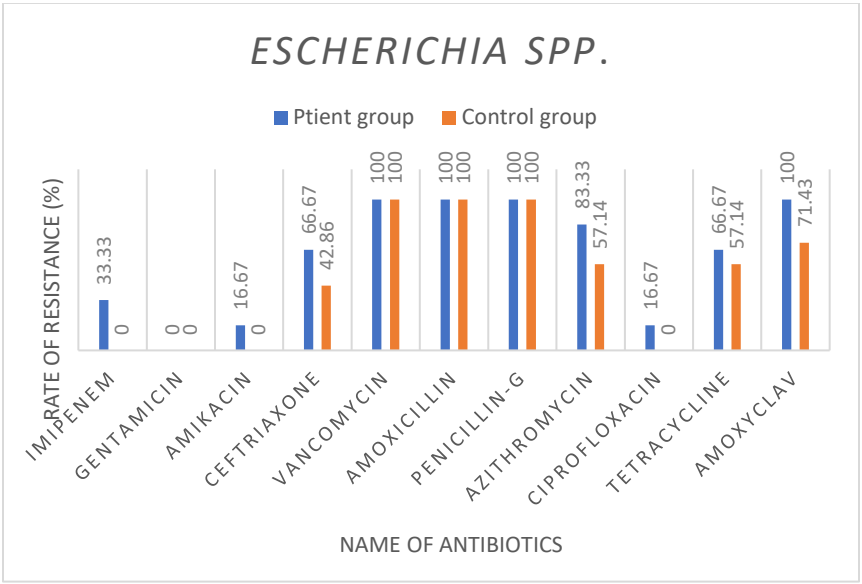

Figure 3.13: Antibiotics resistance pattern for *Escherichia* spp.

Figure 3.13 represents the antibiotics resistance pattern for *Escherichia* spp. From the bar graph, it's seen similarly to other gram-negative organisms, all the isolates from both groups here show 100% resistance towards vancomycin, amoxicillin, and penicillin antibiotics. Moreover, both patient and control group isolates showed 0% resistance towards gentamicin. Gentamicin isolates from the control group showed 100% sensitivity towards imipenem, amikacin, and ciprofloxacin.

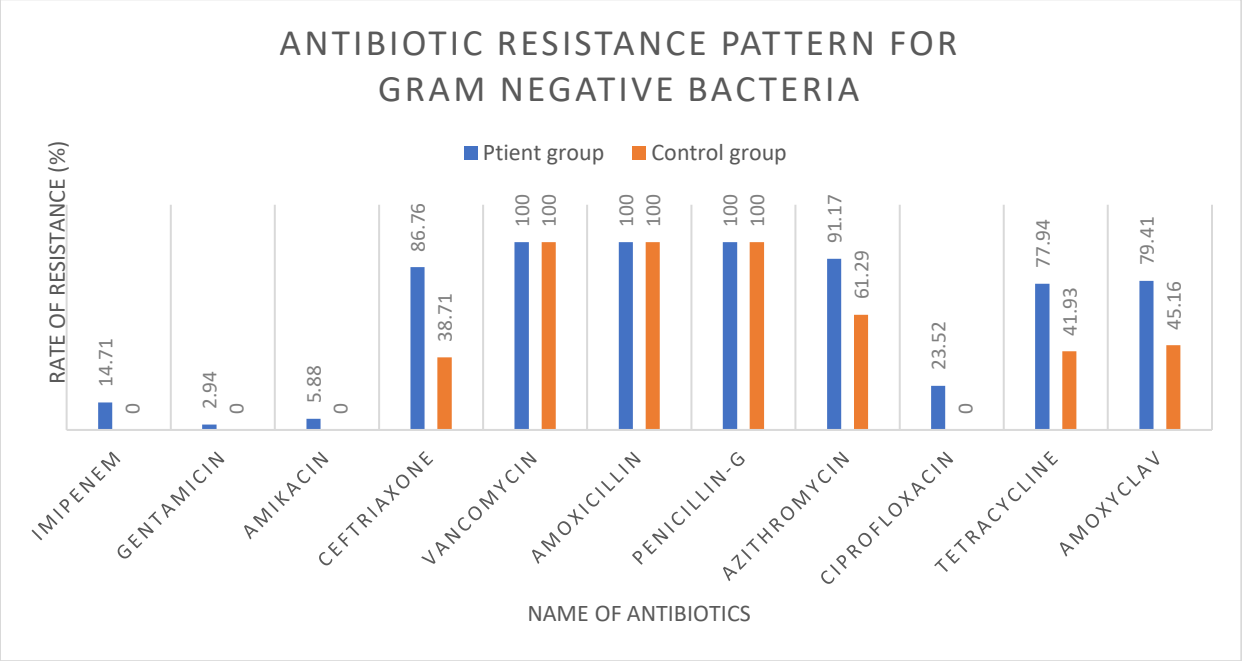

Figure 3.14: Resistance pattern of all four isolated Gram-negative bacteria.

Figure 3.14 represents the antibiotic resistance pattern for all gram-negative isolates in this study. Here, also all the isolates from cancer patients and control groups were 100% resistant to vancomycin, amoxicillin, and penicillin. The resistance of isolates in cancer patients was followed by azithromycin with 91.17% resistance, ceftriaxone with 86.76% resistance, amoxyclav with 79.41% resistance, tetracycline 77.94% resistance, ciprofloxacin 23.52% resistance, 14.71% resistance to Imipenem, 5.88% to amikacin, and minimum resistance of 2.94% to gentamicin.

While, in the case of the control group all isolates showed 0% resistance to imipenem, gentamicin, amikacin, and ciprofloxacin antibiotics.

3.5. Bacterial Species identification by PCR and gel electrophoresis:

The PCR was done for each species with species-specific primers and in specific PCR conditions. Next, The amplified DNA was tested over a 1.5% agarose gel to determine whether the PCR was effective. Using DNA Ladder, the size of the DNA band was determined. In an agarose gel, the ladder permits calculating the size of the unknown fragment by comparing it to the closest band in the ladder lane when run alongside an unknown PCR result. Also, positive controls were used to verify the result.

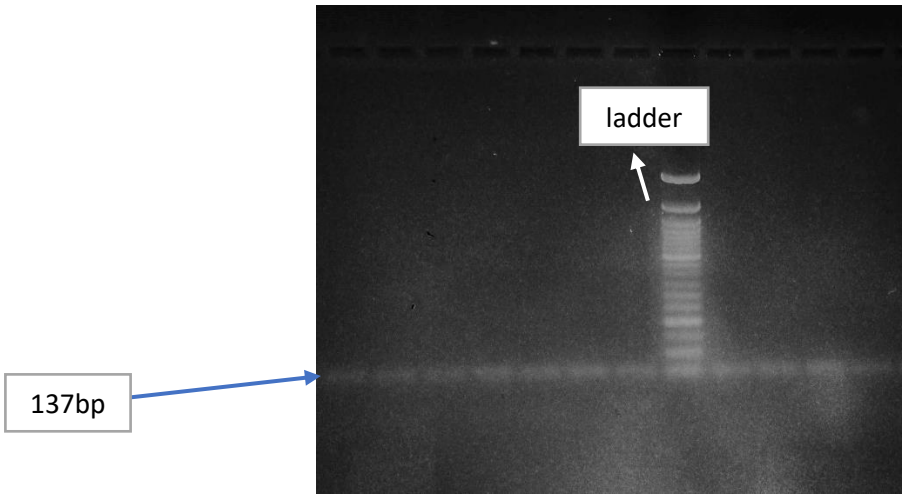

Figure 3.15: Gel electrophoresis of 100bp ladder and PCR product of *Streptococcus spp.*

Here, Figure 3.15 shows the gel electrophoresis of 100 bp ladder and PCR product of *Streptococcus* spp. The band (137 bp) specific for *Streptococcus* genus is shown by an arrow. In

gel run all the probable isolates of *Streptococcus* spp. showed bands proving they are from *Streptococcus* genus.

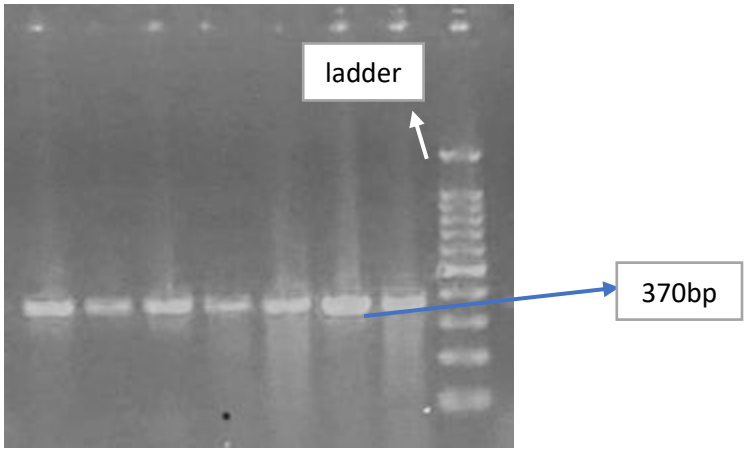

Figure 3.16: Gel electrophoresis of 50 bp ladder and PCR product of *Staphylococcus* spp.

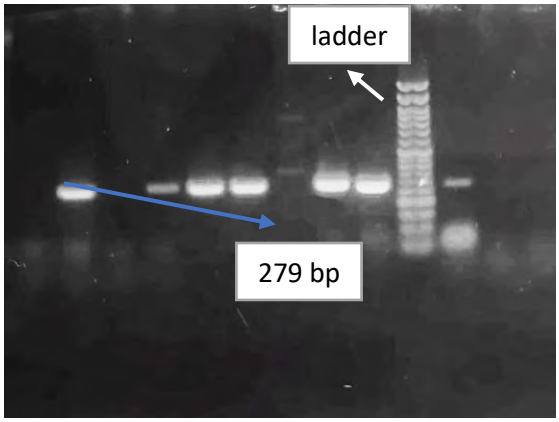

Figure 3.17: Gel electrophoresis of 50bp ladder and PCR product of *Staphylococcus aureus.*

Figure 3.16 shows the Gel electrophoresis of the PCR product of *Staphylococcus* spp (as indicated by the presence of 370 bp band) with ladder and Figure 3.17 shows gel electrophoresis of 50bp ladder and PCR product of *Staphylococcus aureus*(as indicated by the presence of 279 bp band). Here, firstly, all the probable organism *staphylococcus* spp were detected and verified using genus-specific primer. After verifying the *staphylococcus* spp, PCR was again performed with a species-specific primer sequence to verify whether the isolate is S*taphylococcus aureus* or not. Among all the *staphylococcus* spp, 37% of isolates were found *Staphylococcus aureus*.

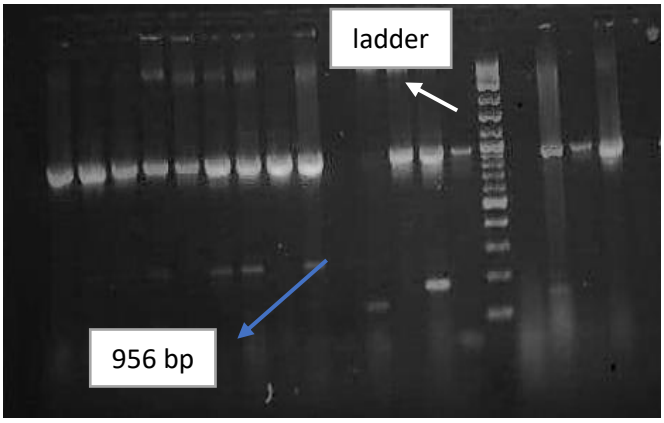

Figure 3.18: Gel electrophoresis of 50bp ladder and PCR product of *Pseudomonas aeruginosa*.

Figure 3.18 shows the gel electrophoresis of PCR product of *Pseudomonas aeruginosa* with 50 bp ladder. After determining the probable *Pseudomonas* spp by biochemical test, all these isolates were used to perform PCR with *Pseudomonas aeruginosa* species-specific primer. Here, about 89% of the isolates were confirmed to be *Pseudomonas aeruginosa* (as indicated by the presence of 956 bp band).

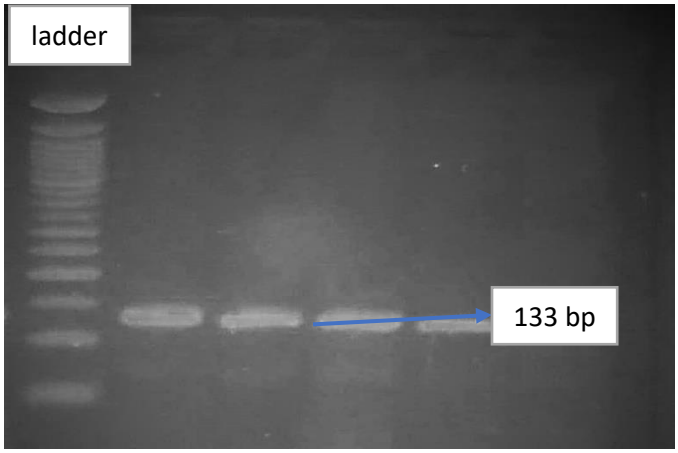

Figure 3.19 shows gel electrophoresis of 50bp ladder and PCR product of *Klebsiella pneumoniae.*

Here, figure 3.19 shows the gel electrophoresis of the PCR product of *Klebsiella pneumoniae* (as indicated by the presence of 133 bp band). Among all the isolates of probable *Klebsiella* spp. about 97% of isolates were found to be *Klebsiella pneumoniae* after the species-specific PCR gel run.

3.6. Identification of antibiotic-resistant gene through PCR:

Numerous organisms were discovered in the phenotypic detection experiments to be resistant to a variety of antibiotics. Therefore, we were curious to find out whether or not such organisms have any genes for antibiotic resistance. To address this issue, focusing on the primary ESBL antibiotic resistance class, we evaluated six distinct ESBL genes NDM, bla-NDM, SHV, bla-CTX-M, bla-TEM, and bla-IMP. In order to detect whether the PCR was successful, the amplified DNA was run in 1.3% agarose gel. The Gene Ruler 1Kb Plus DNA Ladder by Cleaver was used to detect the size of the DNA band.

Of the total of these six ESBL genes examined, three genes (NDM, bla-NDM-1, and bla-IMP) were detected in this study.

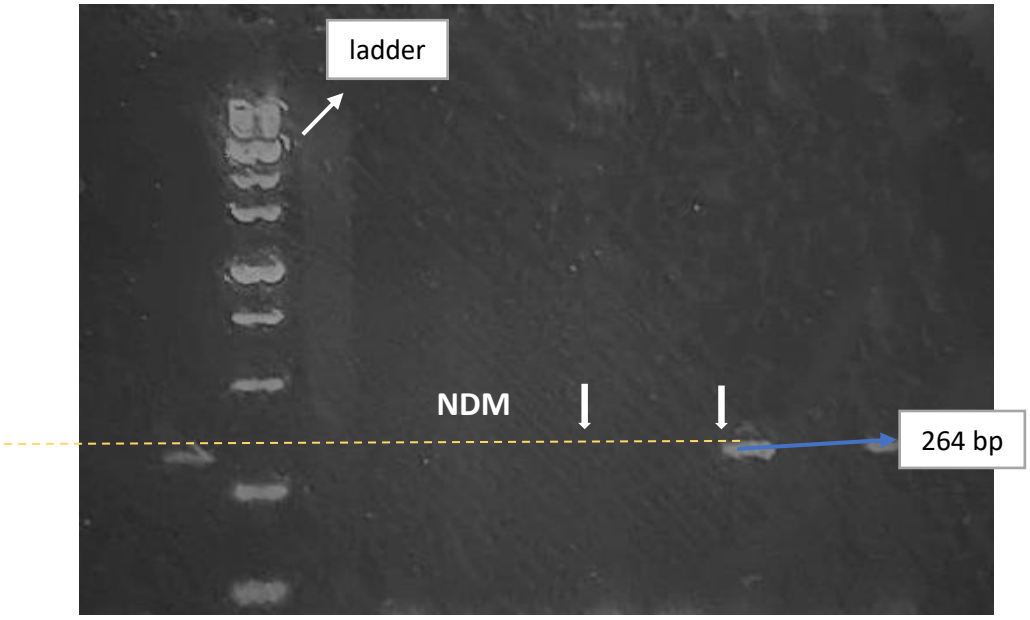

Figure 3.20: Gel electrophoresis of 100bp ladder and PCR product of NDM gene(as indicated by the presence of 264 bp band)

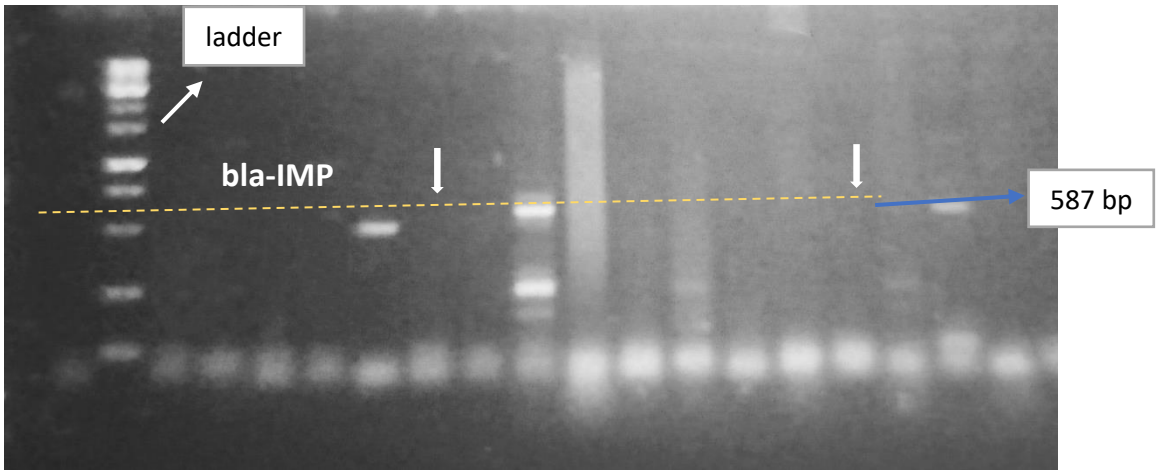

Figure 3.21: Gel electrophoresis of 100bp ladder and PCR product of bla-IMP gene (as indicated by the presence of 587 bp band).

Figure 3.22: Gel electrophoresis of 100bp ladder and PCR product of bla-NDM-1

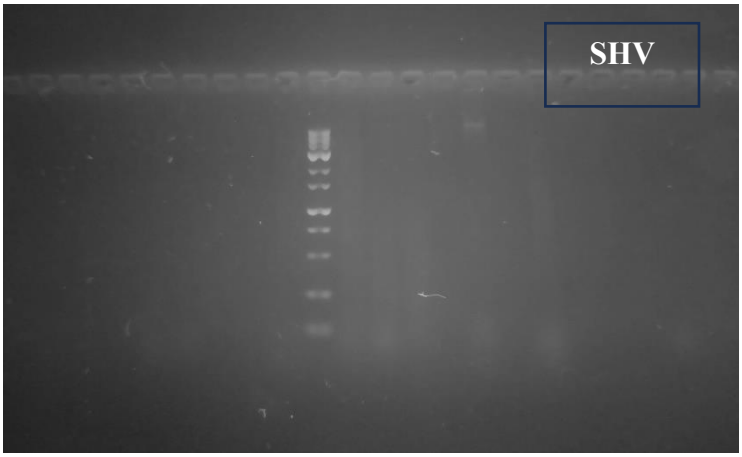

Figure 3.23: Gel electrophoresis of 100bp ladder and PCR product of SHV where no bands could be seen.

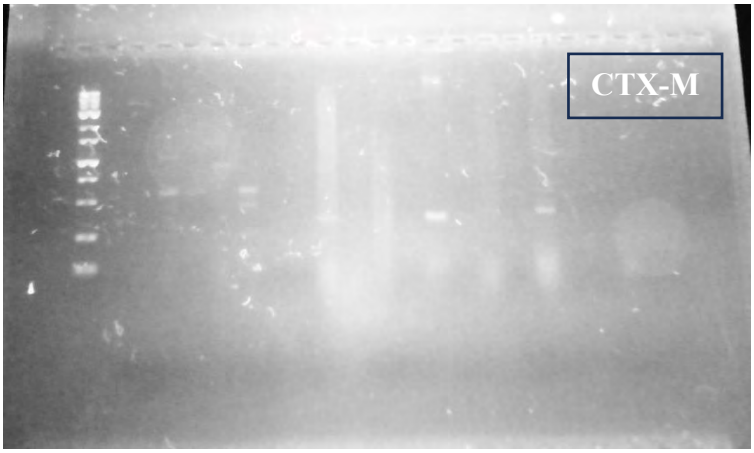

Figure 3.24: Gel electrophoresis of 100bp ladder and PCR product of CTX-M.

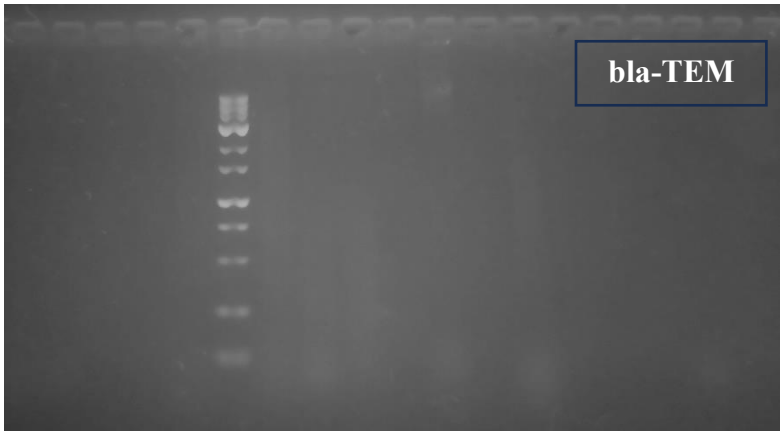

Figure 3.25: Gel electrophoresis of 100bp ladder and PCR product of bla-TEM where no bands could be seen.

3.7. Statistical Analysis of Survey Questionnaire

3.7.1. Gender Distribution

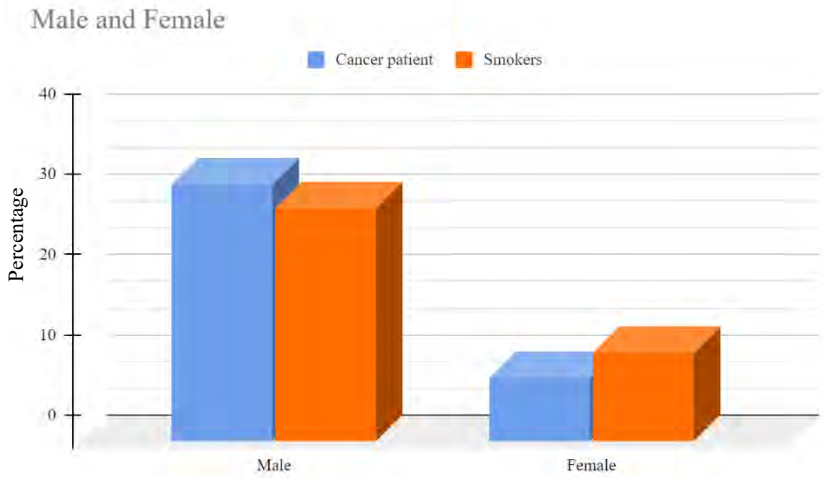

Figure 3.26: gender distribution in cancer patient and smoker control group

In this experiment, the two sample sets had participants of both genders. In both the sample sets of cancer patients and smokers, more men were sampled than women. Over 30 males and over five females were in the cancer patients sample set. In the smokers' sample set, there were over 25 males and over ten females. The number of male participants was more among cancer patients than smokers, while the number of female participants was more among smokers than cancer patients. From the visual interpretation and the observed samples, no solid correlation between gender and the prevalence of cancer or smoking can be concluded.

3.7.2. Age Distribution

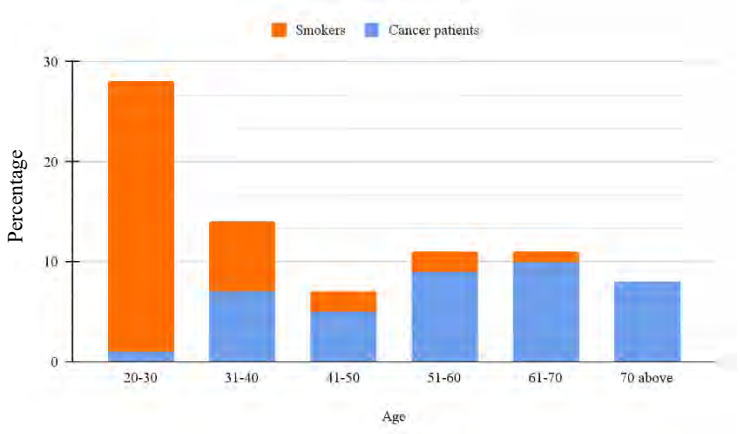

Figure 3.27. Age distribution in cancer patient and smoker control group

The stacked bar chart shows that the participants in both sample sets were from an extensive range of ages. The data for cancer patients is more evenly distributed between 31-70 and above. Only a tiny proportion of the cancer patient's sample was below 31. This is indicative of the onset of cancer coming later in life. Therefore, A correlation between increasing cancer prevalence and age can be established. The data for smokers, however, is largely negatively skewed. Most of the participants belong to the 20-30 age group, and there are no participants in the 70 above age group. This indicates a plausible popularity and normality of smoking among the young-adult population class. It is also worth noting that for the age group 31-40, the number of cancer patients and smokers is the same.

3.7.3. Distribution of Region

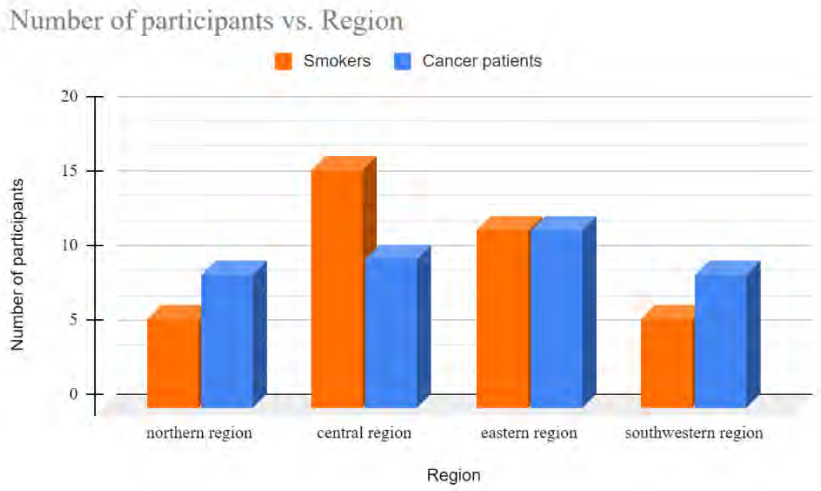

Figure 3.28: Distribution of region in cancer patient and smoker control group.

The participants were observed based on their regions for smokers and cancer patients. A maximum of both Smokers and cancer patients were found to be from the central region of Bangladesh. This could be because the sample was collected from an area where most people of the central region live. Compared side by side, more smokers live in the central region than cancer patients. In contrast, the population of cancer patients is distributed mainly in north and south regions, compared to Smokers. This could indicate particular selection bias regarding where the sample was collected, which gave such skewed results.

3.7.4. Smoking Habits and Duration

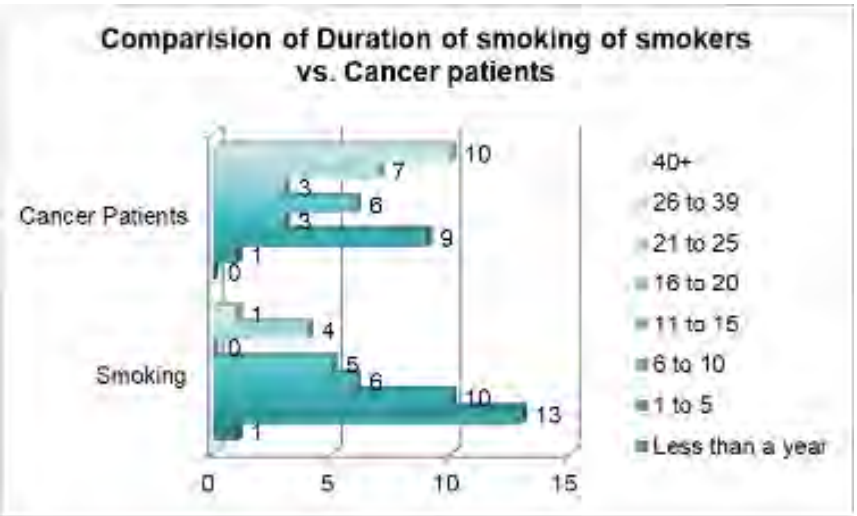

Figure 3.29: Smoking Habits and Duration in Cancer patient and smoker control group.

In the sample of 40 patients of smokers who do not have cancer and smokers who do have cancer, their smoking habits were observed. For both Smokers and Cancer patients, the number of cigarette sticks falls under the range of 1 to 10 sticks a day, While for Smokers, the number of sticks goes up to the range of 30-40, while for cancer patients, the range of cigarette sticks per day stops at the range of 11-20. Regarding the duration of smoking, the ranges for both smokers and cancer patients are skewed. For smokers, the maximum of the sample has been smoking for 6 to 10 years, while for cancer patients, the maximum has been smoking for 40+ years. This may indicate a correlation between their years of smoking and their cancer diagnosis. Most of the Cancer patients have been smoking for many years, while smokers have been smoking in just recent few years. This may indicate why the sample of smokers has yet to have cancer, as they have only started smoking recently.

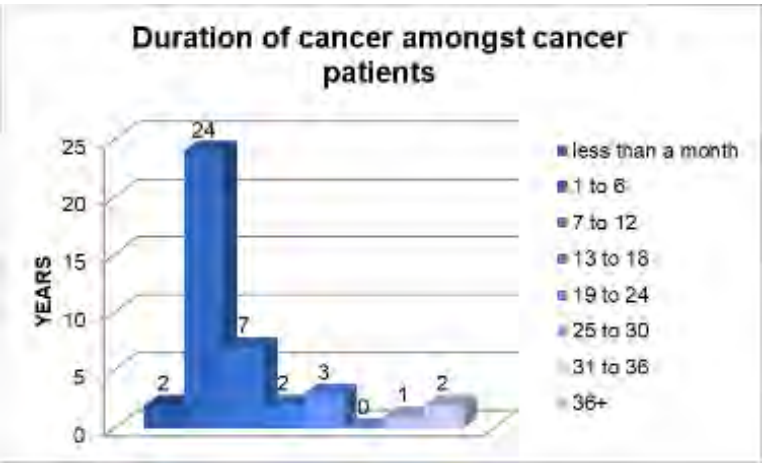

Figure 3.30: Duration of cancer amongst cancer patients.

The duration of cancer of all the Cancer patients is also measured. 60% of the cancer patient sample had their cancer only in the range of the past 1 to 6 months, which means they are still at an earlier stage compared to the rest of the population. The maximum number of years that the cancer patient was shown to be four may indicate that by four years, most patients get themselves checked and treated as early as possible.

3.7.5 Family History of Cancer and Other Diseases Among Patients and Control Group

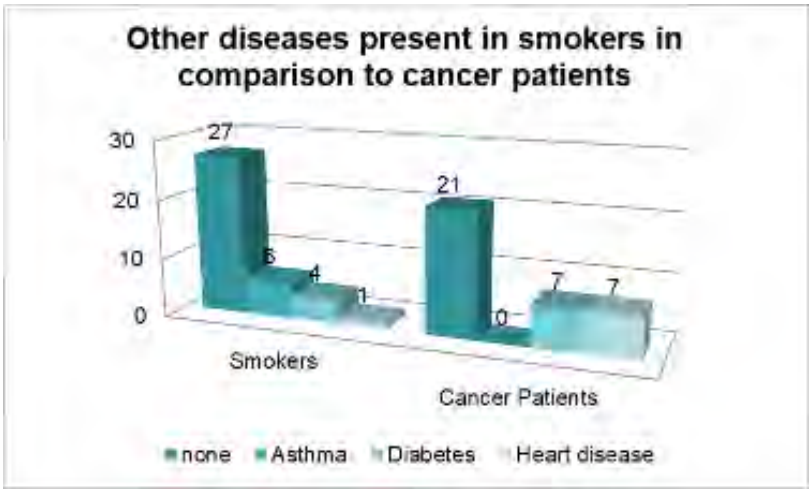

Figure 3.31.: Family History of Cancer and Other Diseases Among Patients and Control Group.

In the experiment, smokers and cancer patients were observed to determine whether or not they had any other complications. Other diseases like asthma, diabetes, and hypertension were observed. It was observed that most of both smokers and cancer patients did not have other diseases. While for smokers, the common disease among these 3 was asthma, for cancer patients, it was diabetes and hypertension. This may indicate that because smokers are in just an early stage of their smoking life, they mostly have asthma, while because most cancer patients have been smoking for decades, they have more severe implications like diabetes and hypertension.

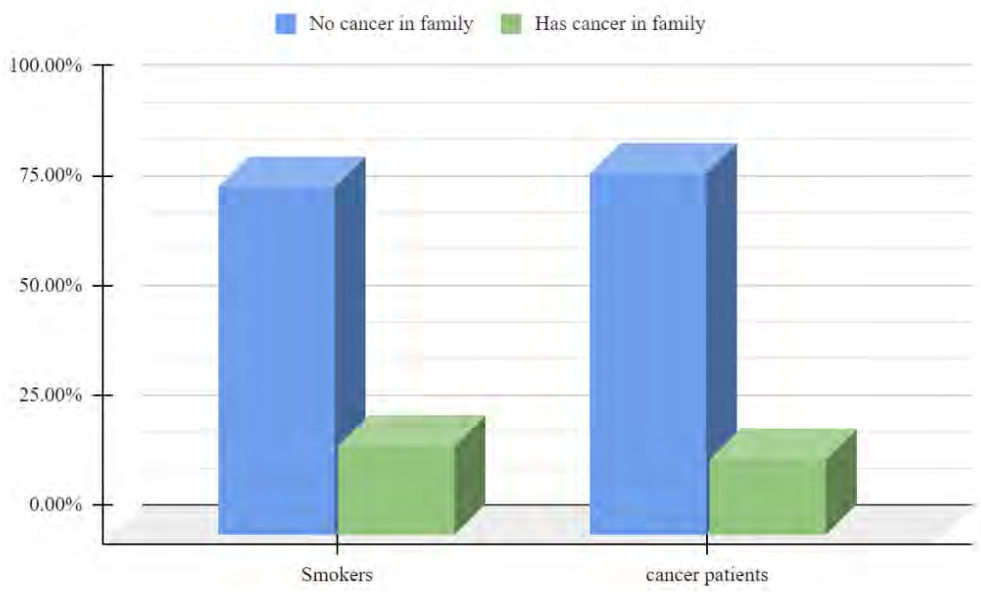

Figure 3.32: Prevalence of cancer in family members in both smokers and cancer patients.

The prevalence of cancer in family members was also observed in both smokers and cancer patients. A maximum of both Smokers and cancer patients did not have any other cancer patients in their family. Although a few more smokers have cancer patients in their families than cancer patients, this may indicate that the sample is too small and skewed to have entirely accurate results.

4. Discussion

The necessity of researching individuals diagnosed with oral cancer arises from this disease's intricate and diverse characteristics and its substantial repercussions on personal well-being and public healthcare systems. The prevalence of oral cancer poses a significant worldwide health concern, characterized by a wide range of causative variables, various clinical manifestations, and complex molecular pathways. Extensive research contributes to a more profound comprehension of oral cancer's fundamental etiology, predisposing factors, and pathophysiology, shedding light on prospective approaches for timely identification, precise assessment, and efficacious intervention. The examination of molecular and genetic changes that contribute to the advancement of oral cancer can result in the discovery of new biomarkers and targets for therapy, thereby facilitating the advancement of individualized treatment strategies. Moreover, comprehending the socio-economic ramifications of oral cancer and its corresponding healthcare burden contributes to developing preventative strategies, public health policies, and healthcare interventions.

This study aimed to identify and evaluate microorganisms present in persons with oral cancer infections and notable history of smoking. The data was then compared to isolates from individuals who smoke but do not display any sickness symptoms. Moreover, examining antibiotic resistance patterns and discovering bacteria resistant to a broad range of drugs in response to the growing occurrence of multi-drug-resistant organisms was also a core objective of this study. Side by side, this study looked forward to identifying the specific genes responsible for providing resistance against a wide variety of antibiotic medicines. The investigation into developing highly effective antibiotics was undertaken to mitigate patient distress. Furthermore, this study undertook a comprehensive survey to assess the epidemiological, etiological, and socio-economic features of individuals diagnosed with oral cancer in Bangladesh. The survey examined a range of variables,

encompassing the length of time individuals had been smoking, the number of cigarettes consumed per day, the duration of cancer diagnosis, and the presence of a family history of cancer, among other factors.

In a past study conducted by Nawar et al. (2021), the bacteria isolated from the infection sites of pre-operative and post-operative patients showed extensive signs of bacterial infection among the pre-operative patients compared to the postoperative patients. The findings outline the fact that pre-operative patients in Bangladesh are more prone to be affected by bacterial infections. The study revealed that 83 (65.4%) Gram-negative and 44 (34.6%) Gram-positive bacteria were isolated. Among these*, Pseudomonas spp*. accounted for the highest prevalence, with 30 isolates representing 54.54% of the total bacteria isolates. However, the present study, solely dedicated to isolates found in pre-operative patients, found 52.94% of isolates to be Gram-negative and the remaining 47.06% of the isolates to be Gram-positive. Thus, the present study slightly differs from the previous study based on the percentage of gram-positive and gram-negative bacteria. However, the present study found that *Pseudomonas spp*. is the most common bacteria, accounting for 22.69% of isolates found in oral cancer patients. This number is followed by 21.85% of *Klebsiella spp.* and 19.32% of *Pseudomonas spp.* By comparing to the previous study conducted by Nawar et al. (2021), it can be seen that the result is consistent if the species of bacteria are considered. However, there is a noticeable change in the percentage. To elaborate, a significant drop in the presence of *Escherichia spp.* (5.04%) was observed in the present study compared to 25.45% *E. coli* in the previous study.

In another study conducted by Ashreen et al. (2020), it was found that the predominant organism observed in the study was *Klebsiella* spp. which accounted for 45% of the total. This was followed by *Pseudomonas* spp representing 34.5% of the total, and *Proteus* spp accounting for 9.5%.

Escherichia coli had the lowest prevalence, accounting for only 6% of the observed cases. Thus, the findings of the previous studies conducted at the exact location are coherent with the present study regarding the bacterial genus, even if there are differences in the percentage of different bacteria found.

Previous research conducted by Ashreen et al. (2020) and Nawar et al. (2021) has provided evidence indicating the presence of noticeable variations in the oral cavity microbiota between patients afflicted with cancer and those in a state of good health. In this current investigation, 40 swab samples were obtained from the control group, leading to the identification and isolation of a total of 38 bacteria. The *Staphylococcus* spp. demonstrated the highest proportion (27.94%) in the specified area, with the second highest proportion detected in *Streptococcus* spp. (23.53%). *Klebsiella* species were observed to comprise 22.06% of the bacterial population, followed by *Pseudomonas* species at 13.23%, *E. coli* at 7.8%, and the least frequent bacterial genus, *Enterococcus*, at 2.94%. The presence of these bacteria in the control group could be explained by an infection in their oral cavity or inadequate oral hygiene practices. In sharp contrast to the cancer patients, the control population exhibited an absence of *Proteus spp*., a very prevalent microorganism among individuals diagnosed with oral cancer.

Various factors, such as poor oral hygiene practices, insufficient saliva production, and reduced mucosal integrity, can potentially disturb the balance, creating favorable conditions for the proliferation of harmful microorganisms and the subsequent development of illnesses. Moreover, specific anatomical characteristics in the local region, such as dental caries, periodontal disease, and oral mucosal lesions, might generate favorable environments that promote the colonization and growth of bacteria. Various systemic diseases, such as immunosuppression, diabetes, and autoimmune illnesses, can exacerbate the vulnerability to bacterial infections by compromising

immune responses and modifying oral microenvironmental factors (Kachlany, 2007). Moreover, various extrinsic factors, such as smoking, inadequate diet, and substance misuse, negatively affect the body's defense mechanisms. As a result, these factors make the mouth cavity more vulnerable to colonization by bacteria and eventual ailments (Sanketh & Amrutha, 2013).

The research employed cheek swabs solely and intentionally refrained from collecting deep swabs to mitigate the potential contamination from germs present in the throat. Nevertheless, a notable discrepancy exists in the occurrence of bacteria among individuals with cancer compared to the control group, suggesting that immune suppression plays a pivotal role in promoting the proliferation of pathogenic microorganisms (Oberoi et al., 2014).

The present study also investigated the antibiotic susceptibility profile of opportunistic bacteria. The antibiotic susceptibility test encompassed the application of 16 different antibiotics selected from a pool of 10 unique groups. The antibiotics were chosen based on their efficacy against Gram-negative and Gram-positive bacteria. After conducting the antibiotic susceptibility test, it was observed that all Gram-positive bacteria demonstrated complete resistance, reaching a prevalence of 100%, against antibiotics classified under the Penicillin group, specifically amoxicillin, cloxacillin, and oxacillin, as well as the Macrolide group, which includes erythromycin. However, in the case of Erythromycin, total resistance was not observed as 7.41% of the isolates were sensitive towards this drug. Notably, there was a sharp distinction in the resistance pattern of Erythromycin when compared with the control group, as only 42.11% of isolates showed resistance, while the others were sensitive to the medication.

According to a study conducted by Yamashita (2013), 69.2% of Staphylococcus species were resistant to oxacillin. Consequently, resistance has significantly increased, which was evident

during the present study. The least resistant drugs were gentamicin and amikacin, while imipenem demonstrated no resistance at all. *S. aureus* showed 100% susceptibility to amikacin in a previous investigation by Bhagwath et al. (2019). The latest study, however, showed that this species is becoming less susceptible to the most potent antibiotics, with a susceptibility rate of 88.89%.

The major microbial community that could be identified consisted of Gram-negative bacteria. The Gram-negative bacteria demonstrated complete resistance, achieving a 100% resistance rate, against the antibiotics classified under the Penicillin group, namely penicillin-G, and amoxicillin. Similar results were observed for the antibiotics classified under the Glycopeptide (vancomycin) category. The primary reason for the natural resistance of the majority of Gram-negative bacteria to vancomycin can be linked to the existence of outer membranes and unique cell wall features that hinder the ability of big glycopeptide molecules to permeate.

Gram-negative microbes have exhibited resistance to many antibiotics, such as azithromycin (with a resistance rate of 91.17%) and amoxyclav (with a resistance rate of 79.41%). The findings indicated that amikacin, gentamicin, and imipenem exhibited the least resistance. In a previous investigation conducted by Bhagwath et al. (2019), it was observed that there was full susceptibility to antibiotics belonging to the Carbapenem group, particularly imipenem. The results of this investigation demonstrate that Gram-negative bacteria displayed a susceptibility rate of 85.29% towards the antibiotic imipenem. The escalating prevalence of carbapenem-resistant Gram-negative bacteria globally is a matter of considerable concern.

The current study demonstrated that both the Gram-positive and Gram-negative isolates showed notable resistance towards amoxicillin. The bacterial strain has exhibited the most limited degree of susceptibility toward the antibiotics amikacin, gentamicin, imipenem, and ciprofloxacin. In a

prior investigation conducted by Bhagwath et al. (2019), it was observed that the antibiotics utilized had a sensitivity rate of 80% when tested against various bacterial strains. Nevertheless, the results of this study suggest a notably low prevalence of sensitivity. Microorganisms have demonstrated a notable degree of resistance to the antibiotics utilized. The observed variability in antibiotic resistance can be ascribed to the demographic attributes of the population under investigation and the impact of geographical and lifestyle determinants.

Antibiotics are pharmacological agents utilized for prophylactic and therapeutic interventions in bacterial diseases. Antibiotic resistance is a condition that occurs when bacteria undergo genetic changes in response to the treatment of specific medicinal drugs. The prevalence of antibiotic-resistant bacteria globally is experiencing a notable escalation, thereby presenting a significant challenge to the efficacy of antibiotics. These pharmaceutical agents have played a transformative role in medicine, preserving innumerable human lives. The global population faces a substantial problem in terms of health, food security, and overall development due to the growth of antibiotic resistance. The rise and worldwide distribution of new resistance mechanisms present a substantial challenge to our ability to efficiently manage common infectious diseases. Presently, a burgeoning phenomenon exists wherein bacteria are progressively acquiring heightened antibiotic resistance due to their exposure to a wide array of medicinal substances.

Furthermore, it is noteworthy to mention that nosocomial microorganisms possess the capability to cause infections in patients, frequently demonstrating resistance to multiple pharmaceutical agents (Breathnach, 2013). Cancer patients frequently undergo various treatments, such as chemotherapies and radiotherapy, which may contribute to developing bacterial drug resistance.

A significant proportion of individuals diagnosed with oral cancer exhibit non-adherence to prescribed antibiotic regimens, while others fail to uphold adequate hygiene practices (Montassier et al., 2013). The occurrence of penicillin resistance is not a recent phenomenon. Reports of penicillin resistance have been documented since the 1950s (Knowles, 1985).

In the context of rising antibiotic resistance, implementing combination therapy using antibiotics offers a feasible alternative for treating opportunistic gram-negative bacteria (Brooks & Brooks, 2014). Additionally, it is imperative to investigate the genetic factors responsible for developing resistance, employing molecular methodologies. One crucial factor is that this would accelerate antibiotic resistance detection. Consequently, it will enhance the efficiency of administering the most suitable drug on time.

In this study, antibiotic-resistant strains were further analyzed for resistant ESBL genes using molecular techniques such as polymerase chain reaction (PCR) followed by PCR product gel electrophoresis. Interestingly, the polymerase chain reaction result revealed the presence of ESBL (NDM-1, bla NDM-1, and bla IMP) genes. In this study, *Klebsiella pneumonia* strains were found to be the predominant bacterial agent carrying almost all of the resistance genes.

According to a previous study conducted by (Noutin Michodigni et al., 2021), 15% of the isolates examined in the study were the combined carriers of the bla-NDM-1 and bla-IMP genes. The study also reported the identification of several other resistant genes, including bla-IMP, bla-VIM-1 bla-SPM-l, bla-NDM-1, bla-OXA-23 bla-OXA-24, bla-OXA-58 and bla-KPC. However, the *Klebsiella* spp. examined in the present study only harbored the NDM-1, bla NDM-1, and bla IMP genes.

In another study conducted by Lindsay (2013), several genes were used to detect multi-drug resistant genes, including blaNDM and blaIMP, among other genes. The study shows the presence of the blaNDM gene in 29 out of the 369 *Pseudomonas spp.* isolates (7.85%). It also found that 34.5% of the isolates carried the blaIMP gene and the blaNDM gene. However, in the present study, it was found that 6 out of 32 *Pseudomonas* isolates (18.75%) carried the blaNDM genes, and only 5 out of the 32 (15.625%) isolates contained the presence of the blaIMP genes. Thus, 83.33% of isolates carry both blaNDM and blaIMP genes according to the present study's findings.

This study elucidates the complex interaction among microbial populations, multi-drug resistance, and the distinctive oral microenvironment in individuals with oral cancer and habitual tobacco users. By employing a methodical approach to gather and examine bacteria that occur by chance in saliva samples, we have obtained valuable knowledge regarding the ever-changing makeup of the oral microbiome within these specific groups. The discovery of multi-drug resistant genes in the bacteria that have been isolated reveals the urgent issue of antimicrobial resistance inside the oral ecology. The comparative investigation conducted between individuals diagnosed with oral cancer and individuals who are regular smokers without the disease has highlighted the possible impact of smoking on microbial profiles and resistance mechanisms. This finding further emphasizes the necessity for focused interventions to address this issue. The results of this study provide significant insights into the dynamics of microorganisms about oral cancer and smoking, establishing a basis for future research endeavors.

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