SCREENING OF ORAL FLORA AND THEIR ANTIBIOTIC SUSCEPTIBILITY

A

Project Work Submitted to

Department of Microbiology

Central Campus of Technology, Tribhuvan University

In Partial fulfillment of the Requirements for the Award of Degree of

Bachelor of Science in Microbiology

Submitted by

Deepa Rai

Department of microbiology, CCT, Tribhuvan University

Hattisar, Dharan

November, 2016

RECOMMENDATION

Project work entitled **"Screening of oral flora and their antibiotic susceptibility."** submitted to Department of Microbiology, Central Campus of Technology, Tribhuwan University, Dharan by Miss Deepa Rai is a record of bonafide project work done by her during the period of her project work under my guidance and supervision and that no part of this research work has found the basis for the award of any degree or other similar titles.

……..

Mr. Suman Rai

Department of Microbiology

Central Campus of Technology

Tribhuwan University

Dharan,

Nepal

CERTIFICATE OF APPROVAL

On the recommendation of **Mr. Suman Rai**, this project work of Miss Deepa Rai entitled **"Screening of oral flora and their antibiotic susceptibility."** has been approved for the examination and is submitted to the Tribhuwan University in Partial fulfillment of the requirements for B.Sc. degree in Microbiology

.....

Mr. Suman Rai
Department of Microbiology
Central Campus of Technology
Tribhuwan University
Dharan,
Nepal

Date: …….

BOARD OF EXAMINERS

Recommended by:

……

Mr. Suman Rai

Supervisor

……..

Ms. Pranita Dangol

Co-supervisor

Approved by:

……..

Mr. Shiv Nandan Sah

(Assistant Professor)

Head of Department

Department of Microbiology

Examined by:

……..

(Internal Examiner)

……..

(External Examiner)

Date: ……..

ACKNOWLEDGEMENTS

I am glad to acknowledge the people without whom our project work wouldn't have been possible.

First and foremost, I would like to thank my respected supervisor **Mr. Suman Rai**, for his supervision on this project work and my co guide **Ms. Pranita Dangol** for her guidance. I would also like to thank my entire teachers from Central Campus of Technology for their guidance, constant support and encouragement over the last 4 years without which I would not have had the opportunity, the confidence, or the ability, to embark on an undertaking such as this.

I would also like to express my gratitude to **Mr. Ain Bahadur Karki,** and **Kamana Bantawa Rai** of the Microbiology laboratory of Central Campus of Technology for their continuous help regarding the equipment and other materials during the study.

My loving and respected parents, **Mr. Bhukendra Rai** and **Mrs. Kapana Rai** for their love, support and encouragement to make me stand here today.

My very dear friends **Tinmaya Rai, Sushmita Phattepuri, Sneha Shrestha, Asmita Basnet, Sushma Rai, Sajina Dhungel, Dipika Paudel, Sajuta Shrestha** and **Smriti Chhetry**, for their great support and encouragement during the study.

Last, but not least, my dear friends, who kindly cooperated with me in the sample collection.

Above, I have gratefully acknowledged people who have been invaluable to me in completing this project work. However, all opinions expressed in this project work, and all errors, are mine.

Date: ……..

ABSTRACT

This study was conducted for the screening of oral flora and their antibiotic susceptibility. The conventional culture method was employed in this study. 50 saliva sample were collected and proceeded first by mixing with Snyder agar tube to observe that whether the organism responsible for tooth decay was present or not. The pure culture was obtained from the subculture on Nutrient agar medium. The isolates were subjected to gram staining and biochemical test. From the biochemical test results, 5 isolated organisms were identified as *Corynebacterium* and the remaining 45 organisms were identified as *Neisseria* spp.

The antibiotic sensitivity test of the isolated bacteria was performed using Mueller Hinton Agar by disc diffusion method. From this test it was observed that ampicillin and amoxicillin were mostly resistance to *Corynebacterium* ssp and *Neisseria* spp while ciprofloxacin and erythromycin were sensitive to *Corynebacterium* ssp and *Neisseria* spp.

Key words: *Corynebacterium* ssp, *Neisseria* spp, oral flora, Snyder test, disc diffusion method.

CONTENTS

Lists of Tables

Lists of Photograph

CHAPTER I

INTRODUCTION

1.1. Background of the study

Human oral cavity is one of the most dynamic habitats for numerous bacterial species where they undergo intense interspecies competition to form multispecies biofilm structure (Rahman et al 2015).The oral flora of man comprises one of the densest and most varied microbial populations in the body (Ross 2016).Various species of the genus *Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, Veillonella* and *Bacteroids* are the prominent bacteria commonly found in the oral cavity. Among the oral bacteria, *Streptococcus* and *Enterococcus* are two important members because they can shift their lifestyle from beneficial microflora on the surface of oral cavity and oropharynx to destructive pathogens when they gain access into the oral tissue and blood stream. Among the diseases caused by oral bacteria include dental caries, periodontitis, endocarditis, pharyngitis, pneumonia, meningitis etc. (Rahman et al 2015)

Dental carries, also known as tooth decay, affecting the vast majority of population (Chamber 2012). Tooth decay is caused by certain types of acid producing bacteria which cause damage in presence of fermentable carbohydrate such as sucrose, fructose and glucose. Oral Streptococci, which are the major member of oral flora, frequently cause bacteremia and infective endocarditis. In a healthy mouth, the pH is around 6.2 to 7.0. A pH of 7 is neutral. Thus, problem starts when pH is less than 5.5. The tooth is now in the acid environment and start to demineralize (lose calcium and minerals from the enamel). As the enamel losses its minerals, it start to break down (Olorunjuwon et al 2013).

It has a complex etiology that carries occurs under conditions relating to the tooth itself, sugar present in the food and drinks, and the oral environment. However, sugar consumption has been identified as the major cause of dental caries.

Dramatic increases in dental caries were seen between the 17th and19th centuries as the availability and consumption of sugar increased. Caries prevalence has been in decline in developed countries since the 1960s, but is increasing in developing countries as diet change and consumption increases. Although key determinant include access to fluoride, either through water supply or from another sources, such as toothpaste or tropical fluoride application (Chamber 2012)

Teeth are made up of 3 layer; enamel, the hard outer layer; dentin, the core structure; and the pulp chamber at the center, which contain blood vessel and nerves. After eating, a combination of bacteria, acid, food debris and saliva collectively referred to as plaque, begins to build up on the teeth. If not removed by regular brushing, flossing and rinsing, the acidic nature of plaque begins to dissolve tooth enamel, creating dental carries. Some of the symptoms of dental caries may be pain after consuming sweet, hot or cold food or drink or visible hole in the teeth. Once the decay has reached the pulp, this is referred to as pulpitis (Olorunjuwon et al 2013). The untreated condition of dental caries can lead to pain, tooth loss, infection, inflammation and finally dead in severe cases. The exhibition of dental caries may be different whereas stages of development and risk factor are similar. In the beginning, it may appear in a tiny area that may progressively develop to a large cavity. Dental caries can be seen directly however some diagnostic method such as radiographic imaging is used to detect the less visible decay and to measure the extent of decomposition. Almost all the types of bacteria found in the oral cavity have efficient pathogenic potential to enhance inflammatory response on teeth and soft tissues. Existence of bacterial flora may be different in various areas of teeth such as dentin enamel junction beneath white spot lesion, gaps between cavity walls and restoration, areas of penetrated caries, fissures, root channels and remaining carious dentin beneath restoration (Borty et al 2015). In highly progressed cases, the infection can spread from the tooth to the surrounding soft tissues which may lead to edentulous mouth. A large number of *Streptococcus, Actinomyces* and *Lactobacillus* species are involved in root caries and periodontal diseases (Fayaz M et al 2014).

Dental are the localized destruction of the tissues tooth by bacterial action. Either enamel or cementum is demineralize by microbial acids. *Streptococcus mutants* are considered to be the main etiological microorganisms in caries disease, with *Lactobacilli* and other microorganism participating in the disease progression. Recent evidence also has supported the role of yeast (*Candida albicans*) as a member of the mixed oral microbiota involved in caries causation (Ozdemir 2014). However, dental caries are usually treated by surgical drainage and antibiotic administration. Random prescribing, inappropriate dosing and duration of treatment, over the counter availability of antibiotic have contributed to the rise of antibiotic resistance among various human pathogens. Antibiotic has become world-wide issue and is the leading cause for therapy failure. Therapy failure may occur if the pathogens involved are resistant to the drug of choice. Antimicrobial susceptibility testing is performed to guide the clinician in decision making (Chengappa et al 2015).

In a country like Nepal the prevalence of different dental disease is not fully explored and documented. But the pattern of dental disease has been changing with the implementation of different preventive procedures and preservation of maximum tooth structure as possible. In spite of this, dental caries and periodontal diseases are still the major cause for extraction, though their relative contribution to tooth mortality varies from place to place (Upadhyaya C 2009)

According to oral health facts (WHO), the most common oral diseases are dental decay and periodontal (gum) disease. Worldwide, 60-90% of school children have dental cavities. According to the Nepal national pathfinder survey 2004, it was found that the prevalence of dental decay is low in adolescents who are studying in school (i.e., 25.6% for 12- to 13-year-olds and 25.6% for 15-to 16-year-olds). The probable reason for this is the use of fluoridated toothpaste. (Dhakal B et al 2015). In India, the prevalence of caries among preschool children was found to be in range of 40-70%. According to the survey conducted in Nepal, 58% of 5-6 year old school children suffer from dental caries (Khanal K et al 2015). The global increase in dental caries prevalence affects children as well as adults,

primary as well as permanent teeth, and coronal as well as root surfaces. A very extensive and comprehensive National Health Survey was conducted in 2004 throughout the entire country of India which reported the prevalence of dental caries among 35-44 years old individuals to be 80.2%. A recent study done in Nepal showed the caries prevalence among 35-44 year olds to be 80.84%. (Shrestha N et al 2015). Oral health is the worldwide problem especially in Asia and Latin American countries. The burden of periodontal problems are more commonly found to be in adolescents which are either the result of poor oral hygiene or habit like tobacco use and alcohol consumption. Nepal is not exception to this problem. (Dhakal B et al 2015). Although children have a basic knowledge of dental health, such as importance of proper brushing and diet in preventing dental caries, many fail to brush their teeth effectively and tend to consume cariogenic foods and may underestimate health risks. (Khanal S et al 2014)

1.2. Statement of the problem:

Dental caries is a multifactorial oral diseases developed by the localized dissolution of the tooth hard tissues caused by bacteria. The frequently encountered bacteria in caries are the different species of streptococci (Subedi et al 2011). Dental caries has become one of the common problems of every individual. Many antibiotics such as Ciprofloxacin, Erythromycin, Cotrimoxazole, Amoxicillin, Penicillin, etc have been introduced against the pathogen involved in dental caries. However, random use of antibiotics has contributed to the rise of antibiotic resistance among various common human pathogens (Chengappa et al 2015). Therefore antibiotic susceptibility test is performed to evaluate sensitivity and resistance to antibiotics used in the treatment.

1.3. Objectives

1.3.1. General objectives

This study has been designed to obtain information about the pathogenic bacteria involved in dental caries and to evaluate the antibiotic susceptibility of microorganism causing odontogenic infection.

1.3.2. Specific objectives

a) To assess the bacterial species associated in dental caries.

b) To evaluate the sensitivity and resistant of pathogenic microorganism involved in dental caries.

c) To isolate the pathogen of dental caries.

d) To determine the antibiogram profile by disc diffusion method.

1.4. RATIONALE OF THE STUDY:

This study is performed to isolate the microbial pathogen responsible for dental caries. Similarly, this study helps. to evaluate the antibiotic resistant and sensitivity of pathogens in dental decay. The antibiotic susceptibility profile can be evaluated by disc diffusion method as well as can identify the microorganisms responsible in the dental carries.

1.5. RESEARCH QUESTION:

a) What is dental caries?

b) Which species is mostly responsible for dental decay?

c) What is the aim of antibiotic susceptibility test?

d) Which method is used for antibiotic susceptibility test?

e) What is the objective of this study?

1.6. LIMITATION OF THE STUDY:

-The study sample included only the students of Central Campus of Technology, therefore the research may not give clear figure about the dental carries of total population.

-Due to less time we cannot collect sample from different places.

- Fluctuation of incubation temperature may delay our.

CHAPTER-II
LITERATURE REVIEW

2.1. Dental caries

Dental caries is a chronic transmissible disease of multifactorial etiology. It has long been accepted that there are a large number of factors involved in the process of carries development. It is, however, the interaction of three principal factors destructive micro flora (plaque) host susceptibility (teeth and saliva) and a substrate (cariogenic diet), as described by keys three circle diagram that determine if the disease will occur. Demineralization of the tooth surface result when the cariogenic biofilm exists in an oral environment that is more pathological than protective. The specific manner in which etiology factor influence the disease process is complex, including host and pathogen adaptations, and is not fully understood. Dental carries is a slowly progressive disease, it is not a consequence of a singular event but rather a sequel of processes occurring over a period of time (Kraglund F 2009).

One of the current approaches to better understanding dental caries is the consideration of its microbiological origin. Bacterial biofilms are ubiquitous in nature and have been found to be involved in a wide variety of microbial infections in the body, including the formation of dental plaque. A biofilm is a structured community of microorganisms encapsulated within a self-developed polymeric matrix that adheres to a living or inert surface. It is a sophisticated ecosystem with its own infrastructure, including metabolic and waste channels, and mechanisms in which bacteria may share genetic material and communicate with one another (Kraglund F 2009).

When the oral environment favours there bacteria, they shift from normal healthy microflora to the acidogenic (acid-forming) and acidoduric (tolerate living in acidic environments) microorganism that are associate with dental carries. Bacteria in biofilms, such as dental plaque are better able to survive and exhibit stronger resistance to various environment factors as they are 1000 times more

resistance to antibodies, antibiotics and antimicrobial products. These attributes lead to persistent bacterial infections that will undoubtly represent a new challenge in the treatment of dental caries (Kraglund F 2009).

2.2. Oral microbiota

The oral cavity is the first part of the gastrointestinal tract and it has a number of features that makes it a distinct microbial habitat. The various surfaces in the oral cavity are continuously bathed with saliva and they represent different ecological niches in which distinct inhabitants exist within this complex environment. The ecological characteristics of the different surfaces found in the oral cavity, each with different key ecological factors such as adhesion ligands, pH, nutrients, redox potential, oxygen tension, and temperature, make it a unique microbial habitat in the human body. The composition of microbiota in the oral cavity is complex and such complicity was noticed in as early as 1683 by Antonie van Leeuwenhoek. The oral microbiota is composed predominantly of bacteria, but fungi, viruses, mycoplasmas, and even protozoa and archaea can be found. It is estimated that more than 700 cultivable and non-cultivable species are present in the oral cavity. Over 400 of the 700 oral species have been identified from the periodontal pocket and 300 species from other locations in the oral cavity. Any particular individual is thought to have approximately 100–200 of these 700 species and is thought to harbor around 50 species in the periodontal pocket (Mohammed Al-Haroni 2007).

Under the conditions in which the human-oral micro flora relationship evolved, the bacteria that inhabit the human mouth appear to have a commensal or even mutualistic relationship with their human host and with each other. The mouth can be considered an ideal environment for the growth of microorganisms, since it is warm and moist and has a constant influx of nutrients through saliva and food intake. In fact, it has been calculated that there are as many as 4 x 1010 organisms in each gram of plaque removed from the teeth. These organisms consist of, on an average, more than 400 species that live together through the exploitation of very specific ecological niches. The ecology of the mouth, however, does not just

involve interactions among microorganisms. In fact, the host plays a large role in maintaining a uniform ecosystem, especially through the saliva. Saliva is a complex mineral- and protein rich solution that delivers nutrients to the many bacterial species within the mouth while also protecting host surfaces. During mastication, increased saliva flow prevents changes in oral pH, because the buffer bicarbonate is present in saliva and acts as an acid sink at a time when acidic products are being introduced into the mouth. Urea and the peptide sialin are both also present in low concentrations in saliva and produce ammonia when hydrolyzed, a basic product capable of raising pH (Dwivedi 2010)

2.3. Pathogenic bacteria

The etiology of odontogenic infections is mixed where both aerobic and anaerobic bacteria are involved. The most commonly found facultative anaerobes belong to the viridians group streptococci and the milleri group streptococci. The viridians group of Streptococci comprises the mitis group, oralis group, salivarius group, sanguinis group and the mutans group. The milleri group comprises the anginosus group, constellatus group, intermedius group. Streptococci are frequently isolated from acute dental abscess and draining sinuses (Chengappa 2015). Dental caries also known as cavity or decay is a disease where bacteria cause damage in hard tooth structure. Lactic acid is produced as a by-product of carbohydrate metabolism by bacterial species which can demineralize enamel, dentin and cementum. The mineral composition of teeth is sensorial to increased acidic condition produced due to lactic acid. The dynamic breakdown of tissues enhanced by *Streptococcus mutans, Staphylococcus aureus* and *Lactobacillus spp*. produce dental caries. Almost all the types of bacteria found in the oral cavity have efficient pathogenic potential to enhance inflammatory response on teeth and soft tissues. Existence of bacterial flora may be different in various areas of teeth such as dentin enamel junction beneath white spot lesion, gaps between cavity walls and restoration, areas of penetrated caries, fissures, root channels and remaining carious dentin beneath restoration (Borty et al 2015). Several Gram-positive and Gram-negative bacterial genera are found in the oral cavity. Among

the Gram-positive ones are *Enterococcus, Peptostreptococcus, Streptococcus, Staphylococcus, Actinomyces, Corynebacterium, Eubacterium,* and *Lactobacillus* species, whereas *Aggregatibacter* (formerly *Actinobacillus*), *Haemophilus, Bacteroides, Campylobacter, Leptotrichia, Prophyromonas, Capnocytophaga, Prevotella, Tannerella, Eikenella, Treponema, Fusobacterium,* and *Wolinella* species are among the Gram-negative one (Mohammed Al-Haroni 2007)

2.4. Dental plaque

The diverse community of microorganisms found on a tooth surface is known as dental plaque. It is defined clinically as the soft, tenacious deposit that forms on tooth surfaces that is not readily removed by rinsing with water. Microbiologically, it can be defined as the diverse community of microorganisms found on a tooth surface as a biofilm, embedded in an extra-celluar matrix of polymers of host, and is of microbial origin. Recently, the classical name of bacterial deposits on tooth surfaces known as "dental plaque" is increasingly substituted by the more appropriate name "dental biofilm (Mohammed Al-Haroni 2007). Tooth has a unique structure and composition so that it is the only organ of the body not subject to metabolic inversion. In early stage bacteria usually brace to the substratum of the teeth. Then the growth and multiplication of bacteria colonize the surrounding area of teeth and initiate the formation of biofilm by agglomerating into long chain. That agglomeration of latent and actively growing bacterial colonies form complex heterogeneous structure with the aid of their enzymes and excretory products. Bacterial colonies heap on the surface of teeth is the primary etiological agent of dental caries. Bacteria interact between them on the dental surface by co-aggregation, metabolic exchange, cell-cell communication and reciprocation of genetic material. These are the mechanisms that make the biofilm difficult to destroy when the therapeutic drugs are used in dental diseases. Dental caries usually demolishes the enamel and dentin by bacterial activity. It is now avowed that the formation of bacterial biofilm is responsible for a variety of human diseases such as osteomyelitis, middle ear infections, dental caries, medical instrument and device related infections, native

valve endocarditis, ocular implant infections and chronic lung infections (Borty Chakra Shuvho et al 2015).

2.5. Responsible factor in dental caries

Human oral cavity is usually having certain temperature and moisture as well as containing different nutritional compound such as carbohydrates, lipids and proteins that shelter the growth of normal flora and sometimes playing as an incubator for some pathogenic bacteria (Borty Chakra Shuvho et al 2015). Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. Specifically, a tooth (which is primarily mineral in content) is in a constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva. When the pH at the surface of the tooth drops below 5.5, demineralization proceeds faster than remineralization (meaning that there is a net loss of mineral structure on the tooth's surface) (Dwivedi 2010)

Adhesion of bacteria species to oral surfaces is the initial event in their establishment as a distinct microbial community in different niches within the oral cavity. The initial adhesion is characterized by the presence of the same bacterial species that later on may modify the surrounding environment, making it suitable for another species to colonize (Mohammed Al-Haroni 2007). Despite the diverse community of microorganisms found within the oral cavity, it is characterized by a high degree of stability. Such a stable community is referred to as climax community. It is maintained in spite of host defense and modest environmental stress, such as, changes in saliva flow, diets, regular exposure to mouth rinses and tooth pastes, challenge by exogenous species and exposure to antimicrobials. This stability, referred to as microbial homeostasis, is of great importance to oral health as it insures that potentially harmful species remain in low numbers. Major environmental perturbations, such as pH or redox potential changes, are necessary to break the microbial homeostasis, resulting in deteriorated oral health and

development of diseases, such as periodontitis and dental caries (Mohammed Al-Haroni 2007).

There is a direct relationship between dental caries and the intake of carbohydrate (Ozdemir 2014). Caries risk is associated with a wide range of factors including high levels of mutans streptococci bacteria, previous caries experience, poor oral hygiene, sugar consumption, low fluoride exposure, reduced salivary flow, and social deprivation. Sugar consumption has been identified as the major cause of dental caries (Chamber 2012). The most cariogenic sugar is sucrose. Sucrose is highly soluble and diffuses easily into dental plaque, acting as a substrate for the production of extracellular polysaccharides and acids. Recent data indicate that high lipid content in saliva enhances caries activity. On the other hand, it is demonstrated that a caniogenic diet becomes less caniogenic when it is combined with cheese or milk products, probably because of the content of calcium phosphate in these product (Ozdemir 2014).

2.6. Antibiotics

Ever since the establishment of dental caries as an infectious disease, antibiotics are constantly in use for treating it. Chlorhexidine digluconate (CHX) had been a gold standard in this field. It has been used with the aim of elimination or suppression of *Streptococci mutans* in the oral cavity (Mohammed Al-Haroni 2007)

Antibiotic susceptibility testing is determination of antibiotics resistance pattern of bacteria. It will be ideal if susceptibility testing could always undertake before the prescription of antibiotics. Previously the most common methods of susceptibility testing were the disk diffusion test, agar dilution, and both micro- and macro-broth dilution. (Dwivedi et al 2010).

2.6.1. Antibiotic resistance

Bacterial resistance to antimicrobial agents can be either natural (inherent, intrinsic) or acquired Mohammed Al-Haroni 2007).

2.6.1.1. Natural (inherent, intrinsic) resistance

In this type of resistance all isolates of a certain bacterial species are not sensitive to the antimicrobial in question. This could be because of a lack of certain structures in bacteria that serve as the target molecules for the antimicrobial or the lack of metabolic processes essential for the activation of the antimicrobial. In agreement with this, bacteria without a cell wall (e.g., the Mycoplasma species) are naturally resistant to antimicrobial agents such as β–lactam antibiotics, having activity against the cell wall. Another example of natural resistance is in the case of enterococci and cephalosporin. There are no penicillin-binding proteins in enterococci that bind these drugs with high affinity, and thus enterococci are intrinsically resistant to these agents Mohammed Al-Haroni 2007).

2.6.1.2. Acquired resistance

In contrast to natural resistance, acquired resistance is found only in some isolates of a certain bacterial species. However, sometimes the percentage of resistant isolates could reach high figures and susceptible isolates are hardly found. Acquired resistance in bacteria is evolved because of genetic alteration that can be achieved by two mechanisms: chromosomal mutation in the preexisting bacterial genome or, most frequently, by horizontal gene transfer between bacteria both within and outside species. Horizontal gene transfer allows bacterial population to develop antibiotic resistance at a rate significantly greater than would be afforded by mutation of chromosomal DNA. Indeed, horizontal gene transfer is the most frequent pathway for the dissemination of antibiotic resistance genes Mohammed Al-Haroni 2007).

2.7. Prevention

The lifestyle of a person and behavioral factors that manipulate the oral hygiene undoubtedly, influence the susceptibility to dental caries (Selwitz et al 2007). The evidence that frequent consumption of food and drinks containing fermentable carbohydrates (sugars) is associated with dental caries is overwhelming. However, the relationship between dietary factors and caries is far from

straightforward, particularly in the current environment where fluoride exposure is widespread. (Whelton H et al 2009)

Dental caries is correlated with sugar uptake in the diet. Numerous studies indicate a linear correlation of sugar consumption with dental caries in populations worldwide (Islam B et al 2007). Eating fibrous food including apples and carrots can reduce caries risk. The frequency of consumption of food and /or drinks containing free sugar should be limited to a maximum of four times per day. In order to minimize the occurrence of dental erosion, the amount and frequency of intake of soft drink and juices should be limited (Moynihan and Petersen 2004). Dairy products have properties that protect teeth against caries, and eating cheese after exposure to sugar rapidly neutralizes plaque acidity. Chewing sugar-containing gum increases caries risk but chewing sugar-free gum after meals can reduce caries risk (Ozdemir 2014).

Oral care, undoubtedly, begins with oral hygiene. Frequent brushing and flossing of teeth helps remove bacteria and fermentable substances. The continuous flow of saliva reduces the cariogenic flora on the tooth. Saliva also acts as a buffering agent during the continuous acid production in the oral cavity. Individuals with dry mouth syndrome are hence more prone to dental caries. This has been observed with people following radiation treatment of head and neck cancers, in narcotics users, and in patients with Sjögren's syndrome (Islam B et al 2007).

Another key determinant includes access to fluoride, either through the water supply, or from another source, such as toothpaste or topical fluoride application. Systematic reviews have suggested that water fluoridation can provide a preventive effect against decay (Chamber 2012). Fluoride has been at the forefront of caries prevention for over 60 years. A series of Cochrane systematic reviews found that topical fluorides (varnish, gel, mouth rinse and toothpaste), used either individually or in combination, significantly reduced caries in children and adolescents compared to placebo or no treatment. However, brushing with fluoride toothpaste is the most important method for delivering fluoride to the tooth surface (Moynihan and Petersen 2004). Fluoride toothpaste is

the most widely used form of topical fluoride throughout the world. It has been suggested that tooth brushing with fluoride toothpaste is close to an ideal public health method in that its use is convenient, inexpensive, culturally approved and widespread (Whelton H et al 2009).

CHAPTER III

METHODOLOGY

3.1. Site of the study

The study was carried out in Central Camus of Technology, Dharan, Sunsari.

3.2. Research methodology

The study was based on the descriptive and quantitative nature. This study was based on the culture method.

3.3. Types of study

The study was of descriptive type.

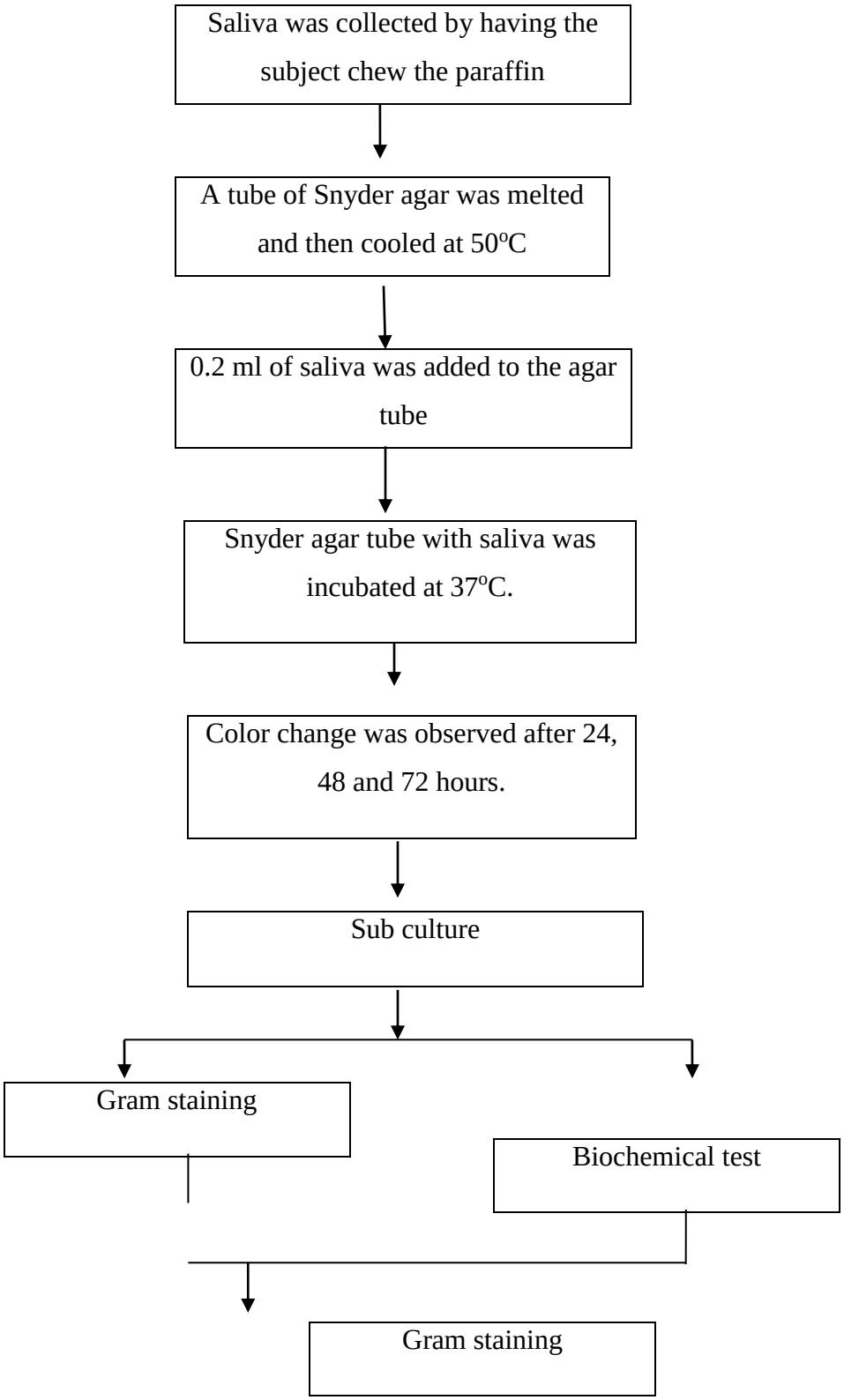
3.4. Sample

Saliva was taken as sample.

3.5. Description of the research

The sample for this study was taken from the student of Central Campus of Technology, Hattisar, Dharan. The laboratory work was carried out from 2073/02/25 to 2073/04/25 in the laboratory of Department of Microbiology, Central Campus of Technology, Dharan. The laboratory was provided with all the necessary materials and equipments that were required to carry out this study.

3.6. Research Design



3.7. Sample collection:

For this study, 50 samples were taken from the students of CCT aged from 21 to25. The students were given to chew chewable paraffin for 2 to 3 minutes and saliva samples were collected in the test tube separately. The sample was processed between three to four hours of collection

3.8. Isolation and identification

3.8.1. Media preparation

For this study, the preliminary work was the preparation of media for the isolation of oral flora. Media like nutrient agar medium and snyder agar medium were prepared. Similarly, biochemical test media were also prepared for the identification of oral microflora. All the ingredients were weighed and dissolved in distilled water. The ingredients were dissolved in water by boiling and shaking. The media was autoclaved at 121^{o}C at 15 lbs/inch for 15 min.

3.8.2. Sample processing:

After the samples were collected, the samples were processed as follows.

I. Snyder agar test:

The Snyder agar tube was melted and then cooled to 50°C. 0.2 ml of saliva was added to the Snyder agar tube and was shaken well. The agar tube along with saliva was incubated at 37°C and the color change was observed after 24, 48 and 72 hours.

II. **Sub Culture:**

After observation of color, from each tube of Snyder agar the sample were further sub cultured on the Nutrient Agar plates by streaking method and were again incubated at 37˚C for 24 hours.

III. **Biochemical test:**

After incubation for 24 hours, the sub culture was tested for the identification of bacteria. The biochemical tests like catalase, oxidase, IMViC, TSI, urease and gelatin liquefaction tests were performed to identify the pathogens.

a. **Gram staining**

It is the first step of the biochemical test which is performed in order to differentiate the bacteria i.e. whether the bacteria is gram positive or gram negative.

b. **Catalase test**

This test is primarily used in order to demonstrate the presence of catalase enzyme in the bacteria.

c. **Oxidase test**

The basic principle of this test is to determine the ability of bacteria to produce oxidase enzyme. Cytochromeoxidase, an enzyme of the bacterial electron transport chain.

d. **IMViC test**

This test consists of four different tests based on the biochemical activities of the organisms, they are: indole production, methyl red, Voges-Proskauer and citrate utilization. Indole test was performed on the trypton broth by using kovac's reagent. Methyl red and Voges-Proskauer test was performed on the MRVP broth by using methyl red as the reagent in the MR test and VP reagent (KOH and alpha naphthol). Citrate test was performed by streaking the organisms in the slant of Simmon's citrate agar media.

e. **TSI Test**

TSI stands for the Triple sugar iron test. TSI test is used for the determination of carbohydrate fermentation and hydrogen sulphide production. This test is carried out in the slant of TSIA media which

consist of three sugars: lactose, sucrose and a very small amount of glucose. This test is generally used to identify enteric pathogen.

f. Urease test

This test is performed for the identification of bacteria based on their ability to hydrolyze the urea.

g. Gelatin Hydrolysis test:

Gelatin hydrolysis test is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin.

3.9. Antibiotic Susceptibility Testing

The susceptibility of isolates to various conventional antibiotics was determined using Mueller-Hinton agar while employing the disk diffusion method. The antibiotics used in this study include Amoxicillin (Amx), Ciprofloxacin (Cip), Erythromycin (Ery), Ampicillin (Amp)

CHAPTER IV

RESULTS

The results of the study are categorized as the result of the culture, subculture and the results of the biochemical tests, separately for each of the sample, which are presented in the tables I and II.

A total of 50 students from Central Campus of Science and Technology aged 21-25 years were studied. Of the total participants, 90% were females and 10% were males. While questioning the student, it was found that all of them had the knowledge about oral hygiene and oral disease. The entire students floss their mouth after meal and brush their teeth twice a day to maintain oral hygiene.

In this study, samples were subjected to Snyder agar test.50 samples were proceeded in this test. The entire sample showed negative test for Snyder agar test which indicates that the entire individual are not susceptible to dental caries.

The organisms were isolated after the subculture followed by gram staining and biochemical test. After gram staining, except sample-1, 11, 21, 31 and 41 which was gram positive rod, other remaining 45 sample were gram negative cocci arranged in cluster which was presented below in table-I. The biochemical test involved in this study were IMViC (indole, methyl red, Voges-Proskauer and Citrate), catalase, oxidase, TSI (triple sugar iron), urease and gelatin liquefaction test. The biochemical test result was shown in the table-I. All 50 samples were indole, MR and VP negative. Citrate utilization test was positive for sample-1, 11, 21, 31 and 41 and negative for the remaining 45 sample. Gelatin liquefaction test was negative for all 50 samples. From the performed test two species of sample

were identified. The sample-1, 11, 21, 31 and 41 were isolated as *Corynebacterium* ssp and the remaining 45 sample were found to be *Neisseria* spp.

The antibiotic sensitivity test of the isolated bacteria was performed and the result was presented in the table-II. According to this table ampicillin and amoxicillin were mostly resistance to *Cornybacterium* ssp and *Neisseria* spp while ciprofloxacin and erythromycin were sensitive to the isolated species.

CHAPTER V
DISCUSSION

This study was conducted to evaluate the oral flora and dental caries status in 21-25 years old population. The oral cavity is colonized by a diverse range of microorganism. This comprises 300-500 species of bacteria, fungi and protozoa, of which only 10% are regularly, isolated using conventional culture techniques (Sweeney et al 2004). Bacteria found in the oral commensal flora include α-haemolytic streptococci, coagulase-negative staphylococci, gram negative cocci belongings to the families Neisseriaceae and Veillonellaceae, lactobacilli, spirochetes, corynebacteria and mycoplasmas which was presented by Sweeney et al (2004) but in the present studies only neisseriae and Corynebacteria were identified.

Bacteria that are potentially pathogenic and that are sometimes found in the oral cavity include *Staphylococcus aureus*, *Enterococcus faecalis*, *S. pneumonia*, *Streptococcus pyogenes*, *Neisseria meningitides*, member of the family Enterobacteriaceae, *Haemophilus influenza* and actinomycetes (Sweeney et al 2004).

According to Okopi et al (2015) dental caries is one of the most common and less attended diseases in the developing countries. There are vast diversities of human bacteria pathogen that are present in the mouth, and each individual has his or her own unique oral flora; but the composition of the oral flora determines the susceptibility of tooth decay. Gauri S et al (2012) mentioned that *Lactobacillus acidophilus, Streptococcus mutans* and *Actinomyces odontolyticus* were the microorganism thought to be responsible for dental caries. These bacteria in the mouth ferment carbohydrate clinging tenaciously to the teeth with the formation of lactic acid and other organic acids, thus reducing the pH of the mouth less than 5 at which decalcification occurs and dental decay begins. However in this study there is absence of *Lactobacillus acidophilus, Streptococcus mutans* and *Actinomyces odontolyticus.*

The result of disk diffusion method revealed that the most isolates were found to be sensitive to the antibiotic tested. Our report does not agree with report found by Okopi et al (2015) that dental caries pathogens were resistance to ciprofloxacin, ampicillin, amoxicillin and erythromycin. In this study ampicillin and amoxicillin are resistance and ciprofloxacin and erythromycin are sensitive to *Corynebacterium* and *Neisseria* spp.

The resistance data reviewed by Sweeney et al (2004) demonstrated that the presence of resistance in oral flora is an international problem. Studies have been performed in countries including Germany, Taiwan and Brazil which show the degree of antibiotic resistance present in particular oral commensal, often those associated with dental infections. The resistance pattern of the isolates towards the tested antibiotics maybe due to the wide spread abuse of the antibiotics (Mussrat et al 2014).

A number of methods are used in oral hygiene to prevent and cure oral diseases. The American Dental Association recommends that the individual must brush twice per day and use floss or other interdental cleaner once per day to effectively remove the microbial plaque. However in this study, almost all student brush twice per day but only 50% of student only now about dental floss (Dixit et al 2013).

Khanal S et al (2014) have suggested that the brushing of teeth twice a day was poor 24%-40% in different Nepali population but in this study the majority of population use toothbrush and toothpaste to brush their teeth twice a day. Rinsing mouth with water after meal is common practice in Nepal, similar practice was observed in the studied population with maximum rinsing their mouth after every meal.

Dhruw C et al (2012) reported that triclosan and fluoride containing toothpastes were found to be highly effective against the acidogenic bacteria. Ayurvedic contents of herbal toothpaste the *Syzgium aromaticum* helps to prevent toothache, *Mentha requienii* helps to prevent bad breathe and kills harmful germs. But in the present study only 10% of students use herbal toothpaste.

Muttaiyah et al (2011) and Mishra et al (2005) demonstrated that most patients with *Corynebacterium diphtheria* endocarditis have underlying cardiac disease, prosthetic valves, or a history of intravenous drug use. In the cases presented here there is presence of corynebacteria but in the patient with this organism do not have any cardiac disease.

Zasada AA (2013) suggested that 22% cases of dental caries were caused by *Corynebacterium diphtheria* but in the present report it was not found to cause dental caries.

Rahman et al (2015) demonstrated that streptococci are the most abundant microflora found in the oral cavity, skin surface upper respiratory and digestive track. But in this study, *Corynebacterium* and *Neisseria* were found as microflora of oral cavity.

Corynebacterium and *Neisseria* were isolated by the Ross PW (2016) from the salivary flora which resembles with this study where these two organisms were isolated from the saliva of the students. It also had been stated by Ross PW (2016) that lactobacilli were always present in the mouth, although in number that varied considerably, but this was not found to be so in the present study.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1. Conclusion:

It was concluded in this study that *Corynebacterium* and *Neisseria* were isolated and identified as normal oral flora. It was further concluded that bacteria isolates were relatively susceptible to the antibiotics used in this study. It was found that ampicillin and amoxicillin are resistance and ciprofloxacin and erythromycin are sensitive to *Corynebacterium* and *Neisseria* spp.

5.2. Recommendation:

- Research can be performed to determine the role of *Corynebacterium* and *Neisseria* spp in dental caries.
- Awareness program should be conducted for oral health education.
- Research can be conducted to evaluate the effects of *Corynebacterium* and *Neisseria.*

REFERENCE

Borty C.S, Hafiz K, Ali Md , Begom K, Ahammed T, Monir S, Islam Md (2015). Isolation, Identification and Antibiogram Profile of Bacteria Isolated from Dental caries patient of Mymensing District of Bangladesh. Asian Journal of Medical and Biological Research. **3** (5):244-245.

Chamber S (2012). Public health and dental carries in young children in deprived communities in Scotland, Scottish University Medical Journal.

Chengappa R, Rakaraddi M, Khan A, Narayanan B, Mekaddath J, Rangaswamy K (2015). Primary Antibiotic Susceptibility against Streptococci in Odontogenic Infection. Internationa Journal of Pharmaceutical Science and Drug Research. 7(6):474-477.

Dhakal B,Humagain M, Kafle D(2015). Prevalence of gingivitis among the patients visiting dental OPD of Dhulikhel hospital. Journal of Nepal Dental Association. 15(1): 24-27.

Dhruw C, Rajmani H, Bhatt R, Verma P (2012). Isolation of dental caries bacteria from dental plaque and effect of toothpastes on acidogenic bacteria. Open journal of medical microbiology.2: 67-68.

Dixit PB, Dixit S, Singh R, Khanal P (2013). Oral hygiene awareness and practices among the Nepalese school children in Bhaktapur. Journal of Nepal Medical Association.13(2): 23-24.

Dwivedi D, Patidar RK, Kushwan S, Kushwan T, Kushwan M, Amdekar S, Singh V(2010). Study of Molecular Mechanism of Antibiotic Susceptibility against Polymicrobial Oral infection. Barkatillah University. Bhopal, India.

Fayaz M, Shiva Kumar KP, Joe M (2014). Prevalence and Antibiotic Susceptibility Pattern of Dental Bioflim forming Bacteria. International Journal of current microbiology and Applied Science. 3(5):46-47.

Gawri S, Shukla P, Chandrakar A (2012). A survey of micro flora present in dental caries and its relation to environmental factors. Recent research in science and technology. 4(3):11-12.

Hillman JD, Brooks TA, Michalek SM (2002). Construction and characterization of effectors strain of *Streptococcus mutans* for replacement therapy of *Streptococcus mutans*. Journal of Dental Research. 68: 543–549

Islam B, Khan NS, Khan UA (2007). Dental caries: From infection to prevention; Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India. 13(12): 196-203.

Khanal K, Shrestha D, Ghimire N, Younjan R, Sanjel S (2015). Assessment of knowledge regarding oral hygiene among patients of pre-school children attending pediatric out patient department in Dhulikhel Hospital. Kathmandu University Medical Journal. 2(2):55-60.

Khanal S, Acharya J (2014). Dental caries status and oral health practice among 12-15 years old children in Jorpati, Kathmandu. Nepal Medical College Journal. 16(1): 84-87.

Kraglund F (2009). Evaluation of Caries Risk Assessment Model in an Adult Population. Master of Science Graduate Department of Dentistry (Dental Public Health). University of Toronto.1-7.

Mishra B, Dignan RJ, Hughes CF, Hendel N (2005). *Corynebacterium diptheriae* endocarditis- surgery for some but not all! Asian Cardiovasc Thorac Ann.13:119-120.

Mohammed Al-Haroni (2007). Antibiotic Resistance in Oral Microbiota; Thesis for the degree Philosopphiae Doctor (PhD) at the University of Bergen: 7-17.

Moynihan P, Petersen EP (2004). Diet , nutrition and the prevention of dental diseases. Public Health Nutrition. 7(1A): 201-226.

Muttaiyah S, Best EJ, Freeman JT, Taylor SL, Morris AJ, Roberts SA (2011). *Corynebacterium diptheriae* endocarditis: a case series and review of the treatment approach. Int J Infect Dis.15:584-585

Okopi A, Okwori J, Michael G, Okojokwu JO, Audu O (2015). Bacterial profile associated with dental caries in Jos, Nigeria. Advances in Research.4 (6): 374-375.

Olorunjuwon B, Hanteefat E, Titilaya A, Adetola A (2013). Antibiotic Sensitivity profile of bacteria isolated from decayed teeth, Scholars Academic Journals of Pharmacy.2 (6): 424-428.

Ozdemir D (2014). Dental Caries and preventive strategies, Journal of Educational and Instructional Studies in the World.4 (4):142-143

Rahman M, Islam Md, Islam NM, Hossain SM (2015). Isolation and identification of oral bacteria and characterization of bacteriocin production and antimicrobial sensitivity. Department of genetic engineering and biotechnology, University of Dhaka.14 (1): 103-109.

Ross PW (2016). Quantitative studies on the salivary flora. J Clin Pathol. 24:717-720.

Selwitz RH, Ismall AI, Pitts NB(2007). Dental caries , Lancet. 369: 51-59.

Shrestha N, Acharya J, Bishet S (2015). Oral health perceptions, practice, dental caries prevalence, severity and related quality of life among adults aged 35-44 years in Jorpati, Nepal. Kathmandu University Medical Journal**.** 17(1-2): 36-42.

Subedi B, Shakya P, KC U, Jnawali M, Paudyal BD, Acharya A, Koirala S, Singh A (2011). Prevalence of dental caries in 5-6 year and 12-13 years age group of school children of Kathmandu valley, Journal of Nepal Medical Association.51 (184):176-181.

Sweeney LC, Dave J, Philip A, Chambers, Heritage J (2004). Antibiotic resistance in general dental practice- a cause for concern? Journal of Antimicrobial Chemotherapy. 53:567-568.

Upadhyaya C, Humagain M (2009). The pattern of tooth loss due to dental caries and periodontal disease among patient attending dental department, Dhulikhel Hospital. Kathmandu University Medical Journal.**7**(25); 59-62.

Whelton H, Beirne P, Clarke M, Farrell M, Ormsby M (2009). Strategies to prevent dental caries in children and adolescents. Iris oral heath services guideline initiative: 19-26. services guideline initiative: 19-26.

Zasada AA (2013). Nontoxigenic highly pathogenic clone of *Corynebacterium diptheriae* , Poland. National institute of public health.19(11): 1870-1871.

APPENDICES

Appendix A

Media composition

Synder test Agar

Trypton:	20g
Sodium Chloride:	5g
Dextrose:	20g
Bromocresol green:	0.02g
Agar:	20g
Distilled water:	1000ml

Nutrient Agar: (pH-7.0)

Peptone:	5.0 g
Beef extract:	3.0 g
NaCl:	5.0g
Agar:	15g
Distilled Water:	1000ml

Mueller Hinton Agar Medium:

Beef extract:	2g
Acid hydrolysate of casein:	17.5g
Starch:	1.5g
Agar:	17g
Distilled water:	1000ml
pH:	7.3

Mannitol salt Agar

Mannitol :	10g
Peptone :	10g
Sodium Chloride:	75g

Beef extract:	0.025g
Agar :	15g
Distilled water:	1000ml

Gelatin agar medium

Gelatin :	40g
Tryptone :	17g
Sodium Chloride:	5g
Dipotassium phosphate:	2.5g
Distilled water:	1000ml
Ph:	7.2

Tryptone broth

Tryptone :	10g
Sodium chloride:	5g
Calcium chloride:	1g
Distilled water:	1000ml
pH:	7.2

MR-VP broth

Peptone:	7g
Potassium phosphate:	5g
Dextrose :	5g
Distilled water:	1000ml
pH:	6.9

Simmons Citrate Agar Medium

Ammonium dihydrogen phosphate:	1g
Dipotassium hydrogen phosphate:	1g
Sodium chloride:	5g
Sodium citrate:	2g

Magnesium sulphate:	0.2g
Bromo thymol blue:	0.08g
Agar:	15g
Distilled water:	1000ml

Urea agar medium

Peptone :	1g
Sodium chloride:	5g
Potassium monohydrogen phosphate:	2g
Agar :	20g
Distilled water:	1000ml
Glucose:	1g
Phenol red (0.2% solution):	6m
Urea (20% aqueous solution):	100ml

Appendix B

Stains and Reagent used

Crystal violet

Solution A

Crystal violet (90% dye content):	2g
Ethyl alcohol:	20ml

Solution B

Ammonium oxalate:	0.8g
Distilled water:	80ml

Gram's iodine

Iodine :	1g
Potassium iodide:	2g
Distilled water:	300ml
Ethyl alcohol:	95ml
Distilled water:	5ml

Safranin

Safranin (2.55 solution in 95% ethyl alcohol):	10ml
Distilled water:	100ml
Hydrogen peroxide:	3ml
Distilled water:	97ml

Methyl red solution

Methyl red:	0.04g
Ethyl alcohol (absolute):	40ml
Distilled water:	60ml

V-P reagent I

α –Napthol:	5g
Ethyl alcohol:	100ml

V –P reagent II

Potassium hydroxide:	40g
Distilled water:	100ml

Oxidase reagent

Dimethyl

P-phenylenediaminedihydrochlloride:	1g
Distilled water:	100ml

Appendix C

Table I: Biochemical test results

Sample No	Gram staining		Biochemical characteristics								
	Gram reaction	Shape	Indole	MR	VP	Citrate	Urease	Catalase	Oxidase	TSI	Gelatinase
1	+	Rod	-	-	-	+	+	+	+	A/A	_
2	-	Cocci	-	-	-	-	+	+	+	A/A	-
3	-	Cocci	-	-	-	-	+	+	+	A/A	-
4	-	Cocci	-	-	-	-	+	+	+	A/A	-
5	-	Cocci	-	-	-	-	+	+	+	A/A	-
6	-	Cocci	-	-	-	-	+	+	+	A/A	-
7	-	Cocci	-	-	-	-	+	+	+	A/A	-
8	-	Cocci	-	-	-	-	+	+	+	A/A	-
9	-	Cocci	-	-	-	-	+	+	+	A/A	-
10	-	Cocci	-	-	-	-	+	+	+	A/A	-
11	+	Rod	-	-	-	+	+	+	+	A/A	_
12	-	Cocci	-	-	-	-	+	+	+	A/A	-
13	-	Cocci	-	-	-	-	+	+	+	A/A	-
14	-	Cocci	-	-	-	-	+	+	+	A/A	-
15	-	Cocci	-	-	-	-	+	+	+	A/A	-
16	-	Cocci	-	-	-	-	+	+	+	A/A	-
17	-	Cocci	-	-	-	-	+	+	+	A/A	-
18	-	Cocci	-	-	-	-	+	+	+	A/A	-
19	-	Cocci	-	-	-	-	+	+	+	A/A	-
20	-	Cocci	-	-	-	-	+	+	+	A/A	-
21	+	Rod	-	-	-	+	+	+	+	A/A	_
22	-	Cocci	-	-	-	-	+	+	+	A/A	-
23	-	Cocci	-	-	-	-	+	+	+	A/A	-
23	-	Cocci	-	-	-	-	+	+	+	A/A	-
25	-	Cocci	-	-	-	-	+	+	+	A/A	-
26	-	Cocci	-	-	-	-	+	+	+	A/A	-
27	-	Cocci	-	-	-	-	+	+	+	A/A	-

28	-	Cocci	-	-	-	-	+	+	+	A/A	-
29	-	Cocci	-	-	-	-	+	+	+	A/A	-
30	-	Cocci	-	-	-	-	+	+	+	A/A	-
31	+	Rod	-	-	-	+	+	+	+	A/A	_
32	-	Cocci	-	-	-	-	+	+	+	A/A	-
33	-	Cocci	-	-	-	-	+	+	+	A/A	-
34	-	Cocci	-	-	-	-	+	+	+	A/A	-
35	-	Cocci	-	-	-	-	+	+	+	A/A	-
36	-	Cocci	-	-	-	-	+	+	+	A/A	-
37	-	Cocci	-	-	-	-	+	+	+	A/A	-
38	-	Cocci	-	-	-	-	+	+	+	A/A	-
39	-	Cocci	-	-	-	-	+	+	+	A/A	-
40	-	Cocci	-	-	-	-	+	+	+	A/A	-
41	+	Rod	-	-	-	+	+	+	+	A/A	_
42	-	Cocci	-	-	-	-	+	+	+	A/A	-
43	-	Cocci	-	-	-	-	+	+	+	A/A	-
44	-	Cocci	-	-	-	-	+	+	+	A/A	-
45	-	Cocci	-	-	-	-	+	+	+	A/A	-
46	-	Cocci	-	-	-	-	+	+	+	A/A	-
47	-	Cocci	-	-	-	-	+	+	+	A/A	-
48	-	Cocci	-	-	-	-	+	+	+	A/A	-
49	-	Cocci	-	-	-	-	+	+	+	A/A	-
50	-	Cocci	-	-	-	-	+	+	+	A/A	-

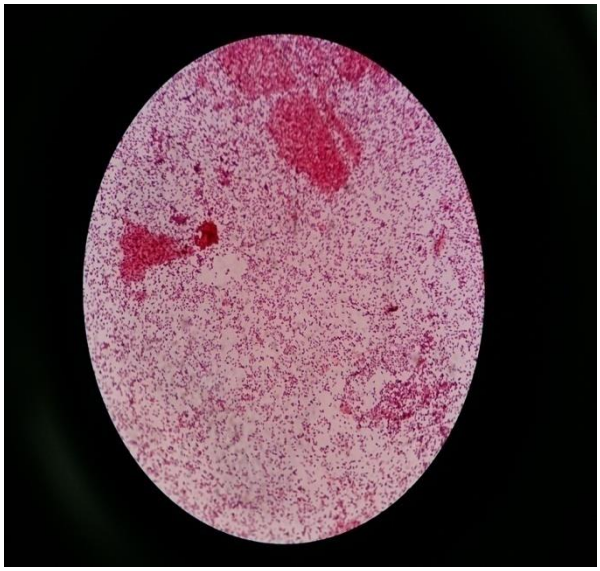
Table II: Antibiotic susceptibility pattern

Sample No	Antibiotics (Diameter zone of inhibition mm)			
	CIP	AMP	AMX	E
1	30	14	14	16
2	20	R	R	10
3	18	R	R	6
4	10	R	R	6
5	20	2	4	6
6	20	R	R	2
7	20	6	8	20
8	20	R	R	4
9	20	R	R	14
10	10	R	R	6
11	30	14	14	16
12	20	R	R	10
13	18	R	R	6
14	10	R	R	6
15	20	2	4	6
16	20	R	R	2
17	20	6	8	20
18	20	R	R	4
19	20	R	R	14
20	10	R	R	6
21	30	14	14	16
22	20	R	R	10
23	18	R	R	6
24	10	R	R	6
25	20	2	4	6
26	20	R	R	2
27	20	6	8	20
28	20	R	R	4
29	20	R	R	14
30	10	R	R	6
31	30	14	14	16
32	20	R	R	10

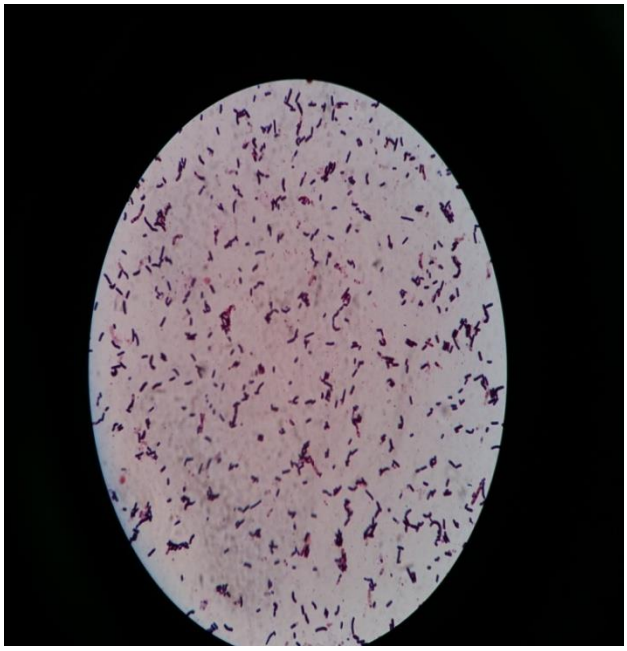
33	18	R	R	6
34	10	R	R	6
35	20	2	4	6
36	20	R	R	2
37	20	6	8	20
38	20	R	R	4
39	20	R	R	14
40	10	R	R	6
41	30	14	14	16
42	20	R	R	10
43	18	R	R	6
44	10	R	R	6
45	20	2	4	6
46	20	R	R	2
47	20	6	8	20
48	20	R	R	4
49	20	R	R	14
50	10	R	R	6

CIP=Ciprofloxacin, AMP=Ampicillin, AMX=Amoxicillin, E=Erythromycin, R= Resistant

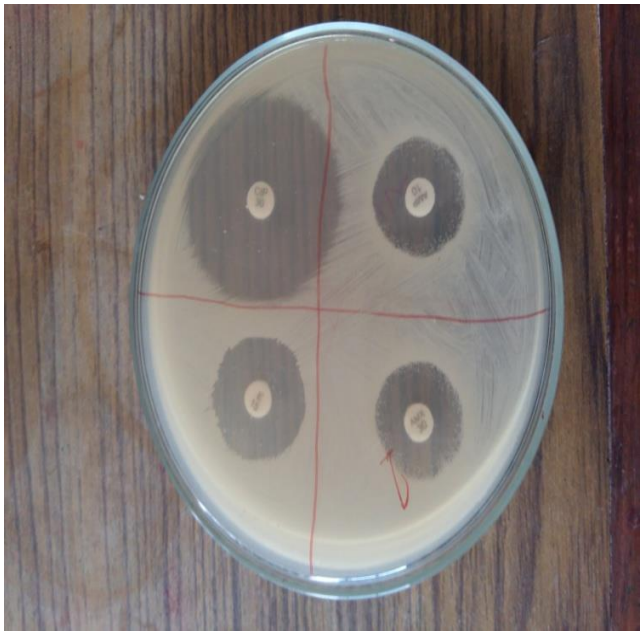
PHOTOGRAPHS



1. Gram negative rod shaped cell of *Neisseria* spp.



2. Gram positive rod shaped cell of *Corynebacterium* spp.



3. Antibiotic sensitivity test of *Corynebacterium* spp.

4. Antibiotic sensitivity test of *Neisseria* spp.