ISOLATION AND IDENTIFICATION OF ORAL BACTERIA CAUSING PERIODONTITIS IN HUMAN WITH ANTIBIOGRAM STUDY

A THESIS

BY

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The author
December, 2017

ABSTRACT

The Presence study was conducted to isolate and identify the oral bacteria causing periodontitis in human by using morphological, cultural and biochemical techniques. However the prevalence of periodontitis was higher in young (65%) in comparing with child(1.3%) and adult(29%) stages. The prevalence of oral bacteria was higher in plaque in comperison with caries (27%) and tartar (23%) respectively. On the other hand the prevalence was also varied with the sugar consumption. In which the highest prevalence were absence in high sugar intake patients (30%) and lowest in less sugar intake patients (25.3%). Out of 150 samples 85% samples were found to be positive for Streptococcus and 75% for Staphylococcus and 65% for *Porphyromonus gingivalis* by using morphological and biochemical techniques. The identified isolates were also subjected to antibiogram study. In which most of the organism were resistant to maximum antibiotic which are available in commercially and sensitive to Ofloxacine. In this study it was observed that most of the oral bacteria were resistant to commonly used antibiotics that is the related for treating the oral infection on the other hand ofloxacine was only the sensitive antibiotic against oral bacteria. From this study it was concluded that determination of antibiotic susceptibility is very important in the determination of first line of choice for drugs in clinical control of periodontitis.

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LIST OF ABBREVIATION AND SYMBOLS

CFU	Colony forming unit
CP	Crude Protein
CO2	Carbon dioxide
E. coli	Escherichia coli
e.g	Example
EMB	Eosin Methylene Blue
et.al	Associated
etc	Etcetra
Fig.	Figure
GDP	Gross Domestic Product
gm	Gram
H2O2	Hydrogen peroxide
H2S	Hydrogen Sulphide
HSTU	Hajee Mohammad Danesh Science And Technology University
lbs	Pounds
Kg	Kilogram
KOH	Potassium hydroxide
Ltd	Limited
MC	Mac conkey
mg	Miligram
Min	Minute
MIU	Motility Indole Urease
mm	Milimeter
ml	Mililiter
MR	Methyl red
NA	Nutrient Agar
NB	Nutrient Broth
No.	Number
PBS	Phosphate buffered saline
S.	Salmonella
SDA	Sabourauds Dexrose agar
Sec	Second
SL.	Serial
SPP	Species
SS	Salmonella-shigella
SSA	Salmonella-Shi
TSI	Triple sugar iron
VP	Voges-Proskauer
+	Positive
-	Negative
%	Percentage
<	Less than
>	Greater than
^{o}C	Degree Celsius

CHAPTER-I

Introduction

Periodontitis disease represents a group of oral inflammatory infections initiated by oral pathogens which exist as a complex biofilms on the tooth surface and cause destruction to tooth supporting tissues. The severity of this disease ranges from mild to moderate and reversible inflammation of the gingiva (Periodontitis) to chronic destruction of connective tissues, the formation of periodontitis pocket and ultimately result in loss of teeth. While human subgingival plaque harbors more than 500 bacterial species like Streptococcus, Staphylococcus, Lactobacillus, considerable research has shown that *Porphyromonus gingivalis*, a Gram-negative anaerobic bacterium, is the major etiologic agent which contributes to chronic periodontitis. This black-pigmented bacterium produces a myriad of virulence factors that cause destruction to periodontitis tissues either directly or indirectly by modulating the host inflammatory response. Here, this review provides an overview of *Porphyromonus gingivalis* and how its virulence factors contribute to the pathogenesis with other microorganism consortium in oral cavity. (*Ref. BL Pihlstrom, BS Michalowicz, NW Johnson - The Lancet, 2005 – Elsevier*)

The present study was aimed to increase the knowledge on the virulence potential of *Porphyromonus gingivalis* as a putative Periodontitis pathogen. *Porphyromonus gingivalis* was selected to be the model species for a Periodontitis pathogen based on its characteristic of expressing a number of significant and unique virulence factors and on the considerable genetic heterogeneity of this species. The hypothesis of the present studies was that the pathogenic potential of *Porphyromonus gingivalis* differs among this species and that certain types of *Porphyromonus gingivalis* have a more pathogenic capacity than others. The over all aim of this thesis was to investigate the phenotypic and genotypic characteristics and virulence properties of the species *Porphyromonus gingivalis. (Ref. VN Stone - 2015)*

Periodontitis disease, a disorder involving bacterial infection of the gums, is very prevalent in the United States today. It is estimated that 35.7 million Americans are living with the disease. It is present when inflammation and infection destroy the tissues that support the teeth, including the gums, the Periodontitis ligaments, and the tooth sockets. Periodontitis, the mildest form of Periodontitis disease, is an inflammatory process confined to the gums. It is caused by a nonspecific, long-term accumulation of plaque on the teeth and is usually reversible. Plaque-induced Periodontitis is the most common form of the disease. Plaque is a

sticky material that develops on the exposed portions of the teeth, consisting of bacteria, mucus, and food debris. If plaque accumulates on the teeth, it mineralizes into tartar, a hard white substance that forms around the base of the teeth. Tartar makes plaque more difficult to remove. The tartar and plaque inflame the gums, which can result in infection. The longer plaque and tartar remain on the teeth, the more they irritate the gingiva and thus Periodontitis occurs. More than 75% of the population experiences Periodontitis at some level. Inflammation of the gums also can occur from injury or trauma caused by overly vigorous brushing or flossing of the teeth. Systemic conditions, such as diabetes, pregnancy, and general illness, can place your gums at risk for Periodontitis. More than 75% of the population experiences Periodontitis at some level. It can be caused by a lack of effective oral hygiene or by the hormonal changes during puberty or early adulthood. Once it develops, Periodontitis may persist or recur throughout life. Although it was once believed that untreated Periodontitis would inevitably lead to periodontitis, a much more serious form of Periodontitis disease, that is no longer the scientific conclusion. Even though periodontitis results from some form of Periodontitis, untreated Periodontitis does not always result in periodontitis. *(Ref. K Feghali, M Feldman, VD La, J Santos…2011)*.

Periodontitis is an inflammation of the gums characterized by a change in color from normal pink to red, with swelling, bleeding, and often sensitivity and tenderness. These changes result when a layer of bacteria accumulates along the gum line and the body's immune system responds to the release of destructive bacterial products.

Symptoms showing Swollen gums, Bright red or red-purple appearance to gums, Shiny appearance to gums, Bleeding gums (blood on toothbrush or floss even with gentle brushing and flossing), Gums that are tender when touched, but otherwise painless, Persistent bad breath or bad taste in mouth.

Risk Factors are Poor oral hygiene, Tobacco use, Systemic diseases, such as diabetes and leukemia, Certain medications, such as steroids, some anti epilepsy and cancer therapy drugs, some calcium channel blockers, and oral contraceptives, pregnancy, decreased immunity, poor nutrition, defective fillings. Because plaque-induced Periodontitis is by far the most common form of gingival diseases, the following sections will deal primarily with this condition.

The cause of plaque-induced periodontitis is bacterial plaque, which acts to initiate the body's host response. This, in turn, can lead to destruction of the gingival tissues, which may progress to destruction of the periodontitis attachment apparatus. *(Ref. DF Kinane, P Mark Bartold - Periodontology 2000, 2007)* The plaque accumulates in the small gaps between

teeth, in the gingival grooves and in areas known as plaque traps: locations that serve to accumulate and maintain plaque. Examples of plaque traps include bulky and overhanging restorative margins, claps of removable partial dentures and calculus (tartar) that forms on teeth. Although these accumulations may be tiny, the bacteria in them produce chemicals, such as degradative enzymes, and dtoxins, such as lipopolysaccharide (LPS, otherwise known as endotoxin) or lipoteichoic acid (LTA), that promote an inflammatory response in the gum tissue. This inflammation can cause an enlargement of the gingiva and subsequent formation. Early plaque in health consists of a relatively simple bacterial community dominated by Gram-positive cocci and rods. As plaque matures and Periodontitis develops, the communities become increasingly complex with higher proportions of Gram-negative rods, fusiforms, filaments, spirilla and spirochetes. Later experimental Periodontitis studies, using culture, provided more information regarding the specific bacterial species present in plaque. Taxa associated with Periodontitis included Fusobacterium nucleatum subspecies polymorphum, Lachnospiraceae species OT100, Lautropia species HOTA 94, and Prevotella oulorum (a species of Prevotella bacterium), whilst Rothia dentocariosa was associated with Periodontitis health. Further study is warranted and may lead to new therapeutic approaches to prevent Periodontitis disease. *(Ref. PE Kolenbrander, RJ Palmer, AH Rickard… - Periodontology …, 2006 - Wiley Online Library)*

The proposed research work upon periodontis will help to know more about of this diseases. In our country the diseases is usually control by antimicrobial drugs administered at prophylactic doses. In discriminated use of antibiotic to control the dieses result drug resistance and limited the therapeutic possibilities in the treatment of the diseases. As a result the immune mechanism of population against periodontitis will develop an ultimately our oral health will be greatly benefited and play an important role in human health.

In Bangladesh in information on periodontitis is very scanty except Professor Liaquat Ali, Director, Bangladesh Institute of I-Iealth Sciences (BII-IS), Dr. Moin Jan, Head of the Department Perodontal Department, City Dental College These investigators focused on epidemological studies. But preliminary isolation identification and pathoganic study for the confirmatory characterization and diagnosis of the ethological agent was not yet performed. For the effective control of a specific disease of a specific host in a country must rely on the geographical epidemiological information based on age, sex, economic condition. As per literature review in the context on Bangladesh was recorded. More over the prevention and control of periodontitis depends on strict maintenance of oral hygiene. Use of antiseptics,

disinfectants and antibiotic. From the avobe sited information and hypothesis in the context of Bangladesh the etiological agent identification bases on age, sex and confirmatory diagnosis of a specific strain of *Porphyromonus gingivalis* by using morphological, cultural, biochemical and molecular technique.

By justifying the research in the context of Bangladesh and neighboring countries of the world. The present study was conducted for the investigation, isolation, identification and characterization of field isolate of *Porphyromonus gingivalis* by using cultural biochemical and antibiogram morphological study. Therefore the present study was undertaken with the flowing specific objectives.

i. To isolate and identify the oral bacteria causing periodontitis from patients based on age, sex, educational, qualification and sociodemographic condition by using cultural and Gram's staining technique.
ii. To characterize the isolated bacteria by using different biochemical techniques.
iii. To evaluate the sensitivity and resistance pattern of commercially available different antibiotics against the field isolates.

CHAPTER-II

Review of literature

2.1 Isolation and identification of oral bacteria causing periodontitis of human oral cavity

Lee Y *et at.* (2006) Studied that certain Periodontitis pathogens potentially transmissible to children from caregivers. By the use of N-benzoyl-DL-arginine-2-naphthy-lamide (BANA) test (BANAMET LLC, Ann Arobor, Mich) results a positive reactious was associated with Periodontitis whose caregiver is positive. The presence of these pathogenie and aerobic bacteria has risk for the children from the caregivers who have Periodontitis and a history of Periodontitis disease.

Tanaka *et at.* (2002) analyzed of patients aged 6 to 69 randomly from a dental outdoor. The high rate of porphyromonas gingivalis analyzed by an immunoslot blot assay (IBA) using their monoclonal antibodies. The following organisms were isolated they are porphyromonos gingivalis (Pg), Prevotella intermedia (Pi), Prevotella II a melaninogenica (Pm) The prevalance of the increase in Probing depth with increasing age was occured by the high rate of orurance of porphy romonas gingivalis. These finding demous trate the association of porphyro monas gingivalis with the inereasing depth of gingivolis.

Shoji T *et al.*. (2002) Studied to determine periodonto pathic bacteria in the supra gingival plaques with differennt oral health conditions. Results revealed that p gingivalis, Pinter media and A actino my cetem comitans i were detected from all children. The group with higher OHI showed a higher prevalence of periodonto pathic bacteria the supra gingival plaque with or with out caries cam harbor periodonto pathic bacteria.

Danser M M (1996) Stated that the conventional Periodontitis treatment on oral mucous membranes in patients with periodontitis decreases the pathogens significantly.

Grossi SG. *et al.*. (1994) Stated that specfic risk indicators associated with either susceptibility or resistamce to severe form of periodontitis Age, Smoking diabetes mellitus and the presence of subgingival P. gingivalis and B. forsy thus are the risk indicators. A wide range of Periodontitis disease experience defined by different levels of attachment loss.

Winkelhoff A. J (2002) Suggested that not all bacteria associated with periodontitis, Particularly P. gingivalis and Actinobacillus A ctinomycetemoco mitaus p. intermedia, P. Jorsythuss, F. nucleatum and P. micros are all significant markers jor destructive Periodontitis disease in adults. After 116 consecutive subjects diagnosed with moderate to severe periodontitis, it is suggested that subjects with moderate to severe periodontitis, it is suggested that subjects with alveolar boneloss shows more bacterial markers.

A. J. Van winkelhoff *et al.* (2002) P. Studied Periodontitis pathogens in subjects with and without Periodontitis destruction. Journal of clinical periodontology, vollume (29) Issue (ii) Nov 2002 pages 1023-1028.

Linden B V T *et al.* (1995) Assessed by clinical, radiogrophic and microbiological examination of Periodontitis conditions was carried out in 2 groups of married couples to assess similarities between husband and wife. By the assessment of plaque, Probing pocket depths, gingival bleeding on probing, Suppuration, supragingival and subgingival calculus.
The results indicates that spouses of patients with advanced periodontitis may also exhibit grequent recovery of suspected periodontal pathogens and have a worse Periodontitis status. then the healthy subjects.

Sanz M *et al.*. (2000) Studied subgingival microbiota of two geographically distinct patient populations both patient groups showed similar clinical characteristics in terms of age, gender and peridontal clinical variables, The sub gingival microbiota from the spanish group shows higher p. gingivalis and the Dutch group shows A. actinomycetem comitans and p. micros.

Winkelhoff A. J. Van *et al.* (2000) investigated the differences in antibiotic use influence the level of anti microbial resistance of the sub gingival micro flora of mutrated patients with adult periodontitis. The widespreacl use of antibiotics in spain is reflected in the level of resistance of the sub gingival micro flora of adult patients with periodontitis.

Haffajec A D *et al.* (1997) exhibited mean loss of attachment following scalling and root planning (SRP), to seek clinical and microbiological differences between subjects who responded well or poorly to SRP, Prior to and 3 months post SRP Clinical assessment was done. The data indicate that SRP is most effective in subjects and sites with high levels of the subgingival species that this therapy affects.

Monzilur Rahman *et al.* (2015) identified eight bacterial strains belonging to the general streptococcus and enterococcus based on colony morphology, biochemical assays, 16S rDNA sequence analysis, and species-specific PCR. Antibiotic susceptibility assay indicated that some of the strains are resistant to one or more antibiotics.

Shamsher Ali *et al.* (2012) Investigated the prevalence of dental caries among 5-14 years old poor locality school children of Lahore Pakistan. Sixteen hundred and seventy three poor locality school children aged 5-14 years; 1113,5-11 years old and 560,12-14 years old were examined for dental caries and oral hygiene status using the World Health Organization criteria for diagnosis of caries and oral hygiene status. The overall caries prevalence among the study group was 71%. The mean df (decayed and filled primary teeth) score in the 5-11 years age group was 2.98. The mean DMFT (decayed, missing and filled teeth) score among 12-14 years old age group it was 3.70. Only 14% of the poor locality school children had good oral hygiene with corresponding mean df of 1.33 and mean DMFT score of 1.97, followed by 31% having fairly good oral hygiene, 26% having poor oral hygiene. 29% of children had a very poor oral hygiene with corresponding mean df score of 2.96 and mean DMFT score of 3.31. The result of the study emphasize the need for re-orientation of oral health services and programs to a more preventive approach. Serious efforts need to be made by focusing on this neglected group of pouplation.

A C.R. Tanner (2015) Studied in advanced periodontitis in the 1970 srevealed microbial complexes that associated with different clinical presentations. Taxono my studies identified species newly-observed in period-ontitis as Aggregatibacter (Actinobacillus) actinomycetemcomitans, Campylobacter (Wolinella) rectus and other Campylobacter species, and Tannerella (Bacteroides) forsythia. Anaerobic culture of initial period-ontitis showed overlap in the microbiota with Periodontitis, and added Selenomonas noxia and Filifactor alocis as putative Periodontitis pathogens.

S Ali *et al.* (2012) investigated the Prevalence of dental caries among 5-14 years old poor locality school children of Lahore Pakistan. Sixteen hundred and seventy three poor locality school children aged 5-14 years; 1113, 5-11 years old and 560, 12-14 years old were examined for dental caries and oral hygiene status using the World Health Organization criteria for diagnosis of caries and oral hygiene status. The overall caries prevalence among the study group was 71%. The mean df (decayed and filled primary teeth) score in the 5-11 years age group was 2.98. The mean DMFT (decayed, missing and filled teeth) score among

12-14 years old age group it was 3.70. Only 14% of the poor locality school children had good oral hygiene with corresponding mean df of 1.33 and mean DMFT score of 1.97, followed by 31% having fairly good oral hygiene, 26% having poor oral hygiene. 29% of children had a very poor oral hygiene with corresponding mean df score of 2.96 and mean DMFT score of 3.31. The results of the study emphasize the need for re-orientation of oral health services and programs to a more preventive approach. Serious efforts need to be made by focusing on this neglected group of population.

S. Hemadneh *et al.* (2011) studied to assess the prevalence of Periodontitis in 6-7 years old children in Jordan. Nine hundred and twenty one 6-7 years old children were examined to assess the prevalence of Periodontitis, using gingival bleeding index at 6 indexed teeth on 2 point mesiobuccal and buccal surfaces. Total 12 teeth surfaces. Sixty nine percent (69%) of the examined children had mild Periodontitis. There were no statistical significant differences between gender of participants and (bleeding proportion, number of bleeding surfaces). Bleeding was not influenced by the location of the teeth whether anterior or posterior. Periodontitis was present, but in most of the cases it was mild. Children were instructed to practice adequate oral hygiene to keep the gums healthy.

N H.A. Al-Mudallal *et al.* (2008) considered to be positive bacterial isolates about (104 bacteria/ml) using selective Ms-agar (Mitis-Salivarius agar) medium. Thirty isolates were considered to be related to the genus Streptococcus and specially to the mutans streptococci of various group; S. sobrinus (serotype D, G), S. mutans (serotype C, F), S. cricetus (serotype A) and S. rattus (serotype B) with percentages of (39.29%), (30.30%), (18.18%) and (3.03%), respectively depending on biochemical and Lancefield grouping identification systems.

P C. Y. Woo *et al.* (2006) showed that it was 70% Actinomyces naeslundii and 30% Bifidobacterium species, whereas the Vitek ANI system and the ATB ID32A Expression system showed that it was “unidentified.” The 16S rRNA gene of the strain was amplified and sequenced. There were 3 base differences between the nucleotide sequence of the isolate and that of Lactobacillus salivarius subsp. salivarius or L. salivarius subsp. salicinius, indicating that the isolate was a strain of L. salivarius. The patient responded to cholecystectomy and a 2-week course of antibiotic treatment. Identification of the organism in the present study was important because the duration of antibiotic therapy would have been entirely different depending on the organism. If the bacterium had been identified as

Actinomyces, penicillin for 6 months would have been the regimen of choice. However, it was Lactobacillus, and a 2-week course of antibiotic was sufficient.

A. Pari *et al.* (2014) Studied indicate that Periodontitis of varying severity is nearly a universal finding in children and adolescents. The shorter life span of the primary dentition may be the reason why in general little attention is given to periodontitis in children. Since early diagnosis is important for successful treatment, it is imperative that children receive a Periodontitis examination as part of their routine dental visit. Furthermore destructive Periodontitis disease occurs in children with certain systemic diseases. Indeed the presence of severe periodontitis may be an early sign of systemic disease. A general medical evaluation to determine if systemic diseases are present should be considered in children who exhibit severe periodontitis, especially if the disease appears resistant to therapy. Though Periodontitis health awareness and therapy are increasing day by day in our country compared to earlier days, it is much restricted to adults rather than children. Oral cavity examination in children is much oriented in hard tissue evaluation than soft tissue health. Hence, this article enlightens about the prevalence of various soft tissue diseases and importance of long term overall oral health maintenance in childhood.

Rajkarnikar J *et al.* (2014) Studied four hundred and seventeen patients were randomly taken from a dental hospital situated in Jorpati during the time period of March 2013 to August 2013. All patients visiting the dental department who fulfilled the inclusion criteria were included in the study. The data included patients overall information along with their chief complaint, gingival bleeding on probing (BOP), probing depth (PD), frequency of brushing, adverse habits and the diagnosis of the patients examined. We found that 52.5% suffered from Periodontitis and 47.5% suffered from periodontitis. Also 28.3% suffered from localized and 18% suffered from generalized form of periodontitis. There was no statistically significant difference in the gender when the prevalence of Periodontitis disease was compared. 51.4% of male and 44.4% of female was seen to be affected with periodontitis. Also habits like smoking and intake of smokeless tobacco was seen to be associated with periodontitis. Regarding the age group more number of patients in the age group of >50 years were seen to be suffering from periodontitis (84.3%) as compared to age group of <35 years (25.9%).

M Rahman *et al.* (2015) Established biofilm lifestyle in the oral cavity by outcompeting other bacteria has been attributed to the production of bacteriocin along with other strategies. The goal of the present study was to isolate and identify oral bacteria and characterize their ability to produce bacteriocin against other oral bacteria as well as their sensitivity to common antibiotics. We have employed deferred antagonism bacteriocin assay for bacteriocin production and disk diffusion assay for antibiotic susceptibility testing. We identified eight bacterial strains belonging to the genera Streptococcus and Enterococcus based on colony morphology, biochemical assays, 16S rDNA sequence analysis, and species-specific PCR. Antibiotic susceptibility assay indicated that some of the strains are resistant to one or more antibiotics. Our study also revealed that the isolated strains are capable of producing one or more bacteriocins against other oral bacteria. Further molecular and biochemical studies are required to understand the nature of observed bacteriocin.

R d S Moreira (2012) studied those using measured values for population groups rather than individuals. In them, a description and analysis are referred to the mean exposure and the prevalence in geopolitical units considered. They have a lower cost, simplicity and easy analytical process in relation of the ethical aspects. It is extremely useful for the evaluation of policies, programs and interventions in health (Peres & Antunes, 2006). The unit of analysis were the WHO member countries. Analyses were performed in each of the six WHO regions: the Americas (AMRO), Africa (AFRO), South East Asia (SEARO), Europe (EURO), Eastern Mediterranean (EMRO) and Western Pacific (WPRO). Figure 1 shows the spatial distribution of the six WHO regions in the world.

N. Suzuki *et al.* (2013) Studied subgingival plaque contains more than 700 bacterial species, and some of these microorganisms have been shown to be responsible for initiation/progression of Periodontitis diseases. The red complex, which includes Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia (formerly Bacteroides forsythus), encompasses the most important pathogens in adult Periodontitis disease. Additionally, Fusobacterium nucleatum, Prevotella species, Eikenella corrodens, Peptostreptococcus micros, and Campylobacter rectus are increased in deep Periodontitis pockets and are implicated as possible periodontopathogens. These bacteria are not usually found alone, but in combination in the Periodontitis pockets, suggesting that some bacteria may cause destruction of the Periodontitis tissue in a cooperativemanner. Studies using animal models have reported the synergistic pathogenicity of mixed infections with P. gingivalis-T. denticola, P. gingivalis-F. nucleatum, P. gingivalis-T. forsythia, P. gingivalis-

Aggregatibacter actinomycetemcomitans, F. nucleatum-T. forsythia, and P. gingivalis-T. denticola-T. forsythia. Furthermore, coaggregation, nutrient effects, and modulation of virulence factors by periodontopathogens or by interspecies interactions between periodontopathogenic and nonpathogenic organisms have been reported to contribute to oral microbial pathogenesis. This paper focuses on interspecies pathogenic interactions within the red complex, in particular the combinations of P. gingivalis-T. forsythia, P. gingivalis-T. denticola, and P. gingivalis-T. forsythia-T. denticola. Potential therapies using normal inhabitants of the oral microbiota that have an antagonistic relationship with the red complex are discussed.

A L. Dumitrescu *et al.* (2010) Studied wide agreement on the etiological role of bacteria in human Periodontitis disease. Studies on the microbiota associated with Periodontitis disease have revealed a wide variety in the composition of the subgingival microfl ora (van Winkelhoff and de Graaff 1991). The search for the etiological agents for destructive Periodontitis disease has been in progress for over 100 years. However, until recently, there were few consensus Periodontitis pathogens. Some of the reasons for the uncertainty in defining Periodontitis pathogens were determined by the following circumstances.

R K Sohi *et al.* (2012) Studied entre maio de 2008 e setembro de 2009. Os dados referentes à experiência de cárie foram coletados em um formato estrutuudiedrado pelo índice cpo-d para 5 anos e índice CPO-D para crianças de 12 de idade. Dentre as crianças de 5 anos examinadas, 46.8% (271) eram do gênero feminino e 53.2% (308) eram do gênero masculino. No grupo de 12 anos, 46.6% (249) eram do gênero feminino e 53.4% (285) eram do gênero masculino. No grupo de 5 anos de idade, 48.3% (579) dos sujeitos foram afetados por cáries; já no grupo de 12 anos de idade, o percentual foi de 30.52% (534). A prevalência de cárie foi maior no grupo de 5 anos.

A. A Rani *et al.* (2016) Studied the cause of dental caries, this study was carried out to isolate the predominant cariogenic organisms from caries samples. A total of 10 caries samples were collected and from the samples 405 microbials isolates were obtained. Among this, 28 morphologically different colonies were identified and from them, 10 predominant were selected for identification. The biochemical and molecular analysis reveals that two organisms belongs to the genus Enterobacter and two belongs to the genus Bacillus. The organisms such as Enterobacter hormaechei, Enterobacter sp., Micrococcus luteus,

Klebsiella pneumoniae, Exiguobacterium sp., Staphylococcus sciuri, Acinetobacter radioresistens, Weissella confusa, Bacillus cereus and Bacillus subtilis were identified as predominant organisms in oral cavity.

R Leroy *et al.* (2009) examined the prevalence of dental caries in and Periodontitis around permanent first molars (PFM) in relation to their timing of emergence. Methods: In a group of 1609 “early emergers” (all PFM present at baseline) and a group of 336 “late emergers” (no PFM present at baseline), caries experience, the presence of plaque, and gingival health were evaluated annually during five follow-up examinations. Results: Even though all children were born in the same year, mean DMFS scores for PFM remained higher in the early emergers throughout the study; mean buccal plaque and Periodontitis scores were comparable in both subgroups. Early emergers had a significantly increased risk of caries experience in the occlusal surface of the PFM for two to four years. No consistent association was observed between timing of emergence and Periodontitis levels. Conclusions: It is recommended that dental age as.

Nelio Jorge Veiga *et al.* (2015) Studied dental caries and Periodontitis diseases, are the most prevalent worldwide nowadays. The World Health Organization (WHO) considers these pathologies important public health issues. It is fundamental to focus on the oral health, since oral diseases can cause problems related to difficulties in chewing, food intake, learning and concentration, sleep and reduced quality of life during childhood and adolescence.

Beena J. P. (2015) Studied the gingival health of human immunodeficiency virus (HIV) infected children and how it correlates to CD4 percentages in vertically infected children with HIV undergoing combination antiretroviral therapy. Two hundred and fifteen HIV positive children on antiretroviral therapy, of both sexes from seven to fifteen years old were evaluated for their gingival status using the Gingival Index. Children were divided into three groups; mild, advanced and severe depending on their absolute CD4 count and CD4 percentage. Statistically significant association was observed between CD4% category (mild, advanced and severe) and Periodontitis ($P < 0.001$). More number of children in mild CD4% category were found to have mild Periodontitis. Based on the results of our study the prevalence and distribution of Periodontitis was high, this data will helps us in formulating.

V Ambarkova *et al.* (2014) cross-sectional studied, adolescents from8 Secondary Schools (first grades, N= 414) were selected. Participants dental status was evaluated using the 2013 World Health Organization caries diagnostic criteria for Decayed, Missing or Filled Teeth (DMFT) by 2 calibrated examiners. The total number of adolescents the sample was 414, comprising 188 (45.4%) females and 226 (54.6%) males. The mean DMFT was 5.77, with standard deviation (SD) of 4.02 and 95% confidence interval (CI) of 5.38- 6.16. Significant caries (SiC) index was 10.22.The prevalence of caries-free adolescentswas9.4%. The percentage of untreated caries or the ration of D/ DMFT was 0.49 (49.05%). The prevalence of dental caries in secondary school adolescents was 90.6%.

G M. EL-Sherbiny (2015) Aime of the study was to investigate the bacterial species from root canal infection (before and after) filled teeth and evaluate susceptibility to antibiotics, antimicrobial agents and plant extracted from ginger (Zingiber officinale).Thirty-three adult patients with symptoms of root canal infection was receiving at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2014, diagnosed by a physician participated in the study. Forty- five bacterial samples were taken from root canals infection, 33 before and 12 after filled teeth. A total 126 bacterial isolates were isolated and identify from this samples. The predominant bacterial isolates were found Enterococcus faecalis 15%, followed by Streptococcus mutans 9.5%, Streptococcus acidominimus 8.7% and Porphyromonas gingivalis 7.93%. The bacterial isolates highly sensitive to amoxicillin-clavulanic acid, vancomycin and erythromycin. About 36.50 % of the isolates were resistant to tetracycline. More than 50 % of all isolates were resistant to metronidazole. The sensitivity of bacterial isolates to antimicrobial agents revealed that sensitive 87.30% to potassium iodide 2.0 %, 73.01 % to calcium hydroxide 2 %, 69 % to chlorxidine 1% and 54% to sodium hypochlorite 0.5%. The in vitro antibacterial activity of Zingiber officinale was studied against bacterial isolates. The aqueous and ethanolic extracts of Zingiber officinale exhibited antibacterial activity against all bacterial isolates with MIC ranged from 0.5 to 1.3 mg/ml and 0.3 to 1.0 mg/ml respectively. In conclusions, amoxicillin-clavulanic acid and vancomycin most potent antibiotics and potassium iodide 2% most antimicrobial agents against bacterial isolates. Zingiber officinale extract has potential antimicrobial action against bacterial isolates.

A M. R. Cardoso *et al.* (2015) Aimed of the present study was determine the prevalence and factors associated with dental caries and Periodontitis disease in Brazilian children and adolescents with cerebral palsy (CP). This is a cross-sectional study conducted with 80 patients ranging in age from 2 to 18 years old. Oral exams were conducted by an examiner with records of DMFT, dmft, Gingival Bleeding Index (GBI) and Community Periodontitis Index (CPI). The statistical analysis used Poisson Regression with robust variance estimation ($\alpha = 0.05$). The prevalence of dental caries was 59.3%, with DMFT and mean dmft of 1.71 ± 2.42 and 2.22 ± 3.23, respectively. The mean GBI was 22.44%, and in the CPI, the prevalence of gingival bleeding, calculus, shallow and deep pockets were 94.73%, 79.62%, 12.90% and 3.22%, respectively. The caregiver's educational level of less than eight years were associated with the dental caries experience (PR = 1.439; 95%CI = 1.09–1.89). The Periodontitis alterations were associated with female sex (PR = 0.82; 95%CI = 0.69–0.97), caregiver's educational level of less than eight years (PR = 1.15; 95%CI = 1.03–1.29), poor oral perception (PR = 0.89; 95%CI = 0.80–0.98), serious communication problem (PR = 0.87; 95%CI = 0.76–0.99) and athetoid type of CP (PR = 0.85; 95%CI = 0.75–0.97). The patients with CP presented high dental caries experience and Periodontitis alterations, which were associated with their demographic, socioeconomic, oral health perception and systemic information.

Subramonian S *et al.* (2016) Studied to investigate the bacterial dominance of dental plaque. For this study, 20 plaque samples were collected from adult humans of the around Marthandam area, Kanyakumari District, Tamil Nadu. The collected plaque samples were inoculated separately into the Basal salt medium and Basal salt medium agar plates. The morphologically different bacterial colonies were selected, identified by studying cultural, morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology. As a result, 7 bacterial genera such as Bacillus sp, Lactobacillus sp, Staphylococcus sp-I, Staphylococcus sp-II, Micrococcus sp, Streptococcus sp, Proteus sp and Pseudomonas sp. were identified. Among these, Streptococcus sp has been found highest incidence (21.57 %) followed by Staphylococcus sp-I (17.65%).

Haydar Barakat *et al.* (2016) Classified gingival biotypes into thin biotype and thick biotype. It is crucial to identify tissue biotype before treatment. Aim of Study: To determine the prevalence of gingival biotypes in a Syrian population, in addition, to study the distribution of gingival biotypes according to gender and tooth shape. Material and Methods: This cross sectional study included 500 volunteers (300 males and 200 females) from the

patients who had visited the department of periodontology-dental faculty at Damascus University. Gingival thickness was assessed to determine the gingival biotype for the maxillary central incisors using the direct measurement technique (Trans-gingival probing). Shapes of the maxillary incisors were recorded. A written informed consent was taken from each patient. Statistical analysis was done using test k2 $p < 0.05$. Results: The mean age was 26.8 ± 4.4 years. Thick gingival biotype was detected in 58.4% of the sample and most of patients are men while the prevalence of thin gingival biotype was 41.6% of the sample. It was also observed that patients with thin gingival biotype had triangular tooth shape in 99.5% ($p < 0.05$). Conclusion: Thick gingival biotype was observed to be more prevalent in a Syrian population than thin biotype. Most Syrian males had thick gingival biotype with square tooth shape while females had thinner biotype and triangular tooth shape.

D Chandrabhan *et al.* (2012) Studied to assess the existence of acidogens potentially causing the dental caries and comparatively evaluation of efficacy of different toothpastes. The dental plaques of fifty persons belong to three age groups (1 - 20, 21 - 40, 41 - 60 year and above) were examined to identify microorganisms by the culture method. Thirty nine bacteria were isolated by spread plate method on BSMY I minimal media. Thirteen out of thirty nine, acidogens colonized in the dental plaques. Seven potentially acidogens CD17, CD26, CD27, CD28, CD29, CD34 and CD35 were treated with five different toothpastes. Inhibition effect of Triclosan and Fluoride con-taining tooth pastes were found more efficient. The results of the present study revealed that bacteria that commonly cause dental caries colonized in dental plaques of children and alcoholic person. Therefore, dental plaques must be con-sidered a specific reservoir of colonization and subsequent dental caries. To reduce the dental problem triclosan and fluoride containing product should be recommended.

Dr Odont (1981) Reviewed some of the commonly used indices for measurement of Periodontitis and Periodontitis disease. Periodontitis disease should be measured using loss of attachment, not pocket depth. The reliability of several of the indices has been tested. Calibration and training of examiners seems to be an absolute requirement for a satisfactory inter-examiner reliability. Gingival and Periodontitis disease is much more severe in several populations in the Far East than in Europe and North America, and Periodontitis seems to increase with age resulting in loss of Periodontitis attachment in approximately 40% of 15-year-old children.

K P Lashkari *et al.* (2016) Studied to assess association between smokeless tobacco consumption and prevalence of dental caries in Dakshina Kannada population. Materials and Methods: In this study 172 elderly dentate and consenting individuals (79 females, 93 males) were included. Patients were subjected to clinical examination under natural light with the aid of mouth mirror, No. 23 explorer and cotton rolls. Age of study group ranged from 20 years to 65 years. Tobacco consumption habit data were collected through validated questionnaire. DMFT (Decayed, Missing, and Filled Teeth Index-WHO modification 1987) was used to assess caries experience of study group. Results: Mean DMFT score of smokeless tobacco chewers was 5.66 ± 1.55 and of non-tobacco chewers it was 3.99 ± 1.6 which showed significant association between smokeless tobacco consumption and dental caries experience in Dakshina Kannada population (p=0.001). Among various forms of smokeless tobacco, tobacco along with paan leaves was the most adopted one (15.1%); but the highest mean DMFT score being 6.00 ± 1.26 was observed in patients who consumed gutka. Conclusion: This study throws light on possible contribution of smokeless tobacco consumption towards increased caries experience and it also reflects potential role of form of smokeless tobacco consumption towards dental caries.

Dr. Prachi Mital *et al.* (2013) Studied to find occurrence of dental caries and Periodontitis among pregnant women and to compare it with those in non-pregnant women. 265 pregnant and 270 non-pregnant women were recruited from Department of Ob –Gy, S. M. S. Method: Medical College Jaipur. Dental caries and Periodontitis was defined clinically according to the World Health Organization (WHO) diagnostic criteria. Over 61.5% of pregnant women had caries, and 52.6% had Periodontitis. There were significant differences between pregnant and non-pregnant women with regard to dental caries (p=0.0001) and Periodontitis (p=0.0008). The pregnant women were 1.97 times more likely to suffer from dental caries (95% confidence intervals (CI), 1.39 – 2.78), and 1.81 times more (95% CI, 1.28 – 2.57) from Periodontitis compared to non-pregnant women. Age less than 25 years (Odd ratio 1.8; 95% CI, 1.09 – 3.03), illiteracy (OR, 2.33; 95% CI, 1.40 – 3.86), and rural dwelling (OR, 1.96; 95% CI, 1.18 – 3.26) were significant predictors for dental caries. Predictors for Periodontitis were similar to dental caries that is age less than 25 years (Odd ratio 2.22; 95% CI, 1.31 – 3.75), illiteracy (OR, 1.79; 95% CI, 1.07 – 2.99) and rural dwelling (OR, 2.14; 95% CI, 1.27 – 3.59). Poor oral hygiene (OR, 1.57; 95% CI, 0.95 – 2.59), poor attitude (OR, 1.51; 95% CI, 0.91 – 2.49) and poor behavior (OR, 2.03; 95% CI, 1.23 –3.35) were important risk factors for dental caries. Similarly, inadequate knowledge (OR, 1.67; 95% CI, 1.01 to 2.75), poor oral health attitude (OR, 1.92; 95% CI, 1.15 – 3.22) and poor behavior

(OR, 2.01; 95% CI, 1.20 – 3.38) were found to be significant risk factors for Periodontitis among pregnant women. In conclusion, dental health awareness programs should be encouraged to improve the dental health of pregnant women.

Dr. A S Alshammari (2017) Evaluated the prevalence of different types of Periodontitis and periodontitis and their distribution in males and females for patients attended to Periodontitis clinic over 15 months from October, 2015 to December, 2016.1)To evaluate the severity and incidence of those disease among group sexes and how specifically affect each sex group.2)To estimate the Periodontitis health condition of the community from the study sample.3)To instruct them about the importance of Periodontitis health and good oral hygiene.4)To make this study a reference study for comparison for other studies. The study was conducted on 1273 patients who attended periodontics clinic, ArAr dental center over 15 months from October, 2015 to December 2016. The sample consisted of 748 male patients and 525 female patients with age vary from 15 to 69. Information on sex, age, systemic diseases, medical history, dental history and Periodontitis status were obtained (probing, radiographs, plaque and gingival indices). According to data analyzed, Periodontitis was more predominant than periodontitis and males are more affected by both Periodontitis and periodontitis.

N Veiga *et al.* (2015) Discussions about oral pathologies and the association with bio-psychosocial factors. Dental caries and Periodontitis disease are one of the major public health problem, therefore epidemiological studies are useful for identifying and monitoring their prevalence among different age and geographical groups, giving new treatment perspectives. It is understood that epidemiology can confer a predictive significance to clinical data, becoming very useful in implementing preventive strategies to reduce the incidence of dental problems. This literature review aims to gather information, in a critical point of view, on the importance of epidemiology in modern dental medicine.

CHAPTER-III
Materials of Methods

3.1 Materials

3.1.1 Study design

Descriptive cross-sectional survey was used to observe, describe and document aspects of exclusive relation between tooth brushing practices, sugar consuming age, sex, employ status and Periodontitis. This design was chosen because the study was concerned with specific prediction and describing characteristics of a particular group (age between sixteen to thirty). The design was preferred as the topic was a social survey under social and behavioral sciences.

3.1.2 Target population

All students who studied the Dinajpur Medical Institute, Dinajpur, District; Dinajpur, Country; Bangladesh.

3.1.3 Area of the study

The study was carried out in Dinajpur Medical Institute, Dinajpur, District; Dinajpur, Country; Bangladesh.

3.1.4 Study population

Students aged 16-30 years were selected from the target population

3.1.5 Inclusion criteria

Students of Dinajpur Medical Institute those were willing to participate in the study

3.1.6 Exclusion criteria

Students of Dinajpur Medical Institute does not willing to participate

3.1.7 Data Collection and processing of sample

The Institute was visited on different days for four weeks. Each visit lasted 4 hours. This is because each Institute had Classes operated from 9:00a.m to 12:00 noon. Simple random sampling was used. Papers with two written choices yes and no were placed in a container.

3.1.8 Sample size determination

Sample size was calculated using the formula below (Fisher *et al..*, 1998):

$$n = \frac{Z2pq}{d2}$$

Where:

n = is the desired sample size

Z = is the standard normal deviation that provide 95% confidence interval (1.96)

P = proportion of exclusive sample

q = 1-p

d = is the absolute precision (error bound 0.05)

The percentage of exclusive breast feeding women in Somalia was 11%. The desired accuracy of results was at 95% confidence.

$$n = \frac{(1.96)^2 x(0.11)x(0.89)}{(0.05)^2}$$

$Z = (1.96)^2$

P = 0.11

q = 1-p = 0.89

d = 0.05

n = 150

Therefore the sample size was 150.

Table 1. Study of epidemic behavior of Age and Sex at Dinajpur Medical Institute as per structured questionnaire and clinical sings and symptom.

Location/Area of institute	No. of institute	Total No. of students observed in the institute as per age (year) group				Sex		Percent	
		less than 17	18-20	20-25	25-30	Male	Female	Age	%
Dinajpur	1	2	94	44	10	84	66	less than 16	1.3
								18-20	63
								20-25	29
								25-30	6.7
								Total	**100**

3.1.9 Data collection procedure

The Semi-structured questionnaire was used for the data collection by using face to face inter-view. The questionnaire and the structured, face - to - face interview schedule were researcher-administered. Following written consent from each student, examination of each student's dentition was performed using natural light, a dental explorer, and dental mirror while lying on a bench, and examiner seated behind the subjects head. Using the World Health Organization (WHO) diagnostic criteria *(Ref. MR Gilbert, B Fiedler - 1964 – ERIC)*, the number of decayed, missing and filled teeth (DMFT) was recorded and when the examiner was in doubt no caries was recorded. For analysis students were categorized according to their caries experience, those without caries experience (DMFT = 0; absence of a decayed, missing tooth/teeth due to caries, or filled tooth/teeth) or those with a caries experience (DMFT $\geqslant$ 1; presence of one or more decayed, missing tooth/teeth due to caries, or filled tooth/teeth). Scoring of supragingival plaque and calculus was done in accordance to a modified version of the WHO diagnostic criteria. The criteria was modified into two scores (absent = 0 and present = 1). The supragingival surfaces of the index teeth (tooth 16, 11, 26, 36, 31, and 46) were assessed for presence/absence of supragingival plaque and calculus. A score = 1 was given when visible plaque remnants were present on any of the index teeth, and a score = 0 was given when there were no visible remnants present on any of the index teeth. In doubtful instances plaque was scored as absent. When supragingival calculus was observed on any of the supragingival surfaces of the index teeth it was scored present = 1, and when no deposits were observed on any of the supragingival surfaces of the index teeth it was scored absent = 0. Subjects were then categorized to be plaquefree or calculus-free (score = 0) when there was absence of supragingival plaque or calculus in all of the index teeth and with plaque or calculus (score = 1) when there was presence of supragingival plaque or calculus in one or more of the index teeth. Infection control was maintained by the use of gloves and masks by the examiners, and the dental mirrors were placed in Steranios 2% (LOT 119113. ANIOS laboratories, Pave du Moulin, France) for 10 minutes, rinsed with water and sterilized using a pressure cooker and gas stove prior to use. The interexaminer variability of the two examiners was assessed by reexamining 10% of the total samples (12) in one institute (Dinajpur Medical Institute) subjects in four schools (Tanga = 2; Lushoto = 2), and kappa values ranged from 0.64 to 1.0 for dental caries, 0.14 to 0.33 for supragingival plaque and 0.1 to 0.39 for supragingival calculus. Data collected was coded, and using the SPSS statistical package analysis was performed. Chi-square tests were used to determine the level of statistical significant difference at $P < 0.05$.

3.1.10 Data analysis

Data was analyzed using the Statistical Package for Social Sciences (SPSS version 16.0). Findings were presented by use of frequency distribution tables, charts and graphs.

3.1.11 Logistical and ethical considerations

Permission letter for data collection was provided by the institute authority .A sample questionnaire was given to the MCH staff in order to clarify issues and obtain their co-operation. Respondents consent (signed) consent for study participation was sought through attaching a brief consent form with the questionnaire that addressed the nature of the study. Informed verbal consent was also sought. During the interviews, students were encouraged to talk freely. The respondents‟ information was treated confidentially.

Table 2. Collection of Sample based on their age, sex, education and socio demographic status:

Age (Year)		Sex		Education			Socio demographic status			Name of Collected Samples	Total Numberof Samples tested
		Male	Female	S.S.C	H.S.C	Graduate	High class	Middle class	Lower class		
Less than 17	2	84	66	2	0	0	0	1	1	2	2
18-20	94			94	0	0	0	88	6	94	53
20-25	44			39	5	0	0	41	3	44	35
25-30	10			4	5	1	0	10	0	10	10

3.1.12 Glass wares and other necessary instruments

In this study work following glass wares and instruments were used:

Test tubes (with and without Durham’s fermentation tube), Petri-culture dishes, Conical flasks, Pipette, Slides, Microscope, Sterilized cotton, Immersion oil, Bacteriological , incubator, Jar, ice boxes, Measuring balance, Hand gloves, Spirit lamps, Match lighter, Bacteriological loops, Glass spreader, Depression slide, Forceps and Scissors, Prob, Mirror, Twiser.

3.1.13 Apparatus/Equipment/Supplies

Biosafety cabinet Class-II, Laminar air flow, Clean Bench, Thermal cycler 96 well, Thermal cycler (G storm), Digital Analytical Balance, Gel documentation system (Uvitec Cambridge Tece), Minispin Centrifuge, Vortex Mixturc, Freezer-20º C , Freezer-80º C, 4º C , refrigerators, Micropipette Tips, Micropipettes (100-1000ul, 20-20ul, 0.1-2.5ul), Gloves Powder Free, Measuring cylinders, Glass bottles (20ml-1000ml)

3.1.14 Laboratory preparation

All item of glassware including test tubes, pipettes, cylinder, flasks, conical flasks, glass plate, slide, vials soaked in a household dishwashing detergent solution (Trix, Recket and Colman Bangladesh Ltd.) for overnight, contaminated glassware were disinfected in 2% sodium hypochlorite solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly and finally sterilized either by dry heat at 160^0 C for 2 hours or by autoclaving for 15 minutes at 121^0 C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven over at 50^0 C. Disposable plastic were (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50^0 C for future use.

3.1.15 Instrument and appliances

Phase contrast microscope, digital pH meter, test tubes, cotton, hand gloves, plastic syringe (5 ml), micropipette (1 ml, 500 μl, 10-20 μl), glass slides, eppendorf tubes, magnifying glass, marker pen, ice-box, spirit lamp, cover slips, inoculating loop and rack, autoclave, refrigerator, conical flask, etc.

3.1.16 Media for culture

3.1.17 Liquid media

- Nutrient broth
- Lactose broth (hi-media, India)
- Peptone broth (Hi-media, India).

3.1.18 Solid media

- Eosin methylene blue (EMB) agar (Hi-media, India).
- MacConkey agar medium (Hi-media, India).
- Sheep blood agar media
- Mitis salivarious agar media
- Manitols salt agar media
- Sabourauds Dextrose agar media (for mycological examination)
- Motility, Indole, Urease (MIU) medium (Hi-media, India).

3.1.19 Chemical and Reagents

Gram's Staining reagent: Crystal violet, Gram's iodine, Acetone and Safranine, Alpha-naphthol solution, Kovac's reagent, Ethyl alcohol (70% and 95%), Sugar media (Dextrose, Maltose, Lactose, Sucrose, and Mannitol) and other chemicals and reagents.

3.1.20 Liquid Media

3.1.21 Nutrient broth

Nutrient broth (NB) was used to grow the organisms from the samples collected from the study areas before performing biochemical test (Cheesebrough, 1984).

3.1.22 Solid Media

3.1.23 Mitis salivarius agar

Mitis Salivarius Agar is recommended for the isolation from mixed cultures of Streptococci especially Streptococcus mitis, Streptococcus salivarius, Enterococcus faecalis showing α and γ haemolytic reactions on Blood Agar.

3.1.24 MacConkey agar

Mac Conkey agar (MC) medium was used to culture the organisms under the family Enterobacteriaceae (Cheesebrought, 1984)

3.1.25 Collection of samples

Samples were collected from Dinajpur Medical Institute, Dinajpur of Dinajpur District of Bangladesh. The samples were collected and brought to Department of Microbiology, Hajee Mohammad Danesh Science and Technology University with necessary precautions for bacteriological examination.

3.1.26 Enumeration of total viable count (TVC)

For the determination of total bacterial count, 1 ml of each ten-fold dilution was transferred and spread on duplicate plate count agar using a fresh pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 30^0C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of lolonies in a praticular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO. The result of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sample.

3.1.27 Enumeration of total *Streptococcus Staphylococcus, Porphyromonas gingivalis* count

For the determination of total *Streptococcus* count 1ml of each tenfold dilution was transferred to EMB agar. For each dilution five test plates containing EMB agar were used. All the agar plates were incubated at 37^0C temperature for 48 hours. The total coliform count was calculated according to ISO. The result of the total coliform count were expressed as the number of organism or colony forming units per gram (CFU/gm) of sample.

3.1.28 Isolation and identification of pathogens

The entire samples were selected for bacteriological culture.

3.2.5 Preparation of culture media and reagents

All the media, broth and reagents used in this experiment were prepared according to instruction of the manufacturer.

3.1.29 Liquid Media

3.1.30 Nutrient broth

Suspend 8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent. (*Ref.* Walsbren, *et. al.* 1951)

3.1.31 Solid media

3.1.32 Nutrient agar

28gms of Bacto-Nutrient agar (Hi Media, India) was suspended in1000ml distilled water and boiled to dissolve completely. The solution was sterilized by autoclaving at l2l^{0}C for 15 minutes at 15 lbs pressure. After autoclaving, the medium was poured in 20ml quantities in sterile petri dishes to form a thick layer and was allowed to solidify. It was kept incubation overnight at 37^0C to check the sterility of the medium. The sterile nutrient agars were stored at refrigerator at 4^0C until used.

3.1.33 Blood agar media

1. Prepare the blood agar base as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.

2. Transfer thus prepared blood agar base to a 50°C water bath.

3. When the agar base is cooled to 50°C, add sterile blood agar aseptically and mix well gently. Avoid formation of air bubbles. You must have warmed the blood to room temperature at the time of dispensing to molten agar base.
   (**Note:** If you are planning to prepare a batch of blood agar plates, prepare few blood agar plates first to ensure that blood is sterile).

4. Dispense 15 ml amounts to sterile petri plates aseptically

5. Label the medium with the date of preparation and give it a batch number (if necessary).

6. Store the plates at 2-8°C, preferably in sealed plastic bags to prevent loss of moisture. The shelf life of thus prepared blood agar is up to four weeks.

3.1.34 Mitis salivarius agar

Mitis Salivarius Agar contains peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose and saccharose are carbohydrate sources. Crystal violet and potassium tellurite (from Tellurite Solution 1%) inhibit most gram-negative bacilli and most gram-positive bacteria except streptococci. Trypan blue gives the colonies a blue color. Agar is the solidifying agent.

3.1.35 MacConkey agar media

An amount of 51.5 gms of Bacto-MacConkey agar (Hi Media India) suspended in 1000 ml of distilled water were taken in a flask. The suspension was heated up to boiling to dissolve the medium completely and then sterilize by autoclaving at 121^0C under 15 Ibs pressure per square inch for 15 minutes. The media was then poured into sterile petridishes (75 mm diameter) in 20 ml quantities to form thick layer. The sterile of the media was checked by incubating at 37^0C over-night and stored at 4^0C.

3.1.36 Brain Heart infusion agar

Add 15 g agar to 1 liter BHI broth. Heat to dissolve agar before dispensing into bottles or flasks. Autoclave 15 min at 121°C. Dispense broth into bottles or tubes for storage. Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. Commercially available BHI is acceptable. (*Ref:* Bacteriological Analytical Manual, 8th Edition, Revision A, 1998.)

3.1.37 Manitol salt agar

Mannitol salt agar is best prepared from ready to use dehydrated powder, available from most suppliers of culture media. The medium is usually used at a concentration of 11.1 g in every 100 ml distilled water (concentration may vary depending on manufacturer).

1. Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at 121^{o}C for 15 minutes.

2. When the medium has cooled to 50-55^{o}C, mix well, and dispense it aseptically in sterile petri dishes. Date the medium and give it a batch number.

3. Store the plates at 2-8^{o}C preferably in plastic bags to prevent loos of moisture. (*Ref:* Tankeshwar Acharya 2013)

3.1.38 Reagents preparation

3.1.39 Procedure of Indole test

2 ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours.0.5 ml Kovac's indole reagent was added, shaked well and examined after 1 minute. A red color ring at the top of the reagent indicated positive test. In negative case there is no development of red ring.

3.1.40 Procedure of Motility Indole Urease Test (MIU)

MIU was prepared in a test tube. Then the organism was inoculated into the media by stabbing method with the help of sterile straight wire. Then the test tube was incubated 37^{0}C overnight. Single stick that is no turbidity throughout the medium indicate gram negative organism (non motile) and turbidity throughout the medium indicate gram positive case.

3.1.41 Procedure of Triple Sugar Iron Test (TSI)

Triple sugar irons contain three sugars (Glucose, Sucrose and Lactose). At first TSI agar slant was prepared in a test tube. Then the organism was inoculated into the butt with a sterilized wire and on the slant with a wire loop producing zigzag streaking. The tube was

incubated for 24 hours at 37^0C.Yellow color of butt and slant of the test tube indicate fermentation of Glucose, Sucrose and Lactose fermentation and butt shows blacking indicate H_2S production.

3.1.42 Methyl Red-Voges Proskaure test

3.1.43 Preparation of MR-VP broth

A quantity of 17 gms of Bacto MR-VP medium (Hi Media, India) was dissolved in 1000 ml distilled water dispensed in 2 ml amount in each test tube and then the tubes were autoclaved at 121^0C maintaining a pressure of 15 pounds/sq. Inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37^0C for overnight to check their sterility and then stored in a refrigerator for future use.

3.1.44 Preparation of MR solution

The indicator methyl red solution was prepared by dissolving 0.1 gm of Bacto methyl-red (Difco) in 300 ml of 95% alcohol and diluting to 500 ml with the addition of 200 ml of distilled water.

3.1.45 Voges-Proskauer solution

3.1.46 Alpha-naphthol solution

Alpha-naphthol solution was prepared by dissolving 5gms of 1-naphthol in 100ml of 95% ethyl alcohol.

3.1.47 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by dissolving 40 gms of Potassium hydroxide crystals in 100 ml of cold distilled water.

3.1.48 Procedure of MR test

The test was performed by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of a bright red color. A yellow or orange color was a negative test.

3.1.49 Procedure of VP test

2 ml of sterile glucose phosphate peptone water were inoculated with the 5 ml of test organisms. It was incubated at 35-37^0C for 48 hours. A very small amount (knife point) of

creatine was added and mixed. 3 ml of 40% potassium hydroxide were added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In negative cases there was no development of pink color.

3.1.50 Gram's staining method for Morphological characterization

The representative *Streptococcus, Staphylococcus, P. gingivalis* colonies were characterization morphologically using Gram's stain according to the method described by Merchant and Packer (1967).

Briefly, a small colony was picked up from SS and BG agar plates with a bacteriological loop, smeared on separate glass slide and fixed by gentle heating. Crystal violate was then applied on each smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine was then added to act as mordant for one minute and then again washed with running water. Acetone alcohol was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, blotted and dried in air and then examined under microscope with high power objective (100X) using immersion oil.

3.1.51 Antibiotic sensitivity tests

All bacterial isolates subjected to antibiotic sensitivity test by Kirby-Bauer Disc diffusion method according to the guidelines of The European Committee on Antimicrobial Susceptibility Testing standard (EUCAST).The antimicrobial discs were applied to the plates. After incubation, the diameter of the zones of complete inhibition (including the diameter of the disc) was measured and recorded in millimeters. The measurements were made with a ruler on the undersurface of the plate without opening the lid. The zones of growth inhibition were compared with the zone size interpretative table provided by EUCAST (Table 1). Antimicrobial testing results were recorded as sensitive, intermediate and resistant according to zone diameter interpretative standards provided by EUCAST.

3.1.52 Morphological characterization of organisms by Gram's staining method

- A loopful of sterile distilled water was placed in the center of a clean sterile slide.
- A small colony was picked up with a bacteriological loop and was mixed with distilled water on the slide.
- The colony was made to thin smear on a slide.
- The smears were fixed by air drying.
- 0.5% crystal violet solution was then applied on the smear for one minute.
- Gram's iodine was then added to act as mordant for one minute.

- Acetone alcohol was then added to decolorize for 1-2 seconds.
- Then the slide was washed with water.
- Safranine was added as counter stain and allowed for one minute.
- The slide was then washed with water.
- Then the slide was blotted with blot paper and was allowed to air dry. The slide was examined under microscope with high power objective (100X) using immersion oil.

3.2 Methods

Experimental layout :

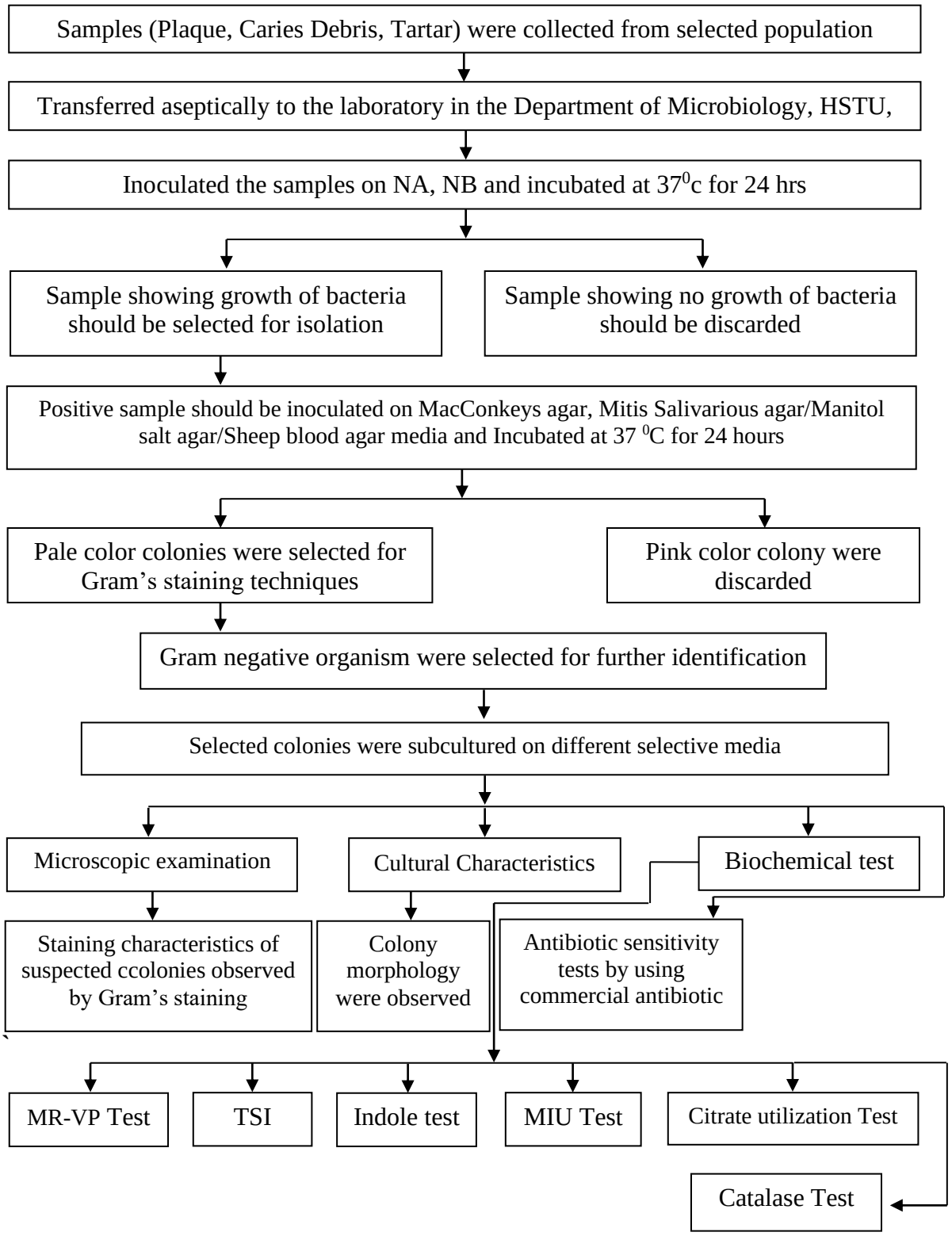


Fig. 1: Schematic Illustration of the isolation and identification of Oral Pathogens from selected sample

3.2.1 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, the mixed culture was inoculated into nutrient agar media by streak plate technique to obtain isolated colonies as per:

Step-1: An inoculum was picked up with a sterile loop and spread on an area of the medium in the petridish.

Step-2: The loop was sterilized by being heated as red hot in a flame.

Step-3: The inoculum was spread over the reminder of the plate by drawing the cooled parallet line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.2.2 Techniques used for the isolation and identification of *Streptococcus*.

3.2.3 Culture into different media

Sterilized platinum loop was used for streaking the lactose broth culture on Mitis Salivarious agar to get isolates in pure culture. All inoculated media were kept at 37^0C for overnight in an incubator.

3.2.4 Mitis Salivarious agar

Materials from lactose fermentation tubes were inoculated into Mitis Salivarious agar placts which after incubation, showed blackish color sheen if positive for *Streptococcus.*

3.2.5 Microscopic study for identification of *Streptococcus* suspected colonies by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram staining reaction was performed according to the methods described by Merchant and Packer (1967)

3.2.6 Techniques used for the isolation and identification of *Staphylococcus*.

3.2.7 Culture into different media

Sterilized platinum loop was used for streaking the lactose broth culture on Manitol Salt agar to get isolates in pure culture. All inoculated media were kept at 37^0C for overnight in an incubator.

3.2.8 Manitol Salt agar

Materials from lactose fermentation tubes were inoculated into Manitol Salt agar plates which were kept at 37^0C for overnight in an incubator.

3.2.9 Microscopic study for identification of *Staphylococcus* suspected colonies by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram staining reaction was performed according to the methods described by Merchant and Packer (1967)

3.2.10 Techniques used for the isolation and identification of *Porphyromonas gingivalis*.

3.2.11 Culture into different media

Sterilized platinum loop was used for streaking the lactose broth culture on Sheep blood agar to get isolates in pure culture. All inoculated media were kept at 37^0C for overnight in an incubator.

3.2.12 Sheep blood agar

Materials from lactose fermentation tubes were inoculated into Sheep blood agar placts which after incubation, showed blackish color sheen if positive for *Porphyromonas gingivalis.*

3.2.13 Microscopic study for identification of *Porphyromonas gingivalis* supected colonies by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram staining reaction was performed according to the methods described by Merchant and Packer (1967)

3.2.14 Identification of *Streptococcus* isolates by biochemical test

3.2.15 Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while.

3.2.16 Triple sugar iron (TSI) agar slant

The test organisms were culture into TSI agar slnt by stab streak method.

3.2.17 Methyl red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 370C, a drop of methyl red solution was added. A red coloration is positive and indicates and acid pH of 4.5 or less resulting from the fermentation of glucose (Cheesbrough, 1984).

3.2.18 Voges-Proskaure test

2 ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organisms. It was incubated at 37^0C aerobically for 48 hours. A very small amount (knifepoint) of creative was added and mixed 3 ml of a-naphthol were added and snaked well. The bottle cap was removed and left for and hour at room temperature.

3.2.19 Identification of *Staphylococcus* isolates by biochemical test

3.2.20 Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while.

3.2.21 Triple sugar iron (TSI) agar slant

The test organisms were culture into TSI agar slnt by stab streak method.

3.2.22 Motility, Indole, Urease (MIU) test

Suspected colony was inoculated into the tube containing MIU medium. Then the medium was incubated at 37^0C for overnight.

3.2.23 Methyl red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 370C, a drop of methyl red solution was added. A red coloration is positive and indicates and acid pH of 4.5 or less resulting from the fermentation of glucose (Cheesbrough, 1984).

3.2.24 Voges-Proskaure test

2 ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organisms. It was incubated at 37^0C aerobically for 48 hours. A very small amount (knifepoint) of creative was added and mixed 3 ml of a-naphthol were added and snaked well. The bottle cap was removed and left for and hour at room temperature.

3.2.25 Identification of *Porphyromonas gingivalis* isolates by biochemical test

3.2.26 Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while.

3.2.27 Triple sugar iron (TSI) agar slant

The test organisms were culture into TSI agar slnt by stab streak method.

3.2.28 Motility, Indole, Urease (MIU) test

Suspected colony was inoculated into the tube containing MIU medium. Then the medium was incubated at 37^0C for overnight.

3.2.29 Methyl red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 370C, a drop of methyl red solution was added. A red coloration is positive and indicates and acid pH of 4.5 or less resulting from the fermentation of glucose (Cheesbrough, 1984).

3.2.30 Voges-Proskaure test

2 ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organisms. It was incubated at 37^0C aerobically for 48 hours. A very small amount (knifepoint) of creative was added and mixed 3 ml of a-naphthol were added and snaked well. The bottle cap was removed and left for and hour at room temperature.

CHAPTER-IV

RESULTS

A total of 150 samples were screened by observing their age, sex, education, qualification and sociodemographic status. (Table no: 1) The prevalance were varied in terms of their age group. (Table no:2) and sugar consumption (Table no:3) It indicated that prevalance is very high in 20 to 25 age group of people in compared to other ages.

4.1 Prevalance of periodontitis

streptoccous, staphylococcus, porphyromonas gingivalis were isolated and identified for the oral cavity (buccal fold, interdental spaces) after cultivation on NA, MC, DGA and SS agar medium. The results of morphemically study of characteristics are present in steptoccous was detected from 100 out of 150 samples. Among the positive samples all of are both male and female and age group between 16 to 30. Each of the positive samples was treated as an isolates.

Table 3: Prevalence of periodontitis

Name of district	**Types of samples**	**No. of sample tested**	**No. of positive isolates**	**Prevalence of isolates (%)**	**χ^2 value**	**Level of significance**
Dinajpur Medical Institute	Plaque	60	35	13.89	0.666	0.881 (NS)
	Caries Devrivs	30	27			
	Tartare	10	23			
Total		**100**	**85**	**11.9**		

4.2 RESULTS OF THE STUDY

This chapter covers results starting with socio – demographic characteristics of the students. This is followed by factors that hinder exclusive brushing practices, sugar consuming age, sex, and employ status.

4.3 Socio – demographic characteristics of the students

The sample size of the study population was 150 students. The variables of interest researched on were brushing practices, sugar consuming, age, sex, employ status.

The students‟ ages ranged from 16 to 30 years. The highest percentage of the students 63% was in the age group of 18-28 while 29% was in the age group of 29-30 years, and the least percentage 1.3% was in the age group of 17 years. The mean age of the students was 29.04 years. Most of the students were engaged in some form of employment with only 32% having no employment, more than two quarters 68% were married while 16.7% were single.

Table 4: Age distribution of the respondents

Variable	Frequency	Percent
Age		
Less than 16	2	1.3
18-20	94	63
20-25	44	29
25-30	10	6.7
Mean=29.04 Std. Deviation=8.60		
Total	150	100

The above table show about that the majority of the respondent’s age were 18-20comprising 63% n=150, while few of the respondents were age group between less than 17 comprising 1.3% and 20-25 comprising 29% and the least 25-30 comprising 6.7%

Table 5 : Sugar consuming Status of the respondents

Variable	Frequency	Percent
Sugar Consuming status		
Less sugar intake	38	25.3
Moderate sugar intake	34	22.7
Avoid sugar	30	20
High sugar intake	48	32
Total	150	100

The above table shows that 32% of the study participants were High sugar intake, 25.3% Less sugar intake, and 22.7% were Moderate sugar intake and 20% were Avoid sugar.

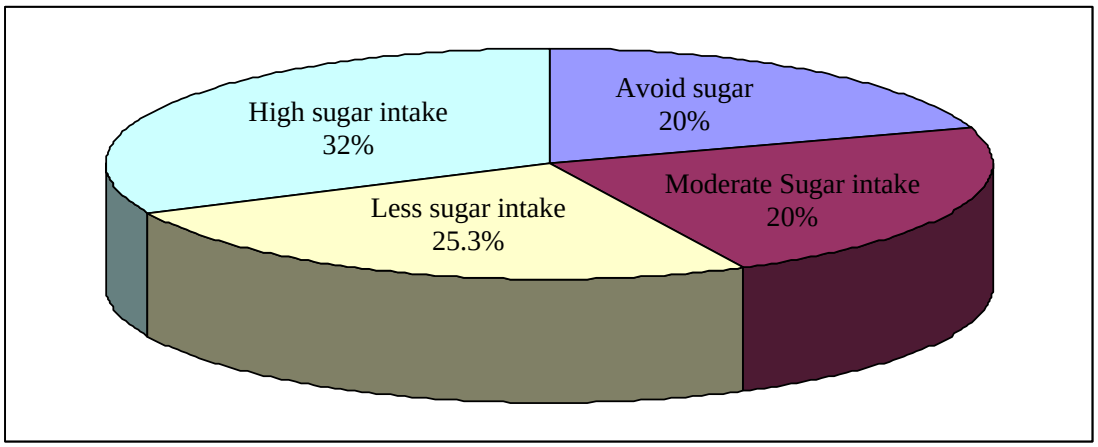


Figure: Sugar consuming Status of the respondents

4.4 Isolation and Identification of oral bacteria

The bacteria were recovered from plaque caries debris, tartar (Plate no: 1) on nutrient broath (The presence of turbidity indicate the positive result), nutrient agar (Bacterial colonies appear white, cream, or yellow in color, and fairly circular in shape). MacConkey agar (The concentration of bile salts, which inhibit gram-positive microorganisms) (The pink colour is due to production of acid from lactose) Sheep blood agar (zones of clear alpha-haemolysis) (Heme from the media is oxidized by the bacteria to produce hemin which accumulates on the cell surface producing a characteristic black pigment). Mitis salivarious agar Medium (pale colour colonies). Manitol salt Agar medium (Yellow colonies surrounded by yellow zone). Brain Heart inffusion medium (Pink color colony due to blood addition). The growth, Morphological and cultural characteristics indicated that the isolated organisms might be streptococcus, staphylococcus and porphyromonus gingivalis. (Table no.....) (Plate no: 20, 21, 22) Which were later confirmed by different biochemical tests. (Table no: 6) The

characteristics of different biochemical test (Plate no: 23, 24, 25, 26, 27, 28, 29, 30) Indicated that the organism might be streptococcus, staphylococcus, *porphyromonus gingivali*.

Table 6: Identification of *Streptcoccous , Staphylococcus, Porphyromonas gingivalis*by cultural and morphological characteristics

Sample (organs)	Colony Characteristics						
	Staining characters	**NA**	**MC**	**MS**	**Mitis agar**	**BHE**	**SBA**
steptococcus,	Gram Positive cocci shaped	Circular, smooth, opaque, translucent colonies	No growth	No growth	Pale color colony against blue background	Pale color colony against colorless background	Green color colony against red background
staphyloccous	Gram Positive cocci shaped	Circular, smooth, opaque, translucent colonies	No growth	Yellow color colonies against yellow field	No growth	Pale color colony against colorless background	Alpha hemolysis against red background
Porphyromonus gingivalis	Gram negative, short rod shaped	Circular, smooth, opaque, translucent colonies	Pink color colonies against red background	No growth	No growth	Pink color colony against red background	Black color colony against red background

Legends

NA = Nutrient agar; **MC** = MacConkey agar; **MS =** Manitol salt agar; **Mitis agar** = Mitis agar Medium, **BHE** = Brain Heart effusion, **SBA =** Sheep blood agar

4.5 Characterization of field isolates by biochemical tests

4.6 Methyl red test

For Streptococcus: **No color change** after addition of reagent is taken as **Negative**.

For Staphylococcus: No **color change** after addition of reagent is taken as **Negative**.

For *Porphyromonas gingivalis:* **No color change** after addition of reagent is taken as **Negative.**

4.7 Voges-Proskauer test

For Streptococcus **color change to red** after addition of reagent is taken as **positive**

For Staphycoccus **color change to red** after addition of reagent is taken as **positive**. For *Porphyromonus gingivalis* **No color change** after addition of reagent is taken as **Negative**.

4.8 TSI slant reaction

For Streptococcus red slant and yellow butt

For Staphylococcus red slant and yellow butt

For *Porphyromonus gingivalis* red slant and yellow butt

4.9 Motility Indole Urea (MIU) tests

For Streptococcus non motile indole negative Urease negative

For Staphycoccus non motile indole negative Urease negative

For *Porphyromonus gingivalis* non motile indole positive, Urease negative

4.10 Indole test

For Streptococcus negative

For Staphycoccus negative

For *Porphyromonus gingivalis positive*

Table 7: Organoleptic characterization of *Oral Pathogens* by biochemical tests

Isolates	Indo	MR	VP	MIU	CA	TSI		
						Butt	Slant	H_2S
streptoccous	_	_	+	_	_	Y	R	_
staphylococcous	_	_	+	_	+	Y	R	_
Porphyromonus gingivalis	+	_	+	_	_	Y	R	+

Legends

Indo = Indole; **MR** = Methyle Red; **VP** = Voges-Proskauer; **SC** = Simmons citrate utilization; **MIU** = Motility Indole Urea; **TSI** = Triple Sugar Iron, **CA**= Catalase test

4.11 Antimicrobial susceptibility of identified isolates.

The result of antimicrobial susceptibility of the isolated bacteria (streptoccous, staphylococcous, Porphyromonus gingivalis) were summarized in (Table no: 7) out of 150 samples 85 samples were identified as streptoccous (85%), staphylococcous (75%), Porphyromonus gingivalis (65%) samples were sensitive to ofloxacine and others were resistance to Cefuroxime, Cefixime, Cefaclore, Nitrofurantoin. (Table No: 8)

Table 8: Antibiotic sensitivity pattern of *Oral Pathogen*

Antibiotics	Zone of inhibition						Perentage of Res. Sen. & Int.		
	Sensitive	Zone of inhibition mm	Intermediate	Zone of inhibition mm	Resistance	Zone of inhibition mm	Sensitive	Intermediate	Resistance
Cefuroxime	-	-	-	-	30	12	00%	00%	100%
Ofloxacin	25	14	5	9	5	-	83.33%	16.67%	00%
Cefixime	-	-	-	-	30	10	00%	00%	100%
Cefaclore	-	-	-	-	30	9	00%	00%	100%
Nitrofurantoin	-	-	-	-	30	10	00%	00%	100%

Table 9: Zone size interpretative chart (EUCAST standard)

Antibiotics	Symbol	Disc content	Zone of inhibition (mm)		
			Sensitive	Intermediate	Resistance
Cefuroxime	CEF	5	21	16-20	15
Ofloxacin	OFL	10	12	11	10
Cefixime	CF	5	17	14-16	13
Cefaclore	CE	30	18	13-17	12
Nitrofurantoin	N	30	19	14-18	13
Kenamycin	K	30	18	14-17	13

Sample of oral bacteria

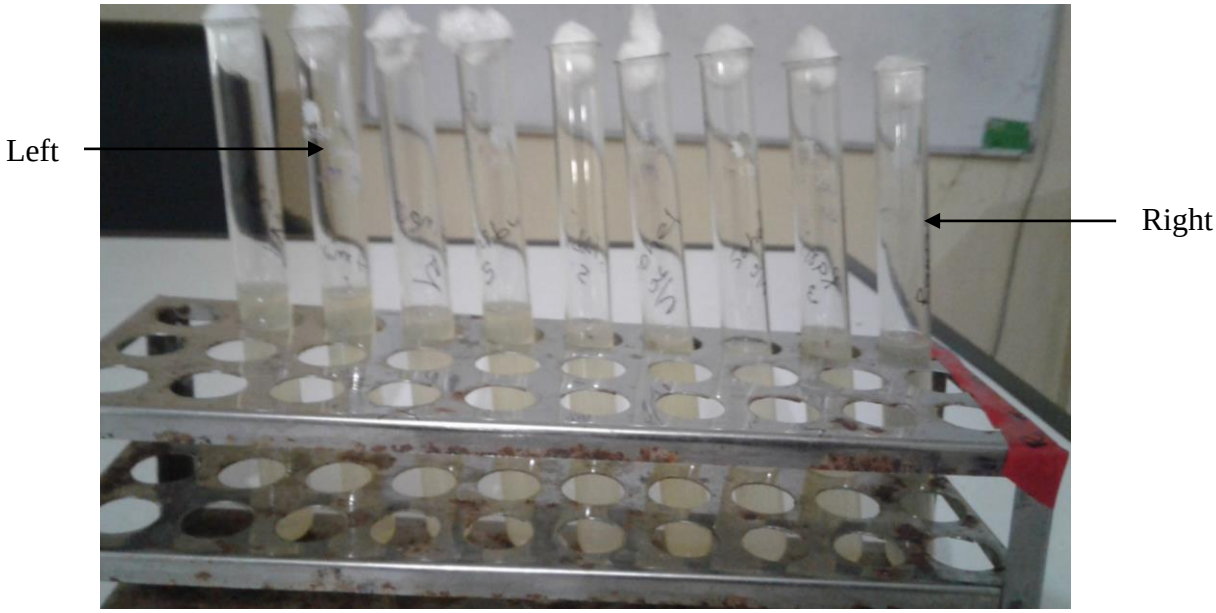


Plate 1: Growth of oral bacteria in nutrent broth showing turbidity (Right) and unincolutaed control (Left)

Plate 2: Growth of *oral bacteria* in nutrent agar medium showing small and round colony (Left)

Plate 3: Un-inoculated control (Right)

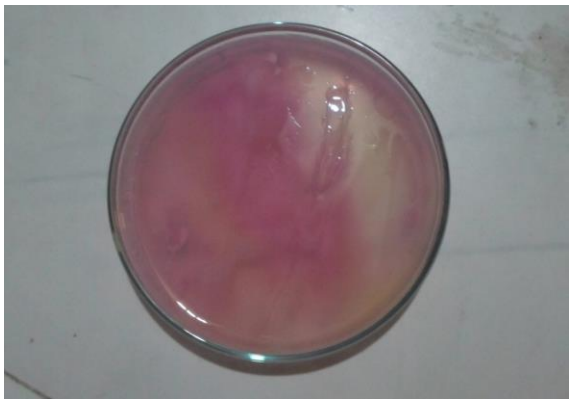


Plate 4: Growth of streptoccous on MacConkey agar showing no growth (Left)

Plate 5: Un-inoculated control (Right)

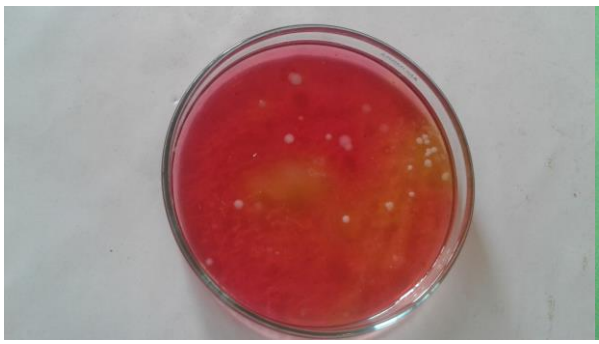


Plate 6: Growth of staphylococcus on MacConkey agar showing no growth (Left)

Plate 7: Un-inoculated control (Right)

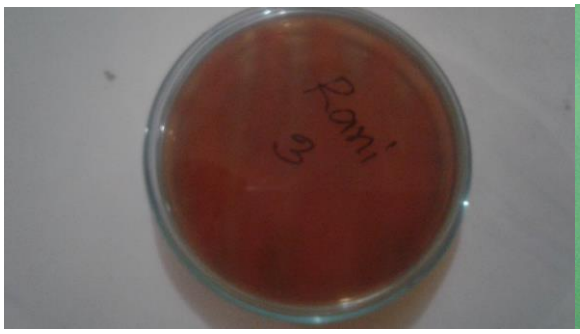


Plate 8: Growth of *Porphyromonus* gingivalis on MacConkey agar showing pink colour colony (Left)

Plate 9: Un-inoculated control (Right)

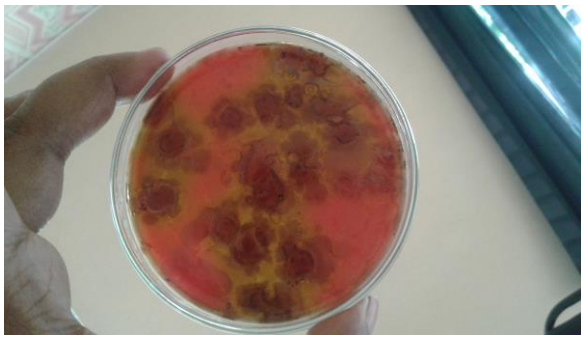


Plate 10: Growth of *streptococcus* on sheep blood agar media showing alpha haemolysis (Left)

Plate 11: Un-inoculated control (Right)

Plate 12: Growth of *staphylococcus* on sheep blood agar media showing green clolour colony (Left)

Plate 13: Un-inoculated control (Right)

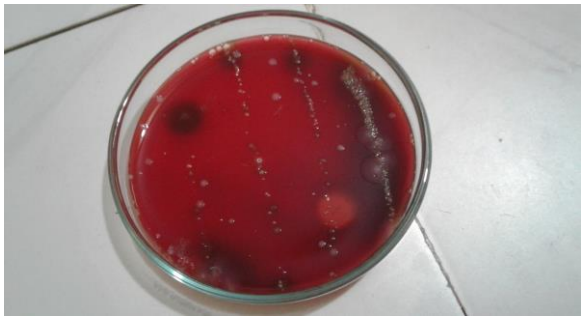


Plate 14: Growth of *Porphyromonus* gingivalis on sheep agar media showing black colour colony (Left)

Plate 15: Un-inoculated control (Right)

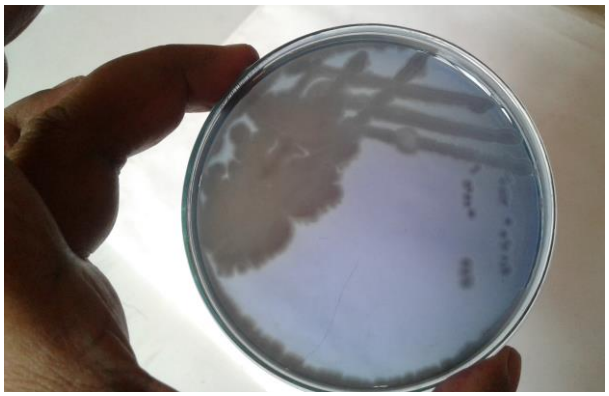


Plate 16: Growth of *Streptococcus* on Mitis Salivaris agar showing translucent pale colour and smooth colony (Left)

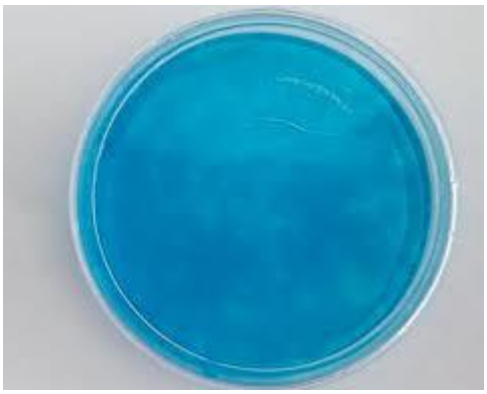


Plate 17: Un-inoculated control (Right)

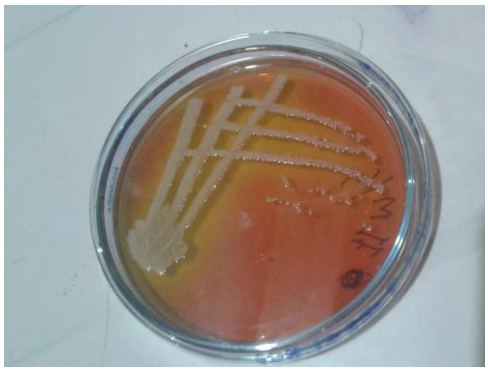


Plate 18: Growth of *staphylococcus* on manitol salt agar media showing yellow colonies surrounded by yellow zone on (Left)

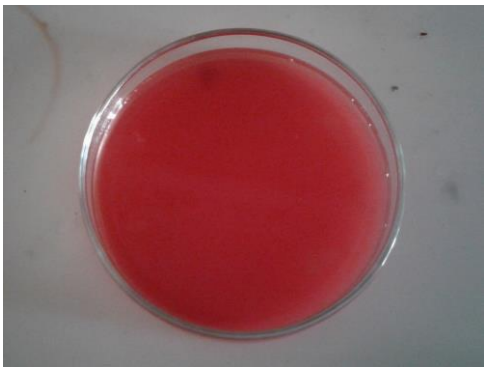


Plate 19: Un-inoculated control (Right)

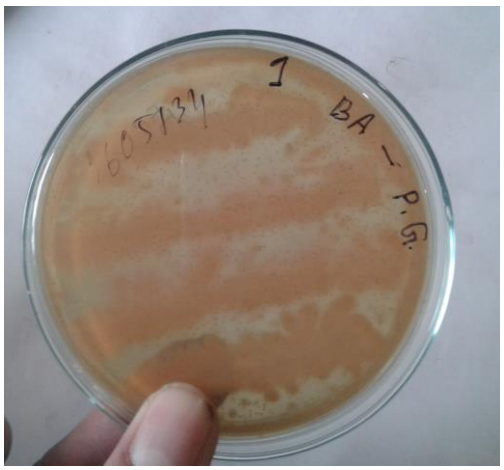


Plate 20: Growth of *porphyromonas gingivalis* on Brain heart infussion agar showing pink color colonies against a pinkish background (Left)

Plate 21: Un-inoculated control (Right)

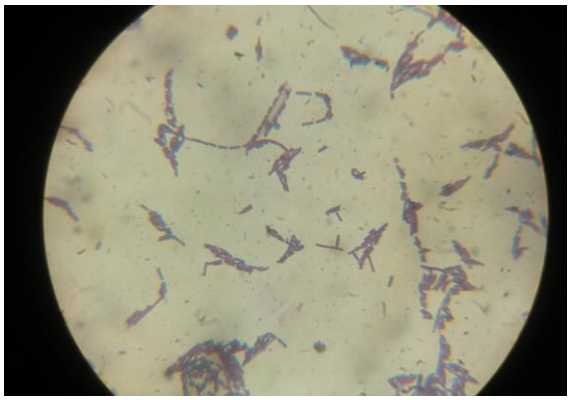


Plate 22: *Streptococcus* showing gram positive cocci in chain form

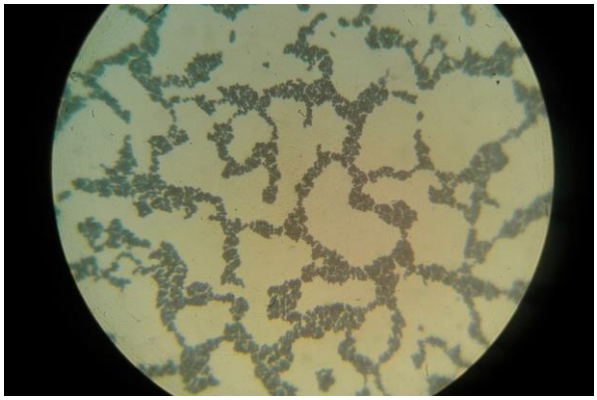


Plate 23: *Staphylococcus* showing gram positive cocci in clusture form

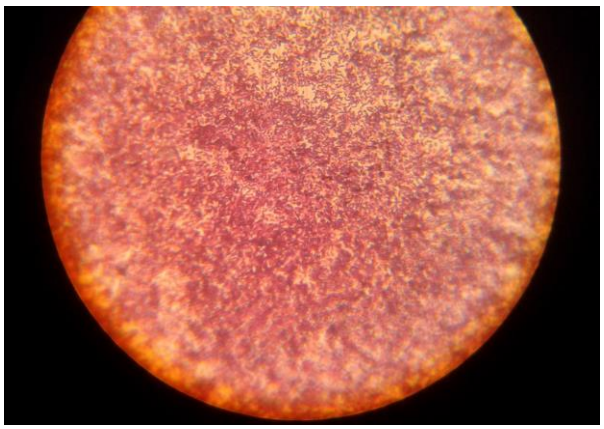


Plate 24: *porphyromonas gingivalis* showing gram negative shaped arranged in single or pair

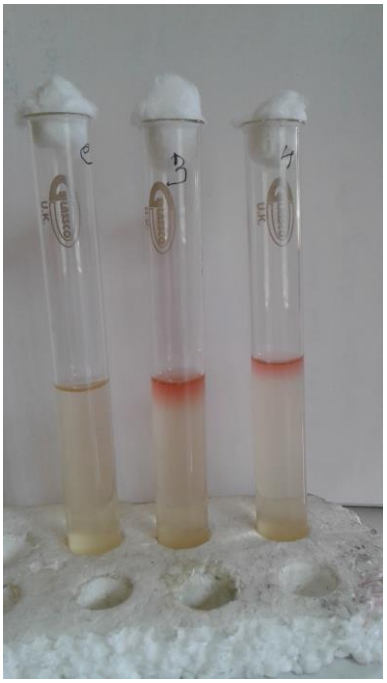


Plate 25: *Oral bacteria* showing negative reaction with light red on the slant in MR Test (Right) and un-inoculated control (Left)

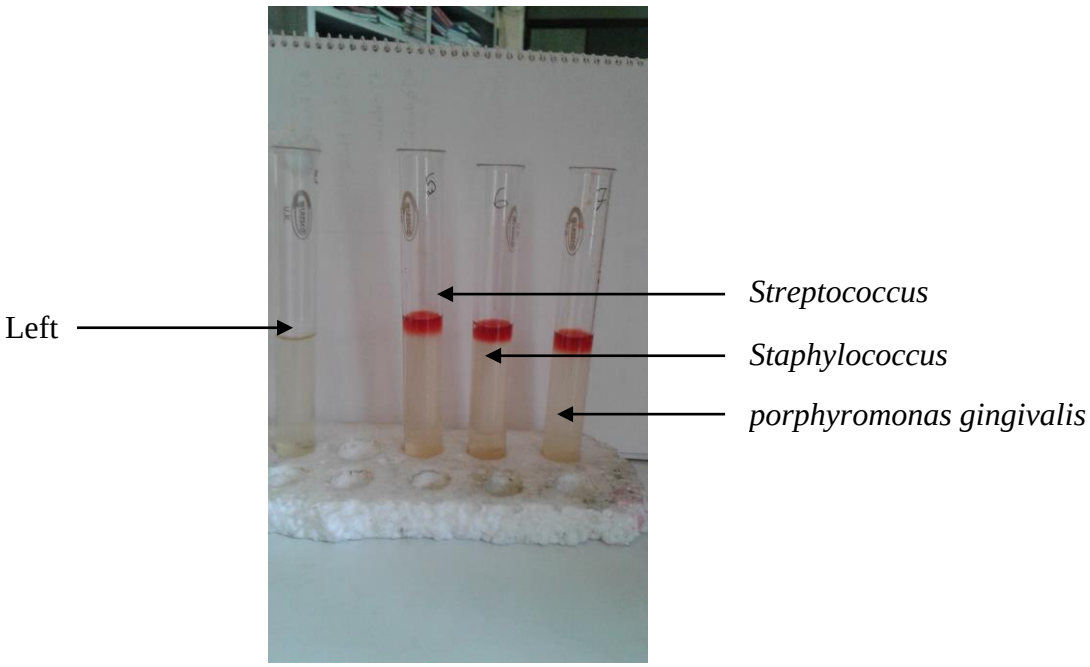


Plate 26: *Oral bacteria* showing positive reaction with Voges-Proskauer Test (Right) and un-inoculated control (Left)

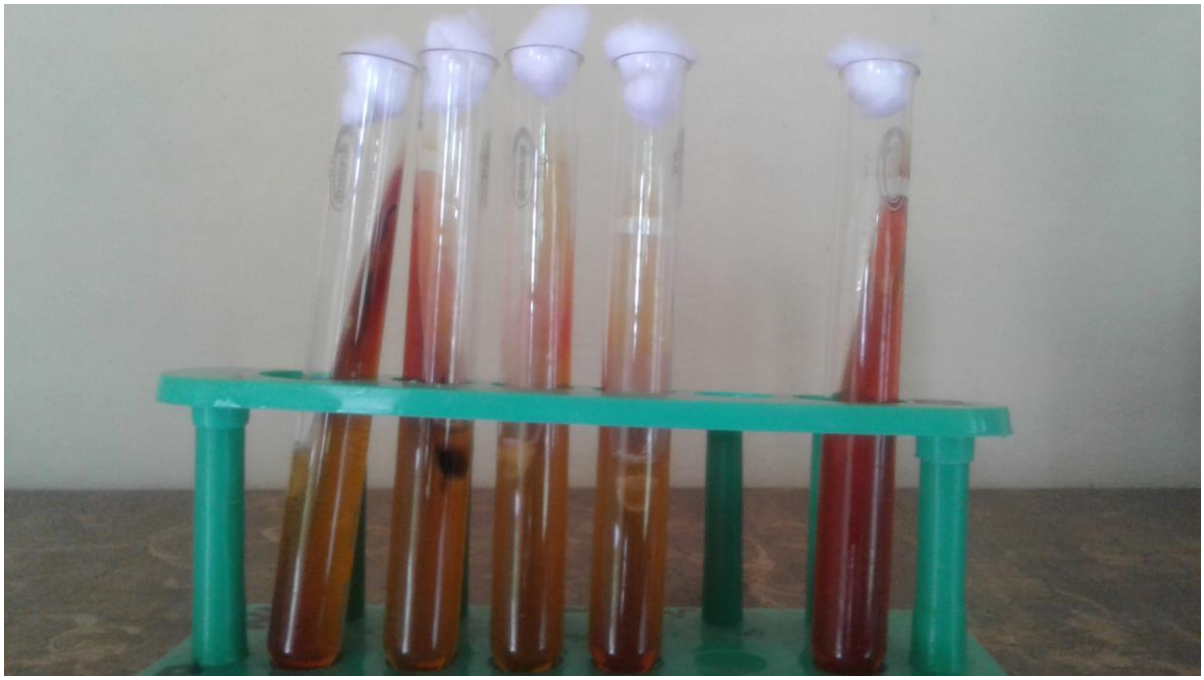
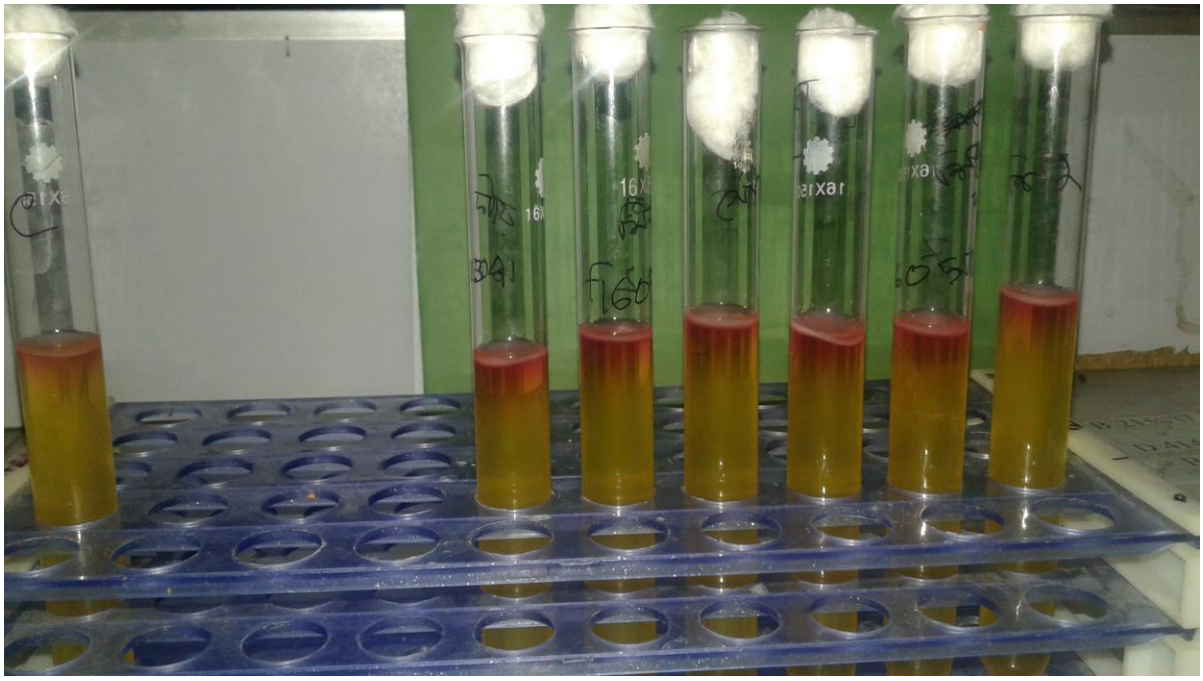


Plate 27: *Oral Bacteria* showing red slant and yellow butt due to sugar farmantation (left) control (Right)



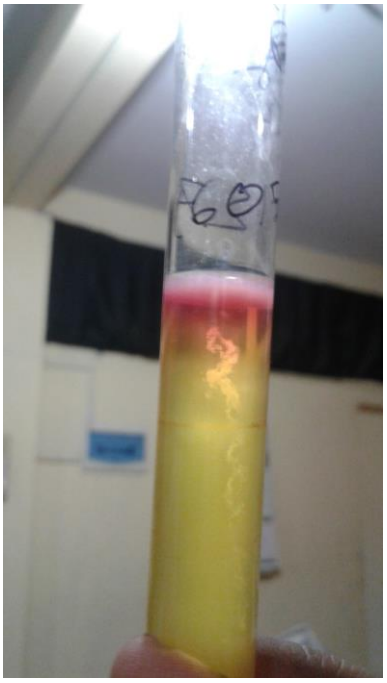


Plate 28: *oral bacteria showing Motility Negetive Indole positive And Urease Negetive*

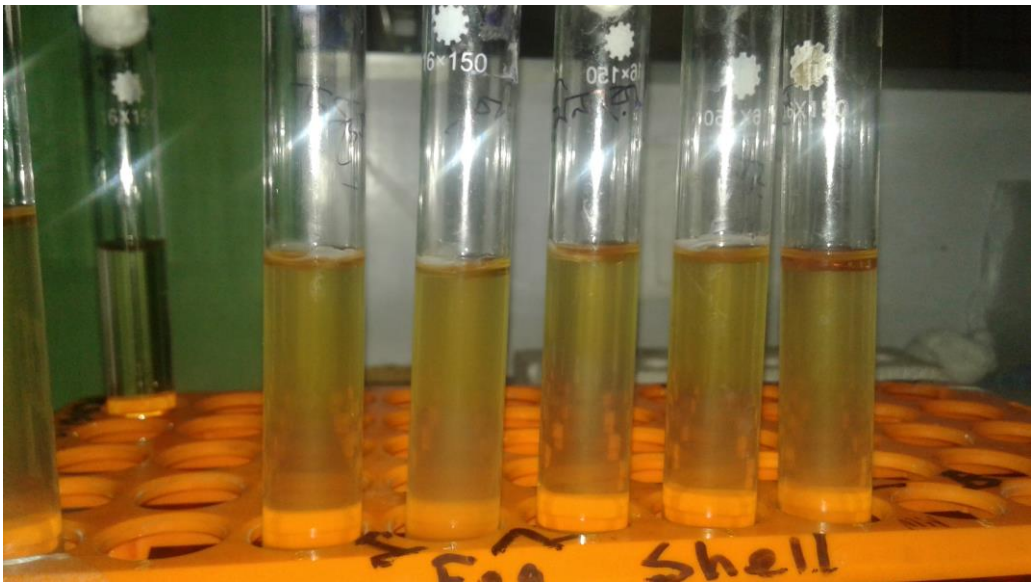


Plate 29: streptococcus and saphylococcus showing Indole negetive

Plate 30: Catalase test showing *Staphylococcus* positive

Plate 31: Catalase test showing *Streptococcus* Negetive

Plate 32: Catalase test showing *Porphyromonas gingivalis* Negetive result.

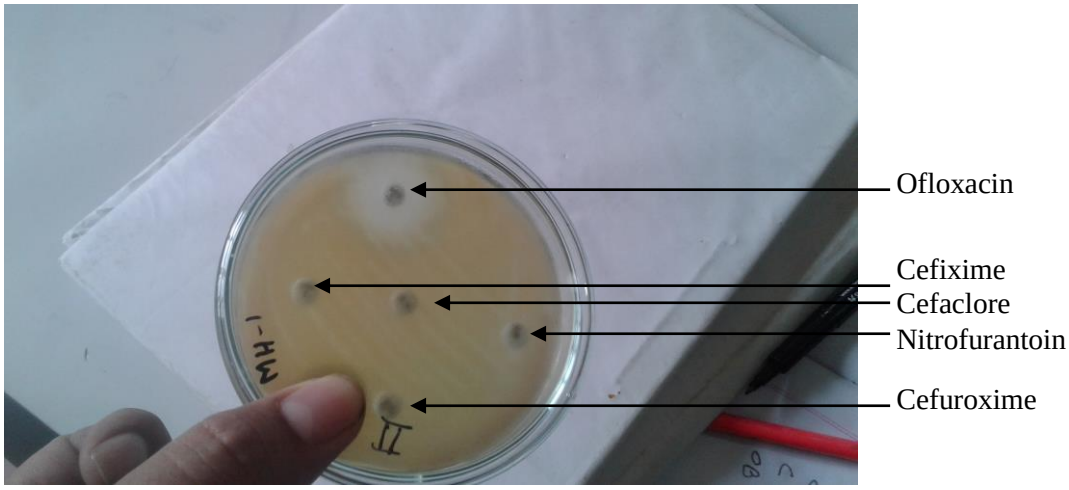


Plate 33: Antibiotic sensitivity test of oral bacreria on Meular hinton agar

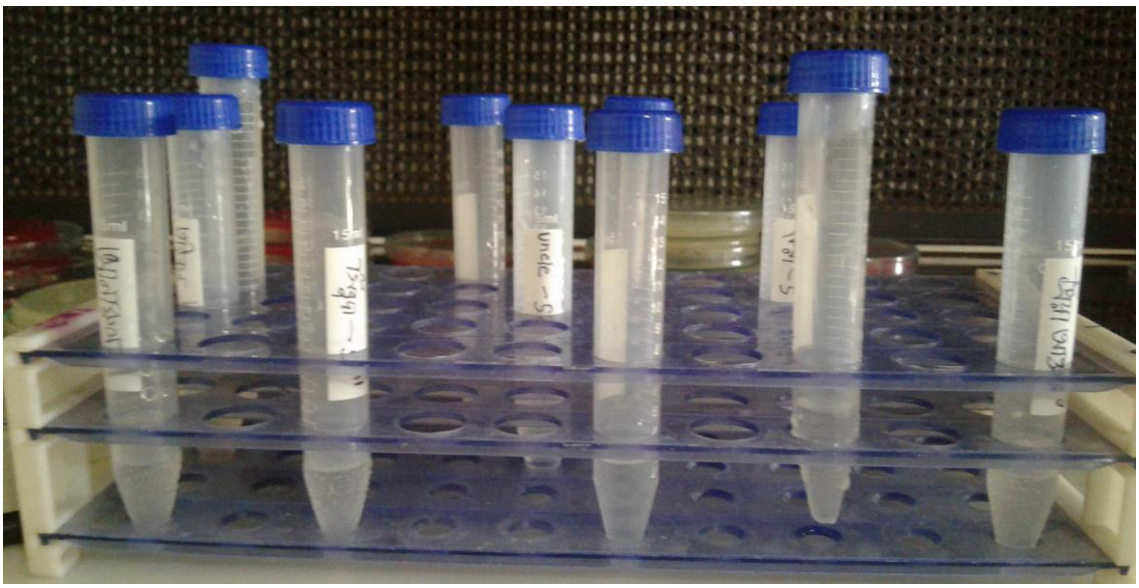


Plate 34: Stock culture

Plate 35: Anaerobic Jar

DISCUSSION

Periodontitis is the inflammation of gingiva causing by oral pathogens is particularly effective at forming biofilms on the gingival sulcus of the human oral cavity. Adherence of *oral pathogens* to gingival sulcus is the first step in the formation of biofilms by these organisms and is mediated by sucrose-dependent and sucrose-independent mechanisms. *Oral pathogens* expresse several surface adhesions that can bind to salivary pellicles formed on the teeth sulcus, whereas sucrose-dependent adherence is mediated by glucan binding proteins and water-insoluble glucans produced from sucrose by glucosyltransferase (GTF) enzymes. (Paraskevas *et al.,* 2008)

Salivary agglutinin (also known as gp340) is a high-molecular-weight glycoprotein in human saliva that mediates the adhesion and aggregation of *oral pathogens* via the cell wall-associated adhesion P1 (a member of the AgI/II family of cell surface proteins, encoded by *spaP*. P1 interaction with fluid phase salivary agglutinin mediates aggregation of *oral pathogens*, whereas adsorption of salivary agglutinin to solid surfaces provides a site for initial adhesion of the organism. Thus, salivary agglutinin is believed to facilitate bacterial clearance from the oral cavity or to promote colonization, depending on whether it is in solution or adsorbed on a surface. (Darveau *et al.,* 2012)

The present study was reflected on the isolation, identification and antibiogram study of oral bacteria from human periodontitis. In this study the collected sample were subjected to morphological cultural and biochemical study. In addition the identified isolates were subjected to antimicrobial susceptibility tests with the commonly used antibiotics by using disc diffusion method followed by EUCAST standard (Zelalem *et al.,* 2011)

In our present study a total of ISO sample comprising plaque (60) dental caries (30) and tartar (10) were collected from patients suffering with periodontitis out of ISO samples 85% were found to be positive for stand 75% staphylococcus and 65% for P. Gingivailis by cultural morphological and biochemical characteristics. These findings supported by other authors (Cohen-Poradosu R, *et al.,* 2007)

In our present findings the prevalence of oral bacteria causing periodontitis were recorded as per information received from the patient by using a structured questionnaire. And the prevalence was higher in young in comprising with child and adults (%). This findings were supported by (IBM SPSS statistics 22)

The above shows that 32% of the study participants were High sugar intake, 25.3% Less sugar intake, and 22.7% were Moderate sugar intake and 20% were Avoid sugar.

This findings supported by several authors like Darveau, R.P.; Hajishengallis, G.; *et al.*. (2012), Fisher *et al..*, *(*1998), Grenier, D; Tanabe, S (2010).

In our present finding it was observed that streptococcus is higher then other organism (85%) this might be due to lack of oral hygiene maintenance or practiced. This findings supported by M, Lamont RJ, Scott DA (2015).

In our present study the antimicrobial susceptibility of isolated bacteria reveled that economically important diseases of oral cavity, *Streptococcus, Staphylococcus and Porphyromonas gingivalis* are the most well recognized bacterial diseases in to Periodontitis causing heavy bleeding, bad odor followed by tooth loss . It is now targeted as a silent killer to our tooth and oral cavity, as the causal organisms transmit from human to human.

In communication of the present study further research work should be performed,

i) Characterization of field isolates covering different serotype techniques like PCR.
ii) The targeted gene PCR product should be further study sequence of the PCR product.

SUMMARY AND CONCLUSION

Among economically important diseases of oral cavity, *Streptococcus, Staphylococcus and Porphyromonas gingivalis* are the most well recognized bacterial diseases in to Periodontitis causing heavy bleeding, bad odor followed by tooth loss . It is now targeted as a silent killer to our tooth and oral cavity, as the causal organisms transmit from human to human.

To prevent the spread of *Streptococcus, Staphylococcus and Porphyromonas gingivalis* in gingival disease management strategies could be undertaken by introducing a continuous monitoring of organism, culling of infected and carrier and implementation of good husbandry practice with plan.

A total of 100 samples from teeth were tested for the presence of *Streptococcus, Staphylococcus and Porphyromonas gingivalis*. Of which 80 samples were found to be positive for Periodontitis. From the present study, it was found that all the tested isolates were highly sensitive to sample suggesting that this sample could be the first choice of drug as an non resistance synthetic antibiotic. Antibacterial sensitivity of sample showed the necessity of in vitro antibacterial sensitivity test prior to treatment. It also emphasizes to have judicious selection of antibacterial agents for effective treatment.

Case processing summary

	Cases					
	Include		Excluded		Total	
	N	Percent	N	Percent	N	Percent
Stepcoccus	30	85.7%	5	14.3%	35	100.0%
Staphyloccous	35	100.0%	0	0.0%	35	100.0%
P. gingivilis	25	71.4%	10	28.6%	35	100.0%

Limited to first 40 cases.

Concluding Remarks:

1. A concise informative prevalence of *Streptococcus, Staphylococcus and Porphyromonas gingivalis* in Dinajpur District of Bangladesh considering study area and organoleptic differences was established for the effective treatment.

2. Isolation and identification technique of *Streptococcus, Staphylococcus and Porphyromonas gingivalis* were successfully performed by using staining, cultural and biochemical techniques in general. However, the strain characterization of field isolates did not be readily used with molecular techniques like PCR.

3. Antibacterial sensitivity study conducted that maintenance of oral hygiene would be the first choice of prevention or treatment.

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APPENDICES

APPENDIX-I

Questionnaire Sheet

Serial No: ☐

Date:.....

1. Particulars of the Patient

i. Name:..... ii. Village:.....

iii. Upazila:..... iv. District:..... v. Mobile:.....

2. According to age

i) Young (2-12) ☐

ii) Adult (13-25) ☐

iii) Old (26-50) ☐

3. According to sex

i) Male ☐

ii) Female ☐

4. According to economic Condition

i) High Class ☐

ii) Middle Class ☐

iii) Low Class ☐

5. According to education

i) Literate ☐

ii) Illiterate ☐

6. According to oral cleanliness

i) Good ☐

ii) Bad ☐

7. According to mode of tooth cleaning

i) Brush with tooth paste

ii) Using Neem, Datun

8. According to dmft score

i) d ☐

ii) m ☐

iii) f ☐

iv) t ☐

9. According to carious teeth

i) Present ☐

ii) Absent ☐

10. According to regularity, frequency, method. Timing and time taken for tooth cleaning.

i) Daily tooth cleaning

a. Regular ☐

b. Irregular ☐

ii) Frequency of cleaning

a. Once daily ☐

b. Twice daily ☐

iii) Method of brushing

a. Correct method ☐

b. Others ☐

iv) Time taken for brushing

a. 1-3 mins ☐

b. Others ☐

11. According to Snacking habit and tooth cleaning habit

i) Tooth cleaning after Snacking ☐

ii) Not cleaning after Snacking ☐

12. According to pregnancy to female patient-

i) Positive ☐

ii) Negative ☐

13. Antibiotics pigmentation

i) Expected ☐

ii) Non Expected ☐

14. According to leaving area

i) Rural ☐

ii) Urban ☐

15. Frequency of dental clinic visit

i) 0, 1, 2, 3, 4 ☐

16. According to occupation

i) Job ☐

ii) Business ☐

iii) Not Work ☐

17. History to accident in maxillofacial region

i) Present ☐

ii) Absent ☐

Signature of investigator

APPENDIX-II

Composition of Different Media

1. Blood Agar Media

Blood Agar Base	-60.0gm
Distilled water	-1000ml
Bovine blood	-5ml
Nutrient agar	-500ml
Sterile defibrinated blood	-25ml

2. Nutrient broth

Ingredients	-g/1
Peptone	-5.0
Sodium chloride	-5.0
Beef extract	-1.5
Yeast extract	-1.5
Final pH(at $25^{O}C$)	-7.4 ± 0.2

3. Nutrient agar

Ingredients	-g/1
Beef extract	-3.0
Peptone	-5.0
Sodium chloride	-5.0
Agar	-20.0
Final pH	-7.4 ± 0.2

4. MacConkey agar

Ingredients	-g/1
Peptone	-117.0
Protease peptone	-3.0
Lactose	-10
Bile salt	-1.5

5. MR-VP media (Himedia, India)

Composition	
Buffered peptone	-7.0
Dextrose	-5.0
Dipotassium phosphate	-5.0
Final pH(at $25^{O}C$)	-6.9 ± 0.2gm

6. Mannitol Salt agar

Proteose peptone	-10gm
Beef extract	-1gm
Sodium chloride	-75gm
D-mannitol	-10gm
Phenol red	-0.025gm
Agar	-15gm
Final pH	-7.4 ± 0.2gm

APPENDIX-III

Preparation of reagents

1. Peptone water

Peptone	-1mg
Distilled water	-1000gm

2. Kovac's reagent for indole preparation

P-dimethyl aminobenzal	-5gm
Amy alcohol	-75gm
Conc.HCL	-25ml

3. V-P reagent-1

5% alpha-napthanol in absolute ethyl alcohol

4. V-P reagent-2

40% potassium hydroxide 0.3% creatine. The ingredient was dissolved by heating gently. Over a steam bath. When in solution, added 0.052gm of cotton blue dye.

5. Methyl red solution

Methyl red	-0.05gm
Ethanol (absolute)	-28ml
Distilled water	-22ml