

**ASSESSING THE ORAL HEALTH RECORDS AND ANTIBIOGRAM OF
ANAEROBIC BACTERIA ISOLATES FROM PATIENTS VISITING DENTAL
UNIT OF AHMADU BELLO UNIVERSITY MEDICAL CENTER, ZARIA**

BY

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FACULTY OF PHARMACEUTICAL SCIENCES,
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FEBRUARY, 2021

DECLARATION

I declare that the work presented in this dissertation entitled “Assessing the Oral Health Records and Antibiogram of Anaerobic Bacteria Isolates from Patients Visiting Dental Unit of Ahmadu Bello University Medical Center, Zaria” has been performed by me in the Department of Pharmaceutical Microbiology under the supervision of Dr. B.A. Tytler and Dr. R.O Bolaji. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Bello Aisha Bisola
Name of Student

Signature

Date

CERTIFICATION

This dissertation entitled “ASSESSING THE ORAL HEALTH RECORDS AND ANTIBIOGRAM OF ANAEROBIC BACTERIA ISOLATES FROM PATIENTS VISITING DENTAL UNIT OF AHMADU BELLO UNIVERSITY MEDICAL CENTER, ZARIA” by Aisha Bisola BELLO meets the regulations governing the award of the degree of Master of Science in Pharmaceutical Microbiology of Ahmadu Bello University, Zaria and is approved for its’ scientific contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this written report to Almighty ALLAH, the most beneficent, the most merciful and source of every true blessing from whom strength, supply and inspiration was received for the work.

Dedicated also to my parents for their support in prayers, finance and their invaluable advice and encouragement all through the period of this thesis.

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ABSTRACT

Oral health can be a good indicator of general health and has been linked to systemic diseases, therefore, maintaining good oral hygiene is important. Oral infections are polymicrobial with dental caries and periodontal disease being the major oral conditions affecting the public. A retrospective study on patients with orodontal conditions in the dental clinic of Ahmadu Bello University Medical Centre, Zaria between January 1st, 2015 and December 31st, 2017 was performed. The medical records of patients diagnosed to have dental conditions were collated. Also prospective study assessed the prevalence of antimicrobial resistance among the common bacterial isolates associated with periodontal disease from oral rinses of patients in the clinic (3months) using standard microbiological methods. The antibiotic susceptibility pattern of bacterial isolates was evaluated using the European Committee on Antimicrobial Susceptibility Testing 2018 recommended guidelines. Data analysis was carried out using descriptive statistics and Chi-square tests was used to determine the significant differences ($P < 0.05$) where appropriate. Out of 10364 visits, prevalence of periodontal disease (65.8%) and dental caries (34.2%) was observed with a significant difference of ($P = 0.000$). A high percentage of male patients 60.5% was observed compared to female patients 39.5% with a significant difference of ($P = 0.004$) was recorded. Most predominate patients were adults. A total of 131 samples of patients with dental conditions was collected with about 40 anaerobic Gram negative bacteria isolated. *Prevotella intermedia* (18%) and *Bacteriodes fragilis* (18%) were the most frequently isolated bacteria, followed by *Fusobacterium nucleatum* (10%), *Fusobacterium varidium* and *Prevotella oralis* (8%) and *Fusobacterium mortiferum*, *Bacteriodes stercoris* and *Porphyromonas asaccharolytica* (6%). High resistance of isolated bacteria to selected antibiotics was observed in metronidazole and chloramphenicol (100%) and amoxicillin-clavulanic acid

(62.5%). About 77.5% of the bacteria isolates were biofilm producers and 22.5% non-biofilm producers. Nine of the anaerobic Gram negative bacteria isolates were characterized genotypically. The resistance gene *bla_{TEM}* was observed in only one bacterial isolate while *cat* gene was conserved in all the nine isolates characterized genotypically. The *nim* gene was virtually absent. This study showed a high prevalence of oral disease among tested patients with adults and male gender preponderance. The antimicrobial resistance rates observed was high and may pose a serious therapeutic challenge to the management of periodontal disease. Preventive measures and sustainable therapeutic strategies for oral health services advocated.

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CHAPTER ONE

1.1 INTRODUCTION

Oral health is an important part of the general health of society and might be a good indication thereof. Monitoring and assessment of attitudes and practice of oral hygiene within a community enables planning for general public health (Jabeen and Umbreen, 2017). It is also an important factor in the organization and management of oral care system (Adeniyi *et al.*, 2012). Oral hygiene implies the maintenance of clear oral cavity free from pathological conditions such as gum disease, cavities, mouth sores, bad breath and ulcers (Jabeen and Umbreen 2017). Poor oral health can be an indication of poor general health as it has been associated with some systemic conditions including cardiovascular disease, diabetes mellitus, respiratory disease like pneumonia, chronic kidney disease, rheumatoid arthritis, e.t.c. (Nazir, 2017).

The healthy human mouth is one of the most heavily colonized parts of the body and contains a diverse range of microorganisms including bacterial, viral, and fungal species (Avila *et al.*, 2009; Sultan *et al.*, 2018). It is estimated to harbour over 700 species of bacteria that colonise the hard surfaces of teeth and the soft tissues of the oral mucosa (Kilian *et al.*, 2016). The teeth enable large masses of microbes to accumulate as biofilms commonly known as plaque (Sultan *et al.*, 2018). Odontogenic infections are some of the most prevalent diseases worldwide, and the principal reason for seeking dental care (Tancawan *et al.*, 2015). Oral infections are polymicrobial and they arise when normal flora changes from commensal to opportunistic pathogens due to a break down in balance within the host in certain circumstances. Oral infections can manifest as acute or chronic forms (Inés *et al.*, 2014). Limited availability of dental care services is a major factor of oral

health in most developing countries especially in sub-sahara Africa (Petersen, 2005; Opeodu *et al.*, 2012). Effective and efficient oral hygiene practices are essential tools to obtaining a good oral health condition (Petersen and Kwan, 2004; Olusile *et al.*, 2014).

Dental caries and periodontal disease constitute the major oral public health problems (Omitola and Arigbede, 2012; Olabisi *et al.*, 2015; Soroye and Braimoh 2017), and are indicators of global burden of oral health and disease (Petersen, 2005). The global distribution of dental infection varies due to geographical locations, socioeconomic pattern and severity of the disease (Joshi *et al.*, 2016). These infections have a major long and short term consequences on the affected population.

Dental caries is the most prevalent oral disease with high morbidity potential. The disease is widespread in every geographical part of the world, affecting people of all ages, gender, race and socioeconomic status (Arora *et al.*, 2016). It is estimated that about 2.43 billion people worldwide suffer from dental caries of permanent teeth and 620 million in baby teeth (Vos *et al.*, 2012). The number of untreated oral condition was estimated at 3.5 billion in 2015 and has likely increased partly due to growing and aging population (Listl *et al.*, 2015; Kassebaum *et al.*, 2017)

Periodontal disease, comprises of both gingivitis and periodontitis, is a chronic inflammatory condition of the periodontium and includes both gingivitis and periodontitis (Tonetti *et al.*, 2017). Its advanced form is characterized by periodontal ligament loss and destruction of surrounding alveolar bone (de Pablo, *et al.*, 2009; Nazir 2017). This oral condition is caused by microorganisms that adhere to and grow on the tooth's surfaces, aggressive host immune response against oral bacteria and dental biofilm (Román-Malo

and Bullon 2017). The dental plaque observed on the surface of the teeth is in fact biofilm formation due to large numbers of colonies (Kimple *et al.*, 2014; Vargas *et al.*, 2015).

Up-to-date information about the economic impact of dental diseases is essential for health care decision makers when seeking to make rational use of available resources. World Health Organization (WHO) estimates that oral diseases are the fourth-most expensive diseases to treat in most industrialized countries (Petersen 2003) with a worldwide costs that was estimated to be \$544.41 billion in 2015 (Righolt *et al.*, 2018).

1.2 Statement of Research Problem

Oral infections are a global public health problem, with periodontal diseases and dental caries estimated to affect about 3.9 billion people worldwide (Murray *et al.*, 2012). This condition is almost always accompanied with increased risk of multiple tooth loss, edentulism and masticatory dysfunction, thereby affecting nutrition, quality of life and self-esteem with attending socio-economic impacts and healthcare costs (Petersen and Ogawa, 2012; Chapple *et al.*, 2015).

Poor oral health and oral disease is one of the many factors that influence various systemic conditions such as cardiovascular diseases, diabetes mellitus, HIV, infective endocarditis and bacterial pneumonia (Li *et al.*, 2000; Mawardi *et al.*, 2015; Masthan *et al.*, 2016; Nazir, 2017; Haque *et al.*, 2019).

Africa, as a continent is identified to have problems, such as crippling poverty, malnutrition and host of other social conditions including poor health-care system (Josefczyk 2015).

Oral diseases such as dental caries and periodontal disease are not generally considered as important when compared with other disease conditions such as Malaria and HIV (Danfillo 2009).

Reports on oral health in Africa are not too widely available. The few available, Ndiaye, (2005) and Abid *et al.*, (2015) have reported an increasing prevalence of oral diseases in the Africa regions.

Nigeria, though acknowledged to be a developing country is still considered relatively well off in the region. Research on oral health in Nigeria have been sporadic with limited resource directed to oral health (Etiba *et al.*, 2015). Dental care was not initially given a lot of considerations as public health planning but quietly gaining great recognitions. In spite of this, general awareness is low and cost is still considered relatively high particularly among low income earners (Tobin and Ajayi, 2017). Information on the utilization of oral health services, oral health awareness and practices of oral hygiene is sparse. Some studies have indicated poor oral health awareness, irregular teeth brushing and slack general oral hygiene practices in Nigeria (Adeniyi *et al.*, 2012; Olusile *et al.*, 2014). It is reported that only few Nigerians visit a dental clinic which may be a result of low numbers of available oral health facilities and other factors related to adequate access to oral health services (Adeniyi *et al.*, 2012). A number of studies have reported varying levels of dental conditions in the country, for example Adegbembo and El Nadeef (1995) reported a nationwide prevalence between 39 to 57% for age 15 and age group 25-29 respectively, Folaranmi *et al.*, (2014) reported a prevalence of dental caries and periodontal disease in Enugu at 68.2 and 91.1% respectively. Other reports on prevalence of periodontal disease include; Kwara state (66%), Edo state (75.7%), Benin City (90.8%) (Umoh and Azodo, 2012; Joshi *et al.*, 2016, Tobin and Ajayi, 2017; Chukumah and Akhionbare, 2017).

The presence of resistance in oral flora specifically those associated with dental disease is an international problem (Sweeney, *et al.*, 2004). Organisms causing dental plaque are

highly resistant to antibiotics making it difficult to treat the infection (Loyola-Rodriguez, *et al.*, 2014). The current knowledge of bacteria causing dental infections and their antibiotic susceptibility profiles are therefore necessary for the prescription of appropriate antimicrobial therapy.

1.3 Justification

The high burden of oral diseases represents a widely underestimated public health challenge for almost all countries worldwide (FDI, 2015). Establishing baseline data on dental conditions and other oral health issues through regular national surveys is crucial for planning and development of intervention programs (WHO, 2012). Most countries in Sub-Saharan Africa focus on high mortality diseases like HIV and AIDS, cancer, tuberculosis, diabetes and malaria. On the contrary, pay little attention to oral health issues yet some have been reported to be associated with some systemic diseases (Mafuvadze, *et al.*, 2013). With the exception of Egwari *et al.*, (2009 and 2016), reports on oral health in Nigeria have mostly been on epidemiological studies.

Despite concerns that dental problem is increasing, there has been little attempt to quantify the burden of dental consultation and associated antibiotics use (Matthews-King, 2013; Cope *et al.*, 2016). The microbial community associated with dental health is highly diverse which may result to increasing antibiotic resistance, especially in dental plaque (biofilm) and change in the susceptibility to effective drugs.

Reports on oral health in Nigeria have primarily focused on the epidemiology within various groups and areas with percentages ranging from 30-91%. The reports have been varied and diverse and only a few have studied the microbiology and resistance patterns to organisms. Of all these reports, only two were done in northern Nigeria. One, Taiwo *et al.*,

(2014) was on facilities in Katsina and the other, Osunde *et al.*, (2017) reported on the reasons for the loss of permanent teeth in Kano were done in northern Nigeria. To the best of my knowledge no report has combined retrospective epidemiological access to facility and microbiological studies in Northern Nigeria.

It is hoped that information obtained from this study will provide current knowledge which would be a guide for management of and prioritizing the preventive measures for oral conditions. The result of the antibiotics resistance pattern should also be a guide to clinicians in the prescribing of the most appropriate treatment.

1.4 Aim

To assess the management of orodental disease in patients and antibiogram of bacteria isolated from patients with orodental disease in dental clinic of A.B.U medical centre.

1.5 Objectives

1. To carry out a three year (2015-2017) retrospective analysis of prevalence and treatment protocol of oral infection in the dental clinic of A.B.U Medical Center Zaria.
2. To determine the mean bacterial load in oral samples of patients visiting the clinic using the viable plate method.
3. To identify bacteria associated with orodental infections among patients attending the Dental unit of A.B.U medical centre
4. To determine the antimicrobial susceptibility profile of the anaerobic gram negative bacteria isolated from the patients.
5. To determine the level of biofilm production by the isolates.

6. To molecularly identify selected resistant genes from anaerobic bacteria isolated from patients.

1.6 Hypothesis

Null hypothesis (H_0): There is no orodontal disease in patients attending dental unit of A.B.U. Medical Centre and there is no antibiotic resistant isolates from the oral rinse obtained.

Alternate Hypothesis (H_1): There is orodontal disease in patients attending dental unit of A.B.U. Medical Centre and there is antibiotic resistant isolates from oral rinse obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Oral Cavity

The oral cavity more commonly referred to as the mouth, is a hollow cavity that permits food and air into the body. It comprises of several uniquely modified tissues, each performing a specific function. The structures include the upper and lower lip, the gum, tongue, teeth, salivary gland and mucosal soft tissues, together these assist in the digestion of food (Abu Naser and Hamed 2016). It is identified as the first point of body defense system and immunity and is the most colonized parts of the body with several habitats distinct for different microbial colonization (Dewhirst *et al.*, 2010; Kilian *et al.*, 2016). The oral cavity forms a diverse ecological environment for the growth and support of various microbial communities such as bacteria, fungi and virus (Xu *et al.*, 2015). These microbial communities compose of complex bacterial flora with about 700 different bacterial species identified and reside in the human oral cavity (Hashim, 2018).

In spite of or maybe because of the vast array of organisms that inhabits the cavity; it is subject to disease, particularly dental caries and the periodontal diseases (Wade, 2013). The oral cavity acts as the starting point for dissemination of pathogenic bacteria to other sites especially in patients who are immune-compromised and suffering from other systemic disease such as malignancies, diabetes, or rheumatoid arthritis or having corticosteroid or other immunosuppressive treatment.

2.1.1 Structure of the Oral Cavity

Anatomically, the oral cavity is the entry point of the digestive system and consist of the lips, oropharynx, hard and soft palates, tongue and floor of the mouth, and buccal mucosa that lines the cheeks, upper and lower gum and teeth. The maxilla constitutes of the upper teeth and the mandible has lower teeth (Lenz *et al.*, 2000; Marur and Demirci, 2014).

The tooth consist of enamel, which is a mineralized soft tissue supported by the dentine, a more resilient hard tissue supported and formed by dental pulp. It occupies about 20% of the mouth area and situated at the entrance of the mouth. Structural composition of the teeth includes a crown, a root and a cervical margin. The teeth play important roles such as masticating food, defense and proper phonetic articulation. The teeth are attached firmly to the jaws, made up of cementum, periodontal ligament and the alveolar bone (Thesleff 2006; Koussoulakou *et al.*, 2009; Souza *et al.*, 2015). The different types of teeth which are incisors, canine, premolars and molar in a normal human number 8,4,8 and 12 respectively. Two sets of dentitions occur over the life span of man, the deciduous occur in early years and are finally replaced by permanent teeth in adulthood.

The tongue is muscular and lined by epithelium which consists of several specialized structures associated to taste sensation. It occupies the floor of the mouth and is associated with striated muscles that are divided into intrinsic muscles within the tongue responsible for alteration of shape and size for speech and swallowing. The extrinsic muscles outside the tongue aids the tongues movement in different directions allowing for protrusion, retraction and elevation (Takemoto, 2001; Iwasaki, 2002; Sanders *et al.*, 2013).

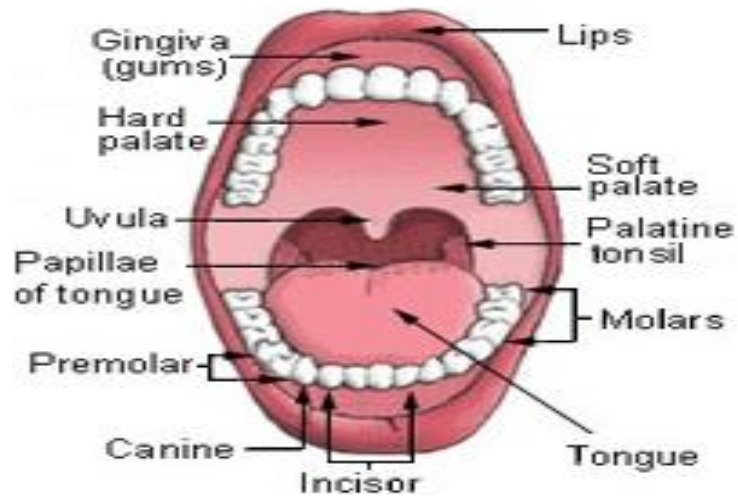


Figure 2. 1: The Diagrammatic Representation of Oral Cavity

Source: https://wellnessadvocate.com/images/anatomy/Mouth_Opened.jpg

2.2 Oral Health

Oral health has been defined as a state of being free from chronic-facial infection, pain, cancer of the mouth and throat, tooth loss, gum disease, dental caries and other oral maladies which have the capacity to hinder biting, chewing, smiling, speaking, and psychosocial wellbeing (WHO, 2012). Oral health is closely linked with general health (Kilian *et al.*, 2016), which may affect well-being and quality of life of individuals (FDA, 2012; Torwane *et al.*, 2014; Taiwo *et al.*, 2014). The organization and management of the oral health system is an important factor influencing the health status of any population (Adeniyi *et al.*, 2012).

World Health Organisation (WHO) and the International Federation of Dentists (FDI) encourage and support the promotion of good oral hygiene (Davies *et al.*, 2003; FDI, 2003; Petersen, 2004) and preventive strategies adopted helps in the reduction of negative effect of oral disease and improve the quality of life.

2.3 Diseases of Oral Cavity

Diseases of oral cavity are often unreported and people accept them as a consequence of aging. They have been classified as the most common non communicable disease which may affect people throughout their life time leading to pain cavity disfigurement and mortality (FDI 2013). Dental disease such as periodontal disease are primarily caused by microorganisms (Sowmya, 2016) where the indigenous bacteria, aerobic Gram positive cocci, anaerobic Gram negative rods and cocci are mostly involved (Kutllovci *et al.*, 2015). Bacteria invasion of the pulp causing this disease occurs through the microtubules of the oral cavity to the vascularized pulp causing pulpitis and acute inflammation which may

spread and destroy the local alveola bone and cause periapical abscess if not treated (Gonzalez-Beicos and Nunez, 2012)

Oral diseases are varied and are of different types with different signs and symptoms. These conditions are common and interventions and preventive measures exist to treat the disease. The seven oral conditions prioritized by WHO include: Tooth decay and cavities (dental caries), gum diseases (periodontal), oral cancers, noma, oral manifestations of HIV and AIDS, oro-facial trauma from accidents and violence, cleft lip and palate. (WHO, 2016).

2.3.1 Periodontal diseases

Periodontal diseases, comprises of both gingivitis and periodontitis. Gingivitis which is the early stage is characterized by swollen, red gum and bleeding and periodontitis is the advanced stage and is characterized by bone lost, gum pulled away from the teeth and the teeth being loosen (Tonetti, *et al.*, 2017). It is the most common oral disease caused by pathogenic bacteria that have biofilm forming ability and triggers inflammatory host response causing gum/tissue destruction and tooth loss (Eke *et al.*, 2012b; Meyle and Chapple, 2015; Vieira-Colombo *et al.*, 2015).

Assessment of global burden of disease between 1990-2010 identified periodontal disease to be the 6th most prevalent oral condition worldwide with a prevalence of 11.2%, affecting about 743 million people. The condition was shown to have increased by to 57.3% from 1990 to 2010 (Murray *et al.*, 2012; Marcenes *et al.*, 2013; Kassebaum *et al.*, 2014a; Jin *et al.*, 2016).

Periodontal disease is mostly seen in adults. From 5-20% of the adults have severe periodontitis and mild to moderate affects majority of adult population (Kassebaum *et al.*, 2014b; WHO, 2016).

Periodontal disease is caused by microorganisms which infect the tissues around the tooth, adhere and grow on the tooth surfaces causing inflammation and overly aggressive host immune response against these microorganisms. The large number of colonies produce large amount of metabolites resulting to dental infection (Sowmya, 2016) and these pathogenic bacteria are present in dental plaque (WHO, 2016). Bacteria organism that have been identified as causing periodontal disease include *Actinomyces actinomycetemcomitans*, *Bacteroides forsythus* (*Tannerella forsythensis*), *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Treponema denticola*, *Peptostreptococcus migros*, *Eikenella corrodens* (Popova, *et al.*, 2013; Santosh *et al.*, 2017).

The dental plaque observed on the teeth surface are in fact biofilm formation due to large numbers of colonies (Kimple *et al.*, 2014; Piano *et al.*, 2014; Bhagya *et al.*, 2014; Vargas *et al.*, 2015). Oral biofilms is important because of the effects oral pathogens have on the overall systemic health. A link has been established between periodontal disease, systemic disease and immune response (Seymour *et al.*, 2007; Arigbede *et al.*, 2012).

2.3.2 Dental caries

Dental caries refers to the destruction of susceptible hard dental tissues and demineralization of tooth enamel by acidic by-products produced by bacteria during the carbohydrates fermentation (Selwitz *et al.*, 2007; Yadav and Prakash 2016). It is a bacterial disease caused by bacteria in the oral cavity which have underlying pathogenic potential.

Dental caries is the most preventable oral disease which causes tooth loss and oral pain and It is a common and major public health disease that obstructs the achievement, protection and maintenance of oral health in all age groups (Thean, *et al.*, 2007).

The pathogenesis of dental caries is the result of three factors which are the host (tooth), bacteria and diet of individuals. The tooth surface is usually colonized by cariogenic bacteria. Sucrose or refined sugar are their dietary source, and when the bacteria ferments the carbohydrate they produce lactic acid which in turns dissolves the hydroxyapatite crystal structure of the tooth leading to dental caries (Watt, *et al.*, 2010; Yadav and Prakash 2016).

Two species of the mutans streptococci ; *Streptococcus mutans* and *Streptococcus sobrinus* are the principal agents of enamel caries (van Houte, 1994;Yadav and Prakash, 2015; Yadav and Prakash 2017). *Streptococcus mitis* are also associated with dental caries (Bello *et al.*, 2013). *Streptococcus mutan* is the main bacteria that have strong association to dental caries (Anil and Anand, 2017).



Figure 2.2: An Infected Tooth Showing Dental Caries

Source: <https://qph.ec.quoracdn.net/main-qimg-465aff9121f8494f6c47d7d3ea77d929-c>



Figure 2. 3: Stages of progression in Periodontal Diseases

Source: <https://hawthorndentalnj.com/images/periodontal.jpg>

2.4 Epidemiology of Dental Infection

Assessment of available data is important in order to understand the clinical and public health impact of oral diseases (Santosh *et al.*, 2017). Dental caries and periodontal disease are major public health problems; therefore epidemiology of these diseases is important for monitoring and identifying their prevalence worldwide (Veiga and Coelho, 2015). These oral conditions have been reported to affect about 60-90% of school children and 5-20% of middle-aged adults (Petersen and Kwan, 2004; Petersen, 2009).

Dental caries condition increases with age and increase in severity in permanent teeth as a result of untreated caries. A global prevalence of dental caries 29% and 59% for adults over the age of fifty has been reported (Yadav and Prakash, 2016). In Europe, some reports have indicated a prevalence of between 72 and 78% (Kamberi *et al.*, 2016; Andegiorgish, *et al.*, 2017) while the prevalence rate in Africa was put between 37.4 to 59.5% (Mafuvadze *et al.*, 2013; Msyamboza *et al.*, 2016). In Nigeria, dental caries prevalence has been reported to be between 4 and 40% (Akpata, 2004; Denloye, *et al.*, 2005; Adekoya-Sofowora, *et al.*, 2006; Omitola and Arigbede, 2012; Braimoh, *et al.*, 2014; Akinyamoju *et al.*, 2018).

Periodontal disease is the 6th most prevalent disease worldwide with an overall prevalence of 11.2% and an increased global burden of 57.3% was observed from 1990 to 2010 (Murray *et al.*, 2012; Marcenes *et al.*, 2013; Kassebaum *et al.*, 2014a; Jin *et al.*, 2016). The severity, prevalence and the burden of this dental condition increases with age and a growing population (Kassebaum *et al.*, 2014b; Jepsen *et al.*, 2017). Carasol *et al.*, 2016 reported a prevalence of 10.1% in a Spanish population. National representative survey conducted in Germany in 2014 recorded prevalence of 10.4% and 8.2% in 35-44 years old and 24.6% and 19.8% for 65-74 years old (Jordan and Micheelis 2016). Lorenzo *et al.*,

2015 recorded an overall prevalence of 9.1% between 2010–2011 in Uruguay (Lorenzo *et al.*, 2015). In Nigeria, a survey reported a prevalence of 75.4% and 15.4% of gingivitis and periodontitis respectively among adults male (Umoh and Azodo, 2012). Similarly a prevalence of 39% and 57% was reported for age 15 and age group 25 – 29 was reported for periodontal disease (Adegbenbo *et al.*, 1995). Folaranmi *et al.*, 2014 reported a prevalence of dental caries and periodontal diseases in Enugu to be 68.2% and 91.1% respectively.

In 2015, about 3.5 billion people of the global population were reported to have untreated oral conditions where 2.5 billion were adults, 573 million were children with untreated dental caries, 538 million had severe periodontal disease and 276 million people with tooth loss. With the continuous growing and aging of the population, the number of affected people is likely to increase. The cost of treatment for oral conditions in 2010 was estimated at US\$298 billion worldwide which corresponds to about 4.6% of global health expenditure (Listl *et al.* 2015; Kassebaum *et al.*, 2017).

2.5 Microorganism Responsible for Dental Disease

Most diseases of the oral cavity are caused by endogenous microorganism acting as opportunistic pathogens (Hashim 2018). Several microorganisms may be involve in periodontal disease and their colonization of the peridontium resulting in inflammation of the subgingival region (Jenkinson and Lamont, 2005). The complex interactions between bacteria and the host defenses affects host-bacteria balance which significantly determines the occurrence of periodontal disease (Hajishengallis *et al.*, 2012).

A number of studies have investigated the prevalence of periodontal pathogens in different parts of the world. Studies in Brazil reported the prevalence of *P. gingivalis* (17-90%), *B.*

forsythus (33.3-100%), *A. actinomycetemcomitans* (23-90%), *T. denticola* (33.6-60%) (Avila-Campos and Velásquez-Meléndez, 2002; Avila-Campos 2003; Cortelli *et al.*, 2005; Imbronito *et al.*, 2008; Kantorski *et al.*, 2006; Klein and Gonçalves, 2003; Victor *et al.*, 2008; Farias *et al.*, 2012). Organisms like *P. gingivalis* (75.8-92%), *P. intermedia* (54.5-82%), *A. actinomycetemcomitans* (23-41.7%), *B. forsythus* (92%) and *T. denticola* (100%) have been identified in some reports in Africa. Similarly in Nigeria, Bacteriodes was the most prevalent periodontal isolate followed by *Fusobacterium* and *Prevotella* with *Porphyromonas* as the least prevalent organism (Egwari *et al.*, 2016).

2.5.1 *Porphyromonas gingivalis*

One of the major pathogens associated with the pathogenesis and progression of periodontal disease is *P. gingivalis* (Hajishengallis *et al.*, 2012). It is an anaerobic gram-negative oral bacteria found in periodontitis. It resides in the subgingival sulcus of the oral cavity and depends on amino acid fermentation to produce energy which is important for survival in deep pocket peridontium where access to sugar is limited (Bostanci and Belibasakis, 2012). The virulence factors and the extracellular protease such as lipopolysaccharide, fimbria, gingipain etc., produced by the bacteria cause the destruction of periodontal tissues (Moon *et al.*, 2013; Decaillet *et al.*, 2012; Hayashi *et al.*, 2012; Perez-Chaparro *et al.*, 2009; Rafiei *et al.*, 2017). The virulence factor aids the invasion of bacteria into the periodontal tissue and evade the host defense mechanisms causing inflammatory response and deregulating innate immune system (Bostanci and Belibasakis, 2012). It is a secondary colonizer which usually adheres to the primary colonizers in dental plaque (How *et al.*, 2016) and its pathogenic ability is attributed to its ability to form

biofilm (Mysak *et al.*, 2014). This organism has been studied extensively because of its ability to evade the immune response (Tribble *et al.*, 2013).

2.5.2 *Fusobacterium nucleatum*

It is an obligate anaerobic Gram negative rod, a member of phylum Fusobacter and part of the microflora of the mouth. It is one of the most abundant organism in the mouth of healthy and infected individuals (Field *et al.*, 2012; Griffen *et al.*, 2012; Loozen *et al.*, 2014). It is said to be a periodontal pathogen because it is always isolated, produces a high tissue irritants, and it can form coaggregates with other periodontal pathogens. It acts as a bridge between early and late colonizing bacteria on the enamel surface (Machuca *et al.*, 2010). It is associated with the various forms of periodontal diseases, mild, advanced, chronic, localized aggressive, and generalized aggressive periodontitis (Saygun *et al.*, 2011; Feng *et al.*, 2014; Liu *et al.*, 2014; Yang *et al.*, 2014; Kistler *et al.*, 2013). Its prevalence increases with increasing severity, progression of inflammation and pocket depth (Yang *et al.*, 2014; Riep *et al.*, 2009). *F. nucleatum* can also be detected in saliva and its quantity is higher in patients with gingivitis and periodontitis than in healthy patients (Saygun *et al.*, 2011; Zhou *et al.*, 2015; Han, 2015).

2.5.3 *Prevotella intermedia*

It is a pathogenic Gram negative, black pigmented anaerobic bacteria belong to the genus *Prevotella* and phylum; Bacteroidetes (Socransky and Haffajee, 2002). It is a member of the orange complex frequently found in subgingival plaque and a core species of the subgingival microbiome (Hong *et al.*, 2015). It is also considered as a major bacterium associated with periodontal disease (Eley and Cox, 2003; Kamma *et al.*, 2004; Zhang *et al.*,

2017). This bacterial pathogen has also been linked to other oral infections including endodontic infection, pregnancy gingivitis and acute necrotizing ulceration (Naito *et al.*, 2016). It possesses various virulent factors including adhesins (Iyer *et al.*, 2010; Sengupta *et al.*, 2014), proteolytic enzymes (Mallorqui-Fernandez *et al.*, 2008) and lipopolysaccharides (Hashimoto *et al.*, 2003) which allow it to colonize oral cavity, evade host defenses and cause tissue damage.

2.6 Risk Factors

Poor oral health is significantly associated with morbidity and mortality (Rabiei *et al.*, 2012; Naseem *et al.*, 2017) especially in immuno-compromised patients promoting life threatening systemic disease (Meurman, and Hämäläinen 2006;WHO 2017a; Haque *et al.*, 2019) . Systemic disease affect oral health and chronic inflammatory oral disease have bad effect on general health. There is a relationship between oral health and general health which has led to oral manifestation, resulting to increased risk of oral disease (FDI, 2015).

Risk factors play a major role in an individual's reaction to oral infection therefore identifying them assist in targeting affected individuals to implement prevention and treatment programmes (Genco and Borgnakke, 2013). The risk factors are classified as modifiable and non-modifiable risk factors. Modifiable risk factors include microorganisms, tobacco smooking, diabete milletus, cardiovascular disease, drug induced stress, nutrition and obesity whereas non-modifiable risk factors include osteoporosis, hematological disorders, host responses, female humornal alteration and pregnancy (Genco and Borgnakke, 2013; Aljehani 2014a; Mehta, 2015).

2.6.1 Smoking

One of the major risk factors associated with dental infection is smoking. A decrease in smoking is reported to be directly proportional to a reduction in the prevalence of dental infection (Bergstrom 2014). Smoking tobacco, cannabis and cigarette has a detrimental impact on periodontal disease (Underner *et al.*, 2009) with adverse effect on the immune system and it alters the oral microbiota leading to an increased level of some particular periodontal pathogens or effect on host response (Bergstrom 2014). Some studies have shown an association between cigarette smoking and common adult forms of periodontitis (Bergstorm and Preber, 1994; Gautam *et al.*, 2011; Borojevic, 2012; Jang *et al.*, 2016).

2.6.2 Microorganisms

The microbiome of the oral cavity consists of over 700 different phylotypes and about 400 species found in subgingival plaque (Paster *et al.*, 2006; Berezow and Darveau, 2011). About a hundred species of bacteria are harboured in the subgingival plaque but only a few are associated with the progression of the disease and are considered causative agents. Bacteria communities causing periodontitis have virulence potentials which causes tissue destruction directly and triggers destruction of immunopathologic host responses which leads to the soft and hard destruction of gum and tooth loss (Armitage and Robertson, 2009).

2.6.3 Diabetes mellitus

This is a clinical condition characterized by hyperglycemia as a result of total or relative deficiency of insulin. It is a systemic risk factor that plays a major role in initiating and progression of periodontal disease (Chávarry *et al.*, 2009; Preshaw and Bissett, 2013;

Casanova *et al.*, 2014). A relationship has been established between diabetes and periodontal disease where diabetic patients are more likely to develop periodontal disease than non-diabetic patients and the severity is proportional to the duration of the diabetes (Li *et al.*, 2000). However, studies have also mentioned the effect of periodontal treatment on glycemic control in patients with diabetes (Al-Mubarak *et al.*, 2002; Janket *et al.*, 2005; Perrino, 2007; Promsudthi *et al.*, 2005; Aljehani 2014b). Therefore, it is important to control periodontal disease not only for good oral health but for general health (Metha 2015)

2.6.4 Poor oral hygiene

Periodontal disease and other oral conditions are linked to poor oral hygiene. The lack of tooth brushing, flossing and other measures to attain good oral hygiene can lead to bacteria formation of dental plaque on the tooth and gum which may lead to the inflammation of the peridontium (de Oliveira *et al.*, 2010). A relationship has been established between poor oral hygiene and accumulation of dental plaque and also increased severity of infection (Albandar 2002). Poor oral hygiene may increase the risk of periodontal disease in patients with type 2 diabetes leading to disease progression (Preshaw and Bissett, 2013; Casanova *et al.*, 2014).

2.6.5 Stress and Medication

Stress and the use of some medications favor periodontal disease. Reduction in salivary flow as a result of use of medication makes an individual vulnerable to dental infections (Güncü *et al.*, 2005) and the most common medications that causes reduction in salivary flow and dry mouth are tricyclic antidepressants, atropine, antihistamine and beta-blockers

(Scully, 2003). There are stress mediated physiological effects which have severe effects on a proper functioning immune system. Stress such as academic stress can lead to poor oral hygiene and inflammation of the gum which is mediated by interleukin-1 β (Reners and Brex, 2007; Degasperi *et al.*, 2018). Other risk factors include age, socioeconomic status, gender and genetics.

2.7 Dental Plaque (Biofilm)

Biofilm is defined as a sessile microbial community characterized by cells adhering to a solid surface or to each other and embedded in a matrix of extracellular polymeric substances and demonstrate an altered phenotype with regard to growth, gene expression and protein production (Donlan, 2001; Saini 2011; Kouidhi *et al.*, 2015). Microbial cells that develop in the biofilm matrix are physiologically different from the planktonic cells of same organisms. They form biofilm matrix in response to certain factors such as cellular recognition of specific and non-specific attachment sites on surface or exposure to a concentration antibiotics. The bacteria forming biofilm are often resistant to antimicrobial treatments because of the ability to form biofilms making them a serious health risk. (Deb *et al.*, 2014).

The ability of microbes to attach to an environment such as the tooth surface and multiply in shielded areas like periodontal pockets and tooth crevices is essential for the organisms to flourish. The accumulation of these microbes on the surface of the tooth results to plaque because of its yellow color (Chandki *et al.*, 2011). The dental plaque observed on the tooth surface are in fact biofilm formation due to large numbers of colonies of microorganisms (Kimple *et al.*, 2014; Piano *et al.*, 2014; Bhagya *et al.*, 2014; Vargas *et al.*, 2015) which produces large amount of metabolites resulting to dental diseases (Sowmya, 2016).

In dental biofilm, bacteria homeostasis is maintained by the host immune system, salivary flow, and healthy dietary intake (Marsh, 2006; Marsh *et al.*, 2011) but a shift in the composition of the bacteria responsible for causing dental disease leads to an increased level of pathogenic bacteria within the dental plaque matrix (Hajishengallis and Lamont 2012; Marsh *et al.*, 2016). The changes in these bacteria composition are due to factors that alter the local host immune responses such as chronic inflammation, unfavorable host genetic predisposition, and the presence of other environmental alterations which favours overgrowth and shift of endogenous species (Bartold and Van Dyke, 2013), and the overgrowth of pathogens will result in increased pathogenicity of the biofilm environment on the teeth (Hajishengallis and Lamont 2012; Marsh *et al.*, 2016).

2.7.1 Formation of dental plaque on the teeth

About 700 different species of bacteria reside in the oral cavity. They are complex and diverse and the environment within the oral cavity is important such as the warmth, neutral pH and moisture which supports the growth of microorganisms (Marsh *et al.*, 2011).

Dental plaque formation is a complex process that involves several stages beginning with adherence to tooth surface (Vasudevan, 2017), they pass through several stages after attachment leading to the formation of mature cells that have the ability to cause infections (Gurenlian 2007; Huang *et al.*, 2011). There is initially a formation of film on a clean dental surface that consists of salivary glycoprotein before adherence (Larsen and Fiehn 2017). The glycoprotein aids in the formation of pellicles which serves as a binding site for the organism. The pioneer bacterial species which are Gram positive adhere to the pellicle followed by gram-positive rods (Vir, 2010) adhere to the pellicle through receptor-pairs

(Marsh *et al.*, 2016) and the stage is known as adhesion phase, attachment phase or lag phase because the metabolic activity is reduced and it is not inert.

During biofilm formation, the bacteria are active metabolically using nutrients in the saliva which results in extracellular matrix formation contributing to co-adherence of bacteria to tooth surface and confer protection on biofilm forming bacteria (Do *et al.*, 2013). The secretion of extracellular matrix which holds the bacteria communities together follows after attachment and then invasion of secondary colonizers which is favored by co-aggregation. Within the extracellular matrix, the bacteria multiplies causing a diverse mixed biofilms (Quirynen *et al.*, 2006).

2.7.2 Implications of microbial biofilms

Biofilm formation by human pathogens have important clinical implication in some areas related to treatment and management of infectious diseases and they are: increased resistance to antimicrobial drug therapy, the ability of cells within biofilm matrix to evade host immune defences, biofilm formation on medical devices causing the failure of the device or serving as a reservoir for recurrent infection and modulation of the host immune system in persistent chronic medical condition (Manavathu and Vazquez, 2014).

Cells within the biofilm matrix are often highly resistant to antimicrobial drugs and difficult to eliminate with standard antimicrobial therapy. These cells are about 1000 fold more resistance to antimicrobial drugs than their planktonic cell counterparts. The extracellular matrix (ECM) was initially believed to be the cause of resistance to antibiotics by acting as a physical barrier which affects drug accessibility to the targeted cells. However, evidence

has shown that the ECM may only be a small part of the drug resistance mechanisms shown by microbial biofilms (Manavathu and Vazquez, 2014).

Bacteria cells within the biofilm matrix acquire transmission of antimicrobial drug resistance determinants by Horizontal Gene Transfer (HGT) which occurs in intra-species and interspecies (Estivill *et al.*, 2011; Juhas, 2013; Seitz and Blockesch, 2013).

2.8 Diagnosis

Diagnosis of a periodontal disease is made by the clinical signs and symptoms together with the medical history of the patient followed by radiographic examination. Effective treatments are easily made by accurate diagnosis and classification of the disease. However the decisions made by the clinicians may be subjective (Ozden *et al.*, 2015). There are factors that must be considered which determine the success of periodontal therapy but the most important factor is the accurate and clear picture of the damaged periodontal bone to be treated (Braúna *et al.*, 2014) information on the amount and type alveolar bone damage is important and can be provided by radiography (Armitage, 2004) and it is also important to determine the extent and severity of the periodontal lesions (Aljehani 2014a). Two dimensional imaging classified into intraoral and extraoral imaging, is a technique that is used routinely to assess the alveolar bone defects in periodontology. The digital intraoral imaging is used in dentistry to show the entire image of tooth/teeth, periapical region and some of the surrounding structures while the extraoral panoramic radiographs are used to view larger areas. Cone beam computed tomography (CBCT) has the ability to diagnose bone craters and can capture images of these areas with no limitations (Acar and

Kamburoğlu, 2014; Akarlsan and Peker 2015). It has better potential of detecting periodontal bone compared with periapical radiographs.

2.9 Prevention and Treatment

2.9.1 Prevention of dental infection

Population-wide prevention and interventions are important and should be universally available and accessible to enable patients register and attend in accordance with individual risk assessments and these interventions include the use of fluoride, and comprehensive oral health care center for patients (Robertson *et al.*, 2015).

Population wide strategies on the reduction of free sugar consumption should be a high and urgent priority to prevent tooth loss, dental caries and other disease which is usually as a result of life long exposure to free sugars (WHO 2017b). Ideally in food and drinks non-sugar sweeteners should be used and in the case where sweeteners are required xylitol should be considered (S.I.G.N 2014).

Brushing the teeth twice-daily with fluoride-containing toothpaste (1000 to 1500 ppm) should be encouraged (O'Mullane et al 2016). Long-term use of this optimal level of fluoride has resulted to a substantially lower incidence and prevalence of tooth decay in all ages combined (Petersen and Ogawa 2016).

Oral health inequalities must be reduced by tackling the broader social determinants through a range of complementary downstream, midstream and integrated upstream policies such as: water fluoridation; regulations on marketing and promotion of food containing sugar and taxes on sugar-sweetened beverages. Promoting a healthy setting such

as healthy cities, workplaces and health awareness in schools are crucial to building a comprehensive support in environments for oral health promotion (WHO 2018).

Personal oral hygiene using tooth brushes is effective in reducing plaque (van der Weijden 2015). The use of tooth brush and tooth paste alone is not enough to accomplish an interdental cleanliness but additional use of floss would be accurate (Chapple *et al.*, 2015). Effective prevention of periodontal diseases produced some guidelines European Federation of Periodontology (EFP) 2015 (GEPPD 2015) where it was stated that there are selective mouth rinse that provide better and effective better management and prevention of dental plaque (Serrano *et al.*, 2015; Herrera *et al.*, 2018).

2.9.2 Treatment

There are several available treatments for dental disease which are in place to stop the progression of the disease or to repair the damage it has caused and the choice of treatment depends on the severity of the disease. The treatment of disease involves various methods which are surgical intervention, mechanical therapy and use of pharmacological agents (Tariq *et al.*, 2012).

Antibiotics are only prescribed to patients who are showing signs of systemic spread or are immune-compromised (Seppänen *et al.*, 2008). There are insufficient guides to promote the use of one treatment regime over the other but the use of low dosage for a short period is consistent with clinical cure and has been effective and also reduces development of resistance (Ellison, 2011). Amoxicillin has always been the first choice of antibiotics and clindamycin as alternative for patients who show allergic reaction to antibiotics in the penicillin group. Where there is no evidence of the infection spreading but there is presence

of pain, antibiotics should not be prescribed even if dental treatment cannot start immediately (Robertson *et al* 2015).

The first treatment approach recommended for the control of periodontal disease is the Non-surgical periodontal therapy (NSPT) which is the basis of periodontal therapy (Drisko, 2001). It is referred to as the plaque removal or plaque control or supragingival and subgingival scaling root planning (SRP). NSPT has advanced over the years but still considered as the gold standard of treatment to which other treatment are compared (Ehizele and Akhionbare, 2013). Its objective is to remove both living and calcified bacteria in the biofilm matrix adherent on the tooth surface and soft tissues. A reduction in the bacteria loads will bring about reduced inflammation of the gum and it also creates an environment where the host can prevent pathogenic microbial colonization effectively using personal oral hygiene treatments. A recent advance which is receiving much attention is the application of lasers, photodynamic therapy (PDT), and hyperbaric oxygen therapy (HBOT) in non-surgical periodontal therapy (Tanwar *et al.*, 2016).

2.10 Antibiotics Used in Dentistry

In dental practice, dentists prescribe antibiotics for prevention, control and treatment of dental infections. Unfortunately this has led to the abuse and misuse of antibiotics in the field (Dar-Odeh *et al.*, 2010). Evidence shows that despite the numerous antibiotics available, only few are useful in treating dental infections. Most infections of dental origin still respond to penicillin group of antibiotics (Brescó-Salinas *et al.*, 2006) and continuous use of new antibiotic will heighten the cost of dental care and also risk of resistance to the antibiotics. Therefore, combination therapy with drugs that possess anaerobic cover such as

metronidazole will provide a synergistic effect for the treatment of dental infections (Bratton *et al.*, 2002; Ramasamy, 2014).

Before a prescription of antibiotic is made, it is important to carry out susceptibility testing to enable the dentist choose the appropriate antibiotics. In the case of minor infections, amoxicillin or amoxicillin/clavulanate is sufficient. A combination of beta-lactamase resistant penicillin group of drug and metronidazole is started in cases of serious odontogenic infections along with appropriate surgical therapy (Daramola *et al.*, 2009).

In clinical practice, antibiotic prescription for the treatment of the infection is empirical because microbiological analysis is not carried out but the clinician is not aware of the particular microorganism responsible for the infections. The epidemiological data on both clinical treatment and bacterial involved has been reported. Therefore, the treatments are based on presumptive decision of the clinicians because the microorganism responsible are only suspected (Oberoi *et al.*, 2015).

About 7 to 11% of all common antibiotics are prescribed by the dentists and the common antibiotics are beta lactams, macrolides, tetracycline, clindamycin, and metronidazole. In dental practice, the most commonly administered group of antibiotic prescribed by dentist is the penicillin group (Al-Mubarak *et al.*, 2004; Ogunbodede *et al.*, 2005; Al-Haroni and Skaug 2006) and amoxicillin being the most used antibiotic in the group, followed by metronidazole and amoxicillin/clavulanate (Palmer *et al.*, 2000; Palmer *et al.*, 2001; Sarka *et al.*, 2004; Poveda Roda *et al.*, 2007; Dar-Odeh *et al.*, 2008). These antibiotics have been advocated for in the treatment of dental infections (Swift and Gulden 2002; Ellison, 2009; Dar-Odeh *et al.*, 2010).

The inappropriate choice of antibiotics and its misuse can lead to the development of resistance to antibiotics and side effects. It is therefore, it is important to ensure that the right antibiotics are prescribed (Akande *et al.*, 2009). There are established guidelines for adequate prescription of antibiotics and they include Dentists' Drug and Prescription Guide, Drug Prescribing for Dentistry, Antibiotic Prescribing Guidelines for Dentists (Scottish Dental Clinical Effectiveness Programme 2011) and National Guideline Clearing house. With these guidelines, it is important to make sure that practitioners comply with them and ensure that the guidelines are accurate (Rachmawati *et al.*, 2014).

2.10.1 β -lactam / β -lactam inhibitor combination

One of the most commonly prescribed antibiotics are the β -lactam antibiotics and they include Penicillins, Cephalosporins, Monobactams and Carbapenems. These β -lactam antibiotics share the same structural feature known as the β -lactam ring and they inhibit bacteria growth by interfering with cell wall synthesis by covalently binding to the penicillin binding protein (PBP) in the cytoplasmic membrane of the bacteria which leads to cell death. The effect of β -lactam antibiotics on Gram negative bacteria is mild because of the outer membrane of their cell wall but it is lethal to Gram positive bacteria (Poole 2004). The mechanism of resistance to β -lactam antibiotics can occur through any of these mechanisms: 1) modification of PBP by mutations or acquiring foreign genes to encode new PBPs, 2) decrease in permeability by alterations of porins and lastly 3) production of β -lactamases inactivating enzymes. The combination of β -lactam and β -lactam inhibitor increases the chances of using β -lactam antibiotics. The β -lactam inhibitor is designed to inactivate β -lactamases and bind irreversibly to them allowing the β -lactam antibiotics to be effective. Clavulanic acid was the first inhibitor to be introduced to clinical medicine and in

1970. Later Sulbactam and Tazobactam were developed as synthetic compounds (Drawz and Bonomo 2010) and used equally.

The groups of penicillins that are used in dental infection treatments are, penicillin V (Swift and Gulden, 2002) amoxicillin (Ellison, 2009) and amoxicillin and clavulanate (Farrier *et al.*, 2007). A combination of amoxicillin and clavulanic acid maintains activity against organism that produce β -lactamase enzymes and are associated with dental infections.

2.10.2 Metronidazole

Metronidazole is a 5-nitroimidazole antimicrobial developed in France. It has a wide spectrum of activity against protozoan and anaerobic bacterial infections. It was initially introduced for treating trichomoniasis in the late 1950s (Maeda *et al.*, 1953) but in 1962 during the treatment of vaginitis associated with the protozoan *Trichomonas vaginalis*, it was realized that it was also effective in the treatment of gingivitis in the same patient (Shinn 1962; Dingsdag and Hunter, 2018).

Metronidazole is a broad spectrum antibiotic commonly used in the treatment of periodontal disease. It is bacteriocidal and targets anaerobic bacteria that are cause periodontal disease (Umeda *et al.*, 2004). It is regularly used alone or in combination with amoxicillin as an empirical treatment for periodontitis.

The actual mechanism of action has not been fully clarified but includes the inhibition of DNA synthesis and damage by oxidation which causes single stranded and double stranded DNA to break leading to DNA degradation and cell death (Land and Johnson, 1999; Löfmark *et al.*, 2010). Mechanisms of resistance to metronidazole are by reduced rate of uptake by efflux or by reducing the rate of metronidazole reductive activation such as

alteration of pyruvate fermentation. Additional resistance mechanisms include rapid repair of DNA and inactivating resistance determinants (Dingsdag and Hunter, 2018).

2.10.3 Lincosamide

The inhibitory effects of this group of antibiotic is on the protein synthesis that binds to the 23S portion of the 50S bacterial ribosomal subunit and the inhibition of this protein synthesis is due to the premature separation of peptidyl-tRNA from the ribosome (Das and Patra, 2017). The only members of this group are Clindamycin and lincomycin. Clindamycin was introduced in 1966 and it is used mostly because of its higher efficacy and superior pharmacokinetic activity (Kumar, 2017).

Clindamycin is an excellent broad-spectrum antibiotic with activity against aerobic, anaerobic bacteria and beta-lactamase producing pathogens (Brook *et al.*, 2005). It is used in the treatment of dental infection because of its susceptibility pattern, great oral absorption and low emergence of resistance (Shweta and Prakash 2013) and it has been used for many years as prophylactic treatment during dental procedures to prevent endocarditis (Brook *et al.*, 2005). Its use has increased due to increasing concern over penicillin resistances (Gilbert *et al.*, 2009) Clindamycin is considered as the antibiotic of choice for penicillin-allergic patient (Kirkwood 2003; Sridhar *et al.*, 2018) and because of its broad spectrum and good clinical efficacy, standard drug guides of antimicrobial therapy has replaced penicillin with clindamycin as the drug of choice in treating odontogenic infections (Sandor *et al.*, 1998). Resistance to clindamycin occurs through: (1) alteration of 23S ribosomal RNA of 50S subunit by adenine methylation (ribosomal protection), (2) Receptor alteration or (3) Drug inactivation (Roland, 2002)

2.10.4 Chloramphenicol

It is a bacteriostatic antibiotic which is highly active against most anaerobic bacteria. Its mechanism of action is by inhibiting protein synthesis in bacteria by reversibly binding to the peptidyl component of the 50s ribosomal subunit. Despite its good oral bioavailability and excellent tissue penetration, in developed countries its use has been abandoned due to reports linking it to serious adverse effects (Sood 2016). Some *Bacteroides* spp have been reported to be resistance to this drug but resistance is rare. This antibiotic has been used for a long time in the treatment of anaerobic infections and bacteremia. It was regarded as the drug of choice for treatment of serious anaerobic infections in the past when the nature and susceptibility of the infecting organisms are unknown (Brook *et al.*, 2013). The most common mode of resistance to chloramphenicol is through the action of chloramphenicol acetyltransferases, enzymes that inactivate the drug.

2.11 Antibiotics/Drug Resistance

A decline has been reached in the often occurrence of untreatable diseases because of the use of antimicrobials in combination with improved sanitation, good housing and nutrition and the proper awareness and administration of immunization programs but this result is currently in jeopardy because of the development, emergence and spread of resistant microorganisms to these antibiotics (WHO, 2002). The emergence and spread of this antimicrobial resistance has become a public health concern. Antimicrobial resistance is a relative term and in its clinical definition a strain is defined as resistant when it survives antimicrobial therapy.

Bacterial resistance has been present before antimicrobials were used. There are intrinsic resistance where the organism have the innate ability to resist the activity of any antimicrobial agent through its inherent structural and functional characteristics. Acquires resistance is as a result of genetic changes in the presence or absence of antimicrobials which could be as a result of mutation or horizontal gene transfer through transformation, transduction and conjugation. Conjugation is a mechanism of horizontal gene transfer, most often with plasmids or transposons through which resistance can be passed on to other species. Transformation and transduction are processes that occur in bacteria that are closely related and belong to same species or genus.

Antibiotic resistance is a worldwide problem and it is spreading rapidly as a result of overuse, self-medication and nontherapeutic use of antimicrobials. It is a serious medical problem because of the fast rise and spread of mutant strains that are not susceptible to medical treatment. (Aluyi *et al.*, 2013)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Incubator (Natural appliance: Aheinicke Company Portland, Oregon, U.S.A. Model-630 Serial), Electronic weighing balance (Top balance digital, U.S.A. Ohaus, PA313-model), Hot-Air-Oven (Baird and Tatlock London limited), Wire loop, Microtitre plate reader (Linbro Scientific, Inc.), Autoclave (Adelphi MFG Co Ltd, Portland autoclave), Microscope (Wild M11, Switzerland), Micropipette, Colony Counters (NAPCO Model 630 Portland, Oregon, U.S.A.), Refrigerator (NAPCO Model 630 Portland, Oregon, U.S.A.), Polymerase Chain Reaction (PCR) Machine, Antibiotic Minimum Inhibitory Concentration Test Strips (Liofilchem®, Italy).

3.1.2 Culture Media

Brain Heart Infusion (BHI) Media (Oxoid Ltd., Basingstoke, Hampshire, England), Nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, England), Brucella agar (Hardy Diagnostics), Columbia Blood agar (Oxoid Ltd., Basingstoke, Hampshire, England), Peptone water (Oxoid Ltd., Basingstoke, Hampshire, England).

3.1.3 Chemicals

Crystal violet (May and Baker Ltd. Dagenham England), Lugol's iodine (May and Baker Ltd. Dagenham England), Acetone, Hydrogen peroxide (SKG Pharma Ltd. Ikeja Lagos, Nigeria), Sterile deionized water, Ethanol, oil immersion (BDH) and Liofilchem® anaerobic identification kit.

3.1.4 Glass wares

Bijou bottles, Microscope glass slides, Petri dishes, Test tubes, Measuring Cylinder, Beakers, Conical flask (Pyrex, England).

3.1.5 Antibiotics used

Amoxicillin-Clavulanate (AMC, 0.016-256 mg/l), Clindamycin (CD, 0.016-256 mg/l), Chloramphenicol (C, 0.016-256 mg/l), Metronidazole (MTZ, 0.016-256 mg/l). (Liofilchem® s.r.l., Roseto degli Abruzzi, Italy).

3.1.6 Genomic characterization materials

DNA Extraction Kit (ZR Fungal/Bacterial DNA MiniPrep™, Made in USA), Primers (Inqaba Biotec West Africa), PCR Thermocycler (Integrated DNA Technology, Germany).

3.1.7 Software

Liofilchem identification software (Liofilchem® s.r.l., Roseto degli Abruzzi, Italy), Statistical Package for Social Sciences (IBM SPSS) Version 21 and Microsoft Excel, 2010.

3.2 Methods

3.2.1 Study Area

This study was carried out in Dental unit of Ahmadu Bello University Medical Centre Zaria Nigeria.

3.2.2 Study Population

Patients of all age groups present at the hospital and diagnosed as having periodontal conditions by the physician.

3.2.3 Retrospective study

Patients record over three year period (January 1st, 2015 to December 31st, 2017) who visited the dental unit, having dental conditions as diagnosed by the clinician were retrieved from the Medical Records Department of A.B.U Medical Centre and reviewed to determine the type of conditions, treatment protocol and the prevalence of oral condition.

3.2.4 Prospective study

Oral rinse samples were collected from patients who visited the clinic and having a dental condition during a three months period (1st August to 30th October 2018). Patient's data such as age, gender, diagnosis and type of dental disease in the hospital were collected.

Inclusion Criteria: Patients with periodontal disease visiting the dental unit and that gave consent for collection of oral rinse samples

Exclusion Criteria: Patients that did not consent nor were in the dental unit.

3.2.5 Sampling method and collection

This study employed convenience sampling of patients with periodontal condition presenting at the dental clinic.

The sample size for the study was determined using the formula by Daniel, 1999;

$$n = \frac{Z^2 \times p \times (1-p)}{d^2}$$
$$n = \frac{(1.96)^2 \times 0.658 \times (1 - 0.658)}{(0.05)^2}$$
$$n = \frac{3.8 \times 0.658 \times 0.348}{0.0025} = 348$$

Where n = sample size (348)

Z= statistics for a level of confidence 95%

p = Prevalence of periodontal condition 65.8% (based on retrospective study results)

D= margin of error (0.05)

Convenience sampling method was used and a total of 131 samples were collected in this study.

3.2.6 Research Limitations

Only samples from patients diagnosed to have periodontal disease in the dental clinic and are available within the three (3) months study period were included. The study only investigated only the prevailing bacterial isolates obtained from the samples.

3.2.7 Ethical consideration

Ethical clearance for this work was obtained from the ethical committee of Ahmadu Bello University, Zaria.

3.2.8 Procedure for Culture Media Preparation

The media used in this study was prepared according to the manufacturer's specifications.

3.2.9 Sample collection

A total of 131 clinical samples (oral rinses) were collected using sterile sample bottles from patients with periodontal disease. The samples were individually sealed and placed in a cooler and transported in a cooler within 5 mins to the Microbiology Laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria where the microbiological analysis was carried out.

3.2.10 Isolation of test organisms

Serial dilution was carried out to 10^{10} dilution factor for the clinical samples using sterile water. One milliliter (1ml) from the 10^7 , 10^8 , 10^9 and 10^{10} dilutions was inoculated unto freshly prepared molten nutrient agar and incubated at 37°C for 24 hours aerobically. Another 1ml from the dilution were inoculated onto molten blood agar supplemented with vitamin K at 37°C for 24-48hours and incubated anaerobically. Colonies from the blood agar were picked and streaked on sterile freshly prepare blood agar supplemented with vitamin K and incubated anaerobically at 37°C for 24-48 hours to obtain purified single colonies. Single discrete colonies were sub-cultured into BHI broth and incubated anaerobically at 37°C for 24 hours. From this, $1\mu\text{l}$ of the sub-culture was inoculated into

sterile 50% glycerol broth and stored for further purposes. Finally, presumptively identified anaerobic gram negative bacteria were stored in a refrigerator at 4°C pending further analysis.

3.2.11 Identification of the Isolates

Gram Staining

This was carried out as described by Chessbrough (2006) to determine the morphology of the organism and as well as classify them into Gram negative and Gram positive bacteria. Smear was prepared from growth colonies, stained and microscopically examined as follows;

A drop of distilled water was placed at the centre of a clean glass slide. A sterile wire-loop was used to pick the colony of the bacteria and made a smear. The smear was allowed to air-dry and heat fixed. Crystal violet (primary stain) was added and allowed to act for 30-60 seconds and rapidly washed off with clean water. Lugol's iodine was added for 30 seconds and the stain washed off with clean water. The slide was decolorized rapidly using acetone and washed with clean water immediately. Then stained with safranin (secondary stain) for 1 minute and rinsed with clean water. It was allowed to air-dry and then observed under the microscope using $\times 100$ oil immersion. The bacterial isolates were further identified using standard commercially available identification kit (Liofilchem anaerobic ID kit).

Procedure:

Anaerobic bacteria isolates was cultured and incubated for 18-24 hours under anaerobic condition using the blood agar plate. The culture was harvested using a swap stick and inoculated into a vial of anaerobic broth (in the kit). The final turbidity was equal to 1.0

Macfarland standard. 0.2ml of the bacterial suspension was dispensed into each well of the system with a drop of Vaseline oil in well 20-URE. The system was covered and incubated at 37°C for 24-48 hours under anaerobic condition.

After incubation, a drop of kovac's reagent was added to well 22-IND for indole test, 2 drops of hydrogen peroxide was added to well 23-CAT for catalase test and a drop of sulphanilic acid and naphtylamine was added to well 24-NIT for nitrate test. Color change was observed and the result was interpreted using the interpretive table. The test results form a 9-digit code for each bacterial isolate which is used to identify the bacteria using the Liofilchem Identification Software.

3.2.12 Determination of antibiotic susceptibility of isolates

The gradient diffusion method using E-Strip was used to determine the antibiotic susceptibility of the isolates (MIC) as recommended by EUCAST 2018. Discrete colonies of isolates sub-cultured on blood agar plates and emulsified in 5 ml of sterile physiological saline and the turbidity was adjusted to 0.5 McFarland standard. The standardized suspension was inoculated on Brucella agar supplemented with sheep blood, Vit K1 and Hemin using a sterile swab to ensure even distribution and confluent growth. The plates were allowed to dry and then the E-Strips of the various antibiotics Amoxicillin-Clavulanic acid, Clindamycin, Chloramphenicol and Metronidazole were aseptically placed using a sterile forceps on the dried inoculated agar surface. After 30 minutes of applying the strips, the plates were then incubated anaerobically in an inverted position at 37°C for 24-48hours. The breakpoints according to the European Committee on Antimicrobial Susceptibility Testing were used to compare the results (EUCAST 2018).

3.2.13 Determination of biofilm-forming capability of the bacteria.

In order to test for biofilm production by the different isolates obtained from the oral rinse samples, the Microtitre plate method (for biofilm detection and quantification) as described by Merrit *et al.*, (2005) was used.

Biofilm Quantification Assay

Biofilm Detection: Biofilm detection was carried out as described by Merrit *et al.*, (2005).

The isolates were grown overnight for 24 hours at 37°C in brain heart infusion broth (BHI) supplemented with 2% glucose and 2% sucrose. The cultures were diluted in 1µl in 10 ml medium and 150 µl of the cell suspension was used to inoculate sterile flat-bottomed 96-well polystyrene microtitre plate and incubated for 48 hours at 37°C. After 48 hours, the suspension was poured off and the wells washed three (3) times in three (3) different trays of normal saline to remove any unfixated microbial cell and leave only those fixed in the well within a biofilm matrix and dried in an inverted position. The dried wells were stained with 250µl of 0.1% crystal violet solution in water and incubated at room temperature for 20 minutes. The excess stain were poured off and wells washed three (3) times in three (3) different trays of normal saline and dried for 30 minutes at room temperature. A positive result was seen as the presence of a layer of stained materials adhered to the inner wall of the wells.

Quantitative Assay of Biofilm: This was carried out as described by Merrit *et al.*, (2005).

The quantitative assay of the biofilm production was performed by adding 250µl of ethanol-acetic acid (95:5 vol/vol) to distain the wells obtained from the preceding test. Then 100µl from each well was transferred to a new microtitre plate and the optical density (OD) of the solution were measured at a wavelength of 630nm using a microtitre plate reader

(ELISA machine). The un-inoculated medium was used, as control, to determine the negative control (OD). The cut-off value (OD_c) [average OD value of negative control + 3× standard deviation of negative control). The experiment was repeated three times separately for each strain and the average values were calculated with standard deviation.

The biofilm ability of the tested strains was classified into four categories based on the OD: non-adherent (OD < OD_c), weakly adherent (OD_c < OD < 2XOD_c), moderately adherent (2XOD_c < OD < 4XOD_c), and strongly adherent (4XOD_c < OD) (Stepanovic *et al.*, 2007).

3.2.14 Bacterial cell preparation

Bacteria cells preparation of biofilm-forming and resistant isolates were carried out using the method described by Lephoto and Gray, (2013). Overnight culture of the selected isolates on Blood agar were inoculated into 5ml Luria-Bertani (LB) broth and incubated anaerobically at 37°C for 24 hours. Bacterial cells were harvested by centrifugation at 4°C, 8000 rpm (6800 ×g) in a microcentrifuge for 2 minutes at room temperature in an Eppendorff's tube, the supernatant were discarded and cells harvested. The centrifugation step was repeated three (3) times to obtain higher yield of cells (Lephoto and Gray, 2013).

Genomic DNA Extraction

Genomic DNA extraction was carried out using method described by Zymo Research Protocol (Lephoto and Gray, 2013). Harvested cell pellets were dislodged by adding 200 µl of deionized water and mixed thoroughly by vortexing. Exactly 400 µl of the lysis solution was added to the mixture and mixed, the mixture was further incubated in a water bath with mild shaking at 70°C for 15 minutes until the cells were completely lysed. Exactly 400 µl supernatant was transferred to a Zymo-spin™ IV spin filter in collection tube and centrifuged at 7000rpm for 1 minute. About 1200 µl of DNA binding buffers was added to

the filtrate in the collection tube from the preceding step. Exactly 800 μ l of the mixture from step above was transferred to a zymo spin IIC Column in a new collection tube and centrifuged at 10000 \times g for 1 minute. The flow through step above in the collection tube was discarded and step above repeated. A measure of 200 μ l DNA pre wash buffer was added to zymo spin column in a new collection tube and centrifuge at 10000 \times g for 1 minute. About 500 μ l of DNA wash buffer was added to zymo spin column and centrifuge 10000 \times g for 1 minute. The zymo spin was transferred to a clean 1.5ml microcentrifuge tube and 100 μ l DNA elution buffer added directly to the column matrix and centrifuge at 10000 \times g for 1 minute to elude the DNA (Lephoto and Gray, 2013).

Polymerase Chain Reaction (PCR)

Amplification of antibiotic resistance and biofilm-forming genes was carried out using PCR after an external optimization of the reaction to ensure a better amplification. The specific primer to recognize each gene was used. The PCR master mix contain 1.0 μ l each of forward and reverse primers, 1X PCR buffer, 1.5 mM MgCl₂, 0.15 mmol/L dNTP, 1.25 IU *Taq* DNA polymerase and 1 μ L of prepared DNA (0.5 μ g) template was added to the final volume. PCR amplifications were carried out using a Bio-Rad DNA Engine thermal cycler with running conditions (denaturation, annealing and extension) as shown in Table 3.1 (Pournajaf *et al.*, 2014).

Agarose Gel Electrophoresis of PCR Products

Exactly 2% agarose gel was used to resolve the PCR genomic DNA fragments with their primers and documented as described above.

Primers used in the Study

The adhesins and resistance genes primers were obtained from Inqaba Biotec Laboratory as represented in Table 3.1

Table 3. 1: Primers, Gene and Cycling Procedure

Gene	Primers Description	Sequence	Amplicon size (bp)	PCR Condition Cycling	Reference
Cat	Forward	GGTGATATGGGATAGTG	349	95°C 3 mins; 34 ×(95°C 1 min; 55°C 1 min; 72°C 1 min) 72°C 5 mins.	Samuel <i>et al.</i> , 2014
	Reverse	CCATCACATACTGCATGATG			
Nim	Forward	ATG TTC AGA GAA ATG CGG CGT AAG CG	458	94°C 10mins; 32× (94°C 30sec; 62°C 1min; 72°C 1min) 72°C 10mins	Trinh and Reysset, 1997
	Reverse	GCT TCC TTG CCT GTC ATG TGC TC			
bla _{TEM}	Forward	TAACCCTGGTAAATGCTTCA	940	940bp 5 min at 95°C, 30×(95°C 1 min; 55°C 1 min; 72°C 1 min) 72°C for 20 min	Di Conza <i>et al.</i> , 2014
	Reverse	CAATCTAAAGTATATATGAG			

Key:

- *cat* (Chloramphenicol resistance gene)
- *nim* (Metronidazole resistance gene)
- *bla_{TEM}* (Beta-lactam resistant gene)

3.2.15 Statistical analysis

Data were presented as diagrams, Pie chart and bar chart d for descriptive analysis and then using inferential statistics. Chi square was used to determine the association between categorical variables. A value of $p < 0.05$ with 95% confidence interval was considered to be statistically significant using the statistical package for social sciences (SPSS) program, Version 21.

CHAPTER FOUR

4.0 RESULTS

4.1 Retrospective Study

Table 4.1 shows the records of visits to the dental clinic in Ahmadu Bello University Medical Centre Zaria between 2015 to 2017. A total of 10,364 visits were recorded, the highest number recorded was in January 2015 and the lowest in December 2017 as shown in Figure 4.1. A significant difference ($p=0.000$) was recorded in the number of diagnosis that occurred in each month during the study period.

Patients diagnosed with periodontal diseases had a high prevalence of 65.8% while a lower prevalence of 34.2% were diagnosed with dental caries with a significant difference ($p=0.000$) between diagnosis. A significant differences in prevalence of periodontal disease between 2015-2017 was recorded. However, there was no statistical significant year on year difference observed regarding dental caries.

As shown in Figure 4.2, a total of 10,364 visitors to the clinic was recorded with about 60.5% male and 39.5% females. The difference in the number between males and females visitors was significant ($p=0.004$). The age distribution of patients showed that the age bracket 26-36 and 48-58 years was higher than the rest with a mean age of 35.47 ± 15.097 as indicated in Table 4.2. The treatment received is as shown in Fig 4.3. All patients diagnosed with periodontal condition (100%) were treated using scaling and polishing/ scaling and root planning methods. About 81.6% patients with dental caries resulted to tooth extraction and 18.4% tooth fillings. Records of antibiotics administered to patients was limited during the study period.

Table 4. 1: Occurrence of Oral Health Conditions in Study Site

Total Visits (2015- 2017)			
Year		Diagnosis	
		Dental Caries freq (%)	Periodontal disease freq (%)
2015	3367	1173(34.8)	2194(65.2)
2016	3611	1194(33.1)	2417(66.9)
2017	3386	1176(34.7)	2210(65.3)
Total	10,364	3543(34.2)	6,821(65.8)

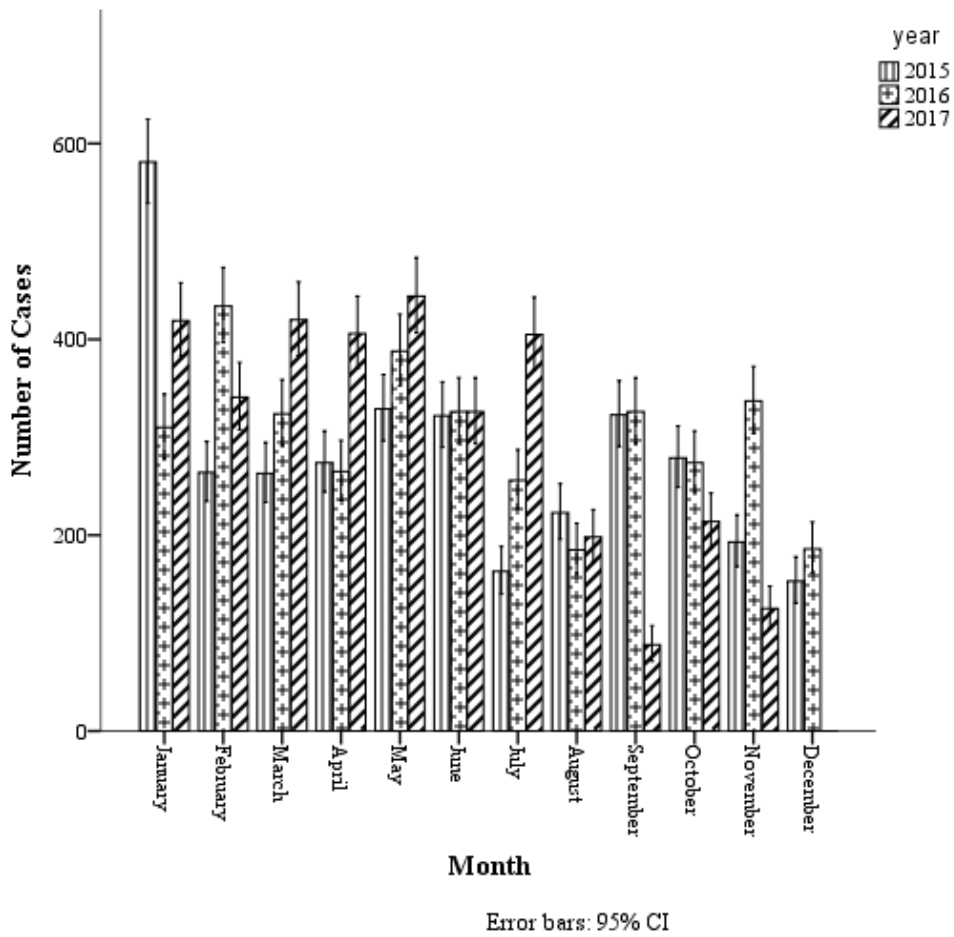


Figure 4. 1: Monthly Distribution of Patients Visits

Table 4. 2: Age and Gender distribution of Study Subjects (2015-2017)

Gender	≤14 years (%)	15-25 years (%)	26-36 years (%)	37-47 years (%)	48-58 years (%)	59 above years (%)	Total (%)
Male	807 (67.1)	956 (57.7)	1443 (59.8)	1169 (54)	1593 (66)	302 (59.5)	6270 (60.5)
Female	395 (32.9)	702 (42.3)	972 (40.2)	997 (46)	822 (34)	206 (40.5)	4094 (39.5)
Total	1202	1658	2415	2166	2415	508	10364

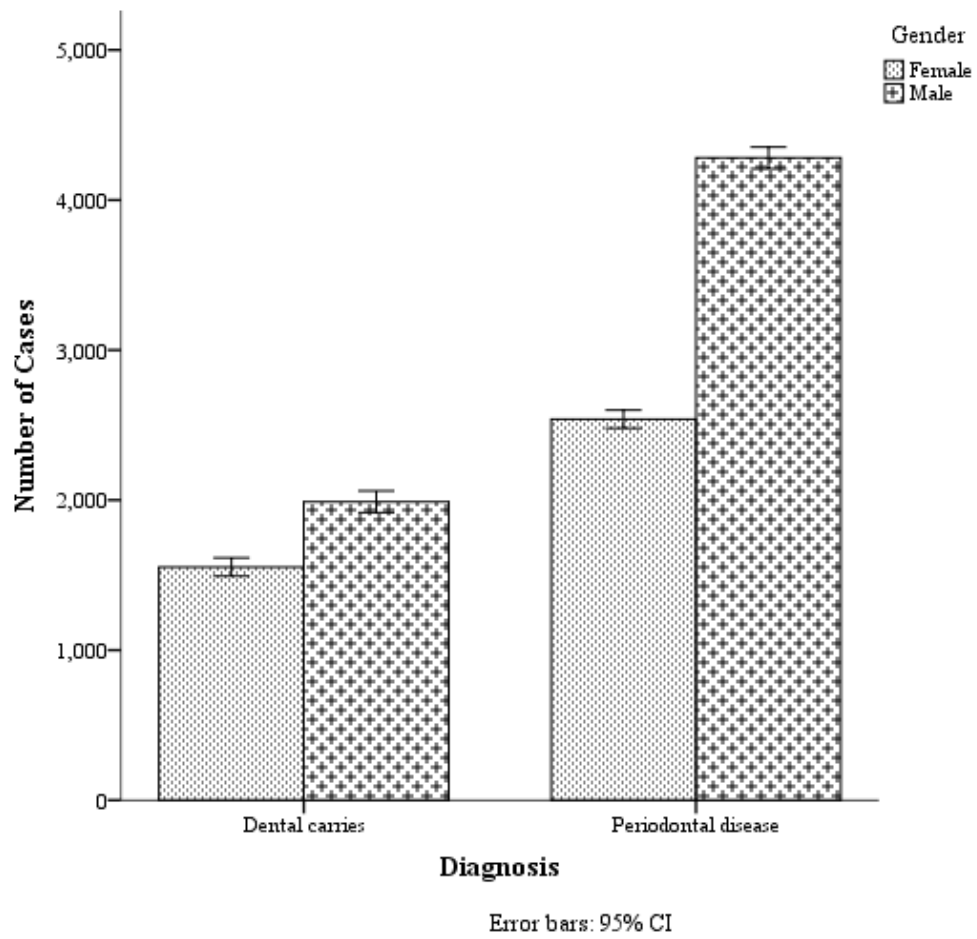


Figure 4. 2: Gender Distribution of Diagnosed Patients

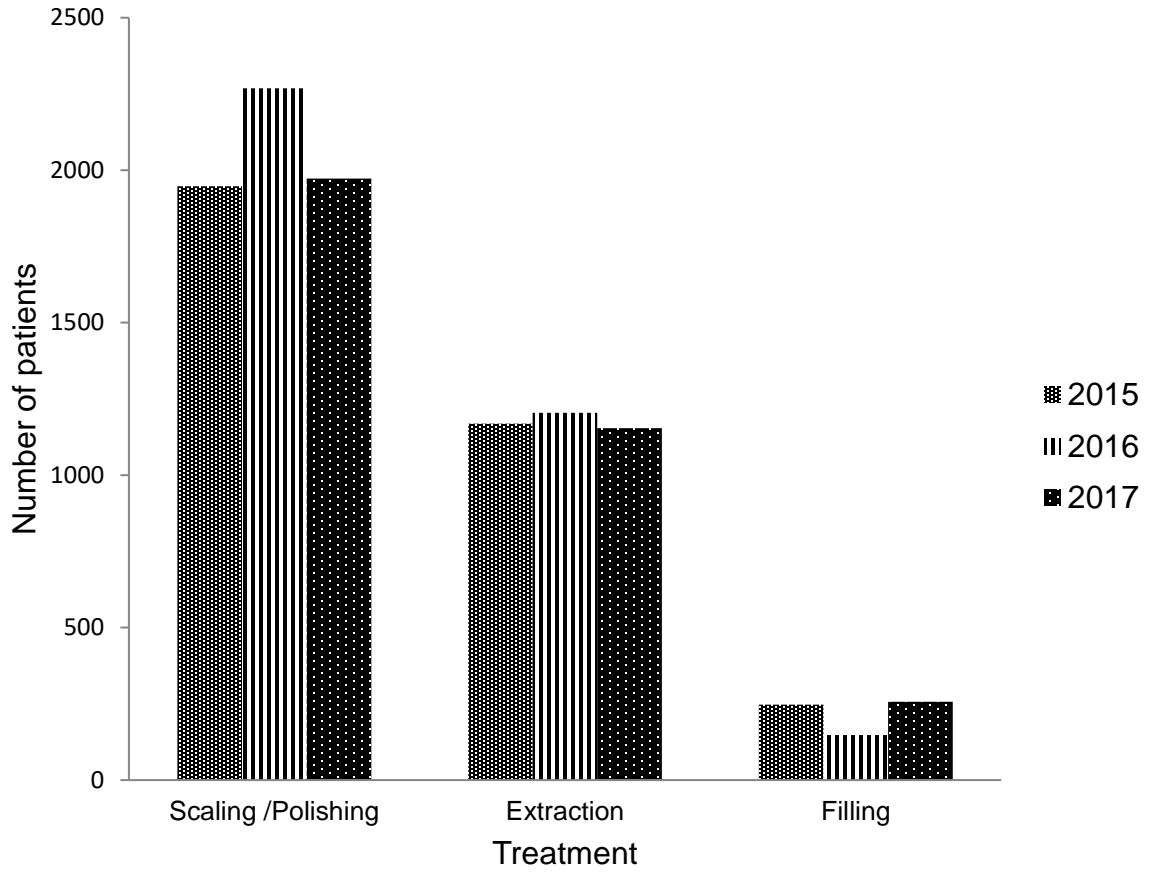


Figure 4. 3: Treatment Conducted During Visit

4.2 Prospective Study

4.2.1: Analysis of Samples Collected

Mouth rinse samples were collected from 131 patients, 74 of which were male and 57 female. The largest groups of patients were those above 48 years and the lowest were those below 14 years with a mean age of 30.8 ± 10.7 as shown in Table 4.3.

Table 4.4 shows the colony count of visible growth of viable bacteria for both aerobic and anaerobic which was recorded as CFU/ML. The mean value for aerobic count was $10.5 \pm 4.72 \times 10^8$ CFU/ML⁻¹ and that of anaerobic was $8.41 \pm 3.92 \times 10^8$ CFU/ML⁻¹.

Preliminary identification by gram staining showed that 36.2% were Gram positive, 32.1% were Gram negative anaerobic cocci and only 31.7% of the isolates were Gram negative anaerobic rods. Final identification confirmed the probable isolates to be *Prevotella intermedia* (22.5%), *Bacteriodes fragilis* (22.5%), *Fusobacterium nucleatum* (12.5%), *Fusobacterium varidium* (10%), *Prevotella oralis* (10%), *Fusobacterium mortiferum* (7.5%), *Bacteriodes stercoris* (7.5%) and *Porphyromonas asaccharolytica* (7.5%) as shown in Table 4.5.

Table 4. 3: Age and Gender Distribution of Patients

Age Groups	Number of Patients (%)	Number of Male (%)	Number of Female (%)
≤14	11 (8.4)	7 (63.6)	4 (36.4)
15-25	31 (23.7)	15 (48.4)	16 (51.6)
26-36	49 (37.4)	26 (53.1)	23 (46.9)
37-47	27 (20.6)	22 (81.5)	5 (18.5)
48-58	13 (9.9)	4 (30.8)	9 (69.2)
Total	131	74 (56.5)	57 (43.5)

Table 4. 4: Mean Total Bacteria Count from Mouth Rinse Samples

Mean \pm SD $\times 10^8$ cfuml⁻¹

Gender	No. of cases	Aerobic	Anaerobic
Male	74	10.5 \pm 4.53	8.23 \pm 3.99
Female	57	10.5 \pm 4.53	8.63 \pm 3.85
Total	131	10.5 \pm 4.72	8.41 \pm 3.92

Table 4.5: Anaerobic Gram Negative Rods Isolated from Mouth-Rinse Samples

Identified bacteria	Number of the isolate
<i>Prevotella intermedia</i>	9
<i>Bacteriodes fragilis</i>	9
<i>Fusobacterium nucleatum</i>	5
<i>Fusobacterium varidium</i>	4
<i>Prevotella oralis</i>	4
<i>Fusobacterium mortiferum</i>	3
<i>Bacteriodes stercoris</i>	3
<i>Porphyromonas asaccharolytica</i>	3
Total	40

4.2.2 Antibiotic Susceptibility of Isolates

The Gram negative anaerobic bacteria from the oral rinse samples isolated were resistant to Metronidazole (100%), Chloramphenicol (100%), Amoxicillin/clavulanic acid (62.5%), and Clindamycin (25%) as shown in Figure 4.4. The values greater than the MIC breakpoint were considered resistance to the antibiotics as shown Table 4.6. The isolates are classified based on the pattern of their antibiotics resistance.

4.2.3 Quantitative Analysis of Biofilm Forming of Clinical Isolates

Biofilm quantification showed that 6 (15%) of the bacterial isolates were moderate biofilm producers, 25 (62.5%) were weak biofilm producers and 9 (22.5%) were non-biofilm producers. There was no significant difference ($p>0.05$) between the adherent biofilm formers and non-adherent biofilm formers. The classification and distribution of biofilm produced by sample type is shown in Figure 4.5.

The antibiotic resistance pattern in biofilm producing and non-biofilm producing bacterial isolates were compared as shown in the Table 4.7. Thirty one (31, 77.5%) resistant to metronidazole and chloramphenicol, 15(37.5%) to clindamycin and 23(57.5%) to Amoxicillin/clavulanic acid were biofilm producers, while 9(22.5%) isolates resistant to metronidazole and chloramphenicol, 6(15%) to Amoxicillin/clavulanic acid were non-biofilm producers.

Table 4.6: Antibiotic Susceptibility of Anaerobic Bacteria Isolated from Patients Attending Dental Clinic

Isolates/ Antibiotics	MIC range	Susceptibility (n)	Resistant (n)	Resistance (%)
<i>Prevotella oralis</i> (4)				
Clindamycin (S≤4, R>4)	0.5-0.75	4	0	0
Metronidazole (S≤4, R>4)	>256	0	4	100
Amoxicillin/clavulanic acid (S≤4, R>8)	0.023->256	2	2	50
Chloramphenicol (S≤8, R>8)	24->256	0	4	100
<i>Prevotella intermedia</i> (9)				
Clindamycin (S≤4, R>4)	0.023-32	6	3	33
Metronidazole (S≤4, R>4)	>256	0	9	100
Amoxicillin/clavulanic acid (S≤4, R>8)	0.023->256	2	7	78
Chloramphenicol (S≤8, R>8)	38->256	0	9	100
<i>Bacteriodes fragilis</i> (9)				

Table 4.6 Contd.

Clindamycin (S≤4, R>4)	<0.016- >256	6	3	33
Metronidazole (S≤4, R>4)	>256	0	9	100
Amoxicillin/clavulanic acid (S≤4, R>8)	0.032->256	5	4	44
Chloramphenicol (S≤8, R>8)	24->256	0	9	100
<i>Bacteriodes stercoris</i> (3)				
Clindamycin (S≤4, R>4)	0.5-12	0	3	100
Metronidazole (S≤4, R>4)	>256	0	3	100
Amoxicillin/clavulanic acid (S≤4, R>8)	0.032->256	1	2	67
Chloramphenicol (S≤8, R>8)	48->256	0	3	100
<i>Fusobacterium nucleatum</i> (5)				
Clindamycin (S≤4, R>4)	0.38->256	0	5	100
Metronidazole (S≤4, R>4)	>256	0	5	100

Table 4.6 Contd

Amoxicillin/clavulanic acid (S≤4, R>8)	0.5-32	2	3	60
Chloramphenicol (S≤8, R>8)	47->256	0	5	100
<i>Fusobacterium varidium</i> (4)				
Clindamycin (S≤4, R>4)	<16	0	4	100
Metronidazole (S≤4, R>4)	>256	0	4	100
Amoxicillin/clavulanic acid (S≤4, R>8)	64->256	0	4	100
Chloramphenicol (S≤8, R>8)	16->256	0	4	100
<i>Fusobacterium mortiferum</i> (3)				
Clindamycin (S≤4, R>4)	48-64	0	3	100
Metronidazole (S≤4, R>4)	>256	0	3	100
Amoxicillin/clavulanic acid (S≤4, R>8)	>256	0	3	100
Chloramphenicol (S≤8, R>8)	>256	0	3	100

Porphyromonas

asaccharolytica (3)

Clindamycin (S \leq 4, R $>$ 4)	>0.38	3	0	0
Metronidazole (S \leq 4, R $>$ 4)	>256	0	3	100
Amoxicillin/clavulanic acid (S \leq 4, R $>$ 8)	>0.023	3	0	0
Chloramphenicol (S \leq 8, R $>$ 8)	>256	0	3	100

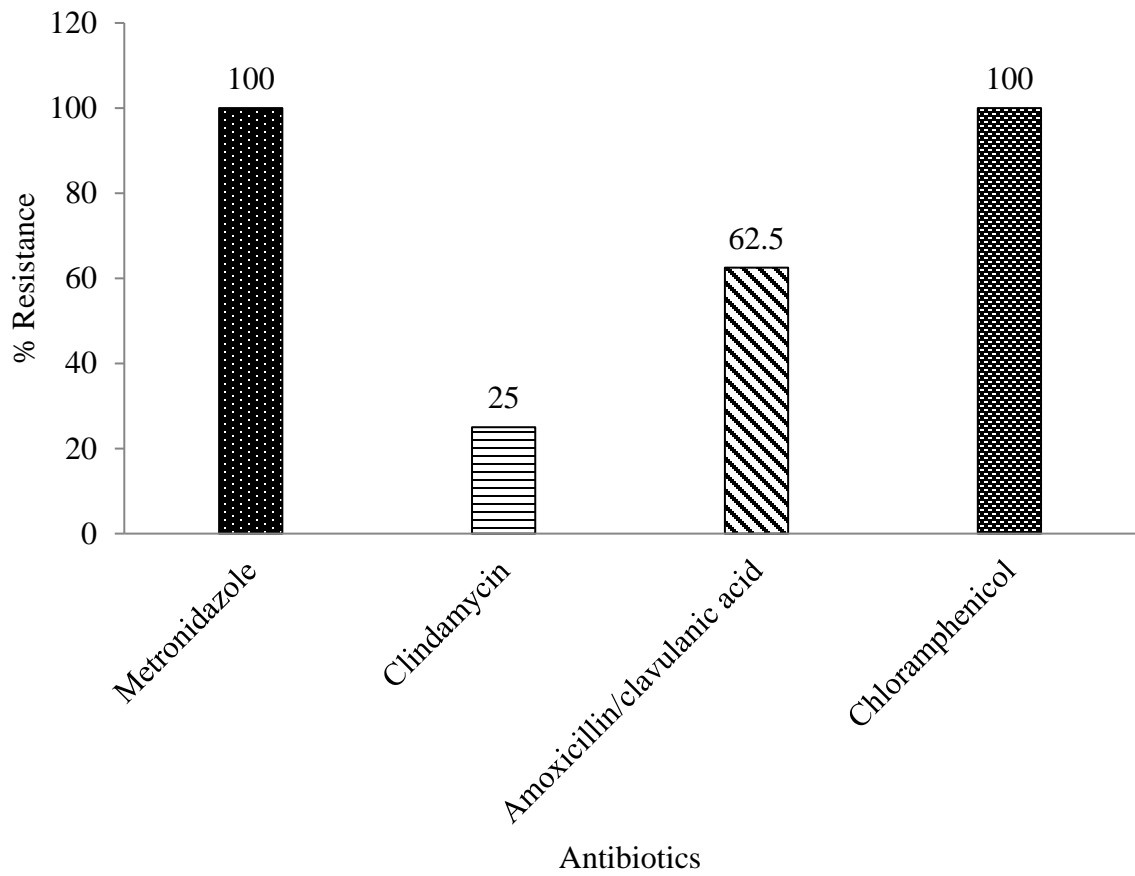


Figure 4. 4: Percentage Resistance of Isolates to Tested Antibiotics

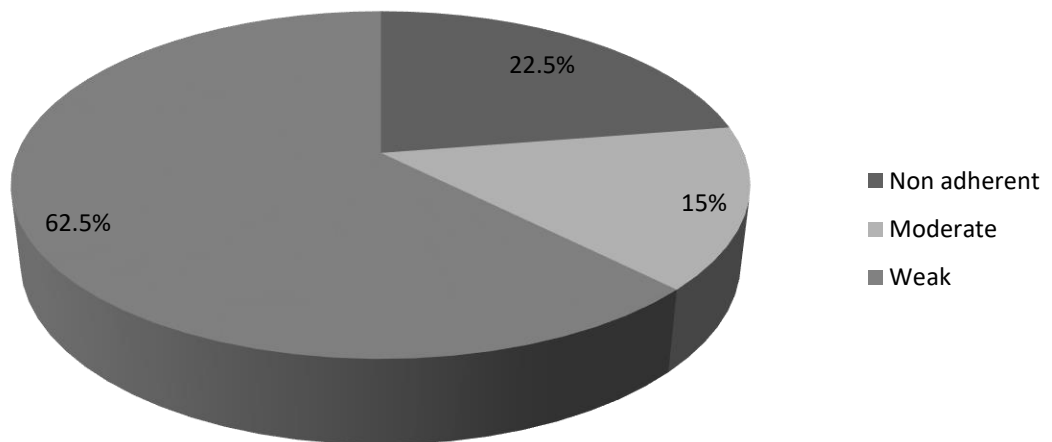


Figure 4. 5: Biofilm Production by the Isolates

Table 4.7: Biofilm Forming Isolates Resistant to Antibiotics

		Biofilm Producers	Non-Biofilm
Antibiotics	Susceptibility	(n=31)	(n=9)
Amoxicillin-Clavulanic Acid	R	23(57.5%)	6(15%)
Metronidazole	R	31 (77.5%)	9 (22.5%)
Chloramphenicol	R	31(77.5%)	9(22.5%)
Clindamycin	R	15(37.5%)	0(0%)

4.2.4 Detection of Resistance Gene Markers in Isolates

Three (3) genes including: *bla_{TEM}*, *cat* and *nim* were detected by polymerase chain reaction as shown in plates 4.1 and 4.2. All (9, 100%) bacteria isolates tested harboured the *cat* gene, 1 (11%) harboured *bla_{TEM}* gene and 0% *nim* gene.

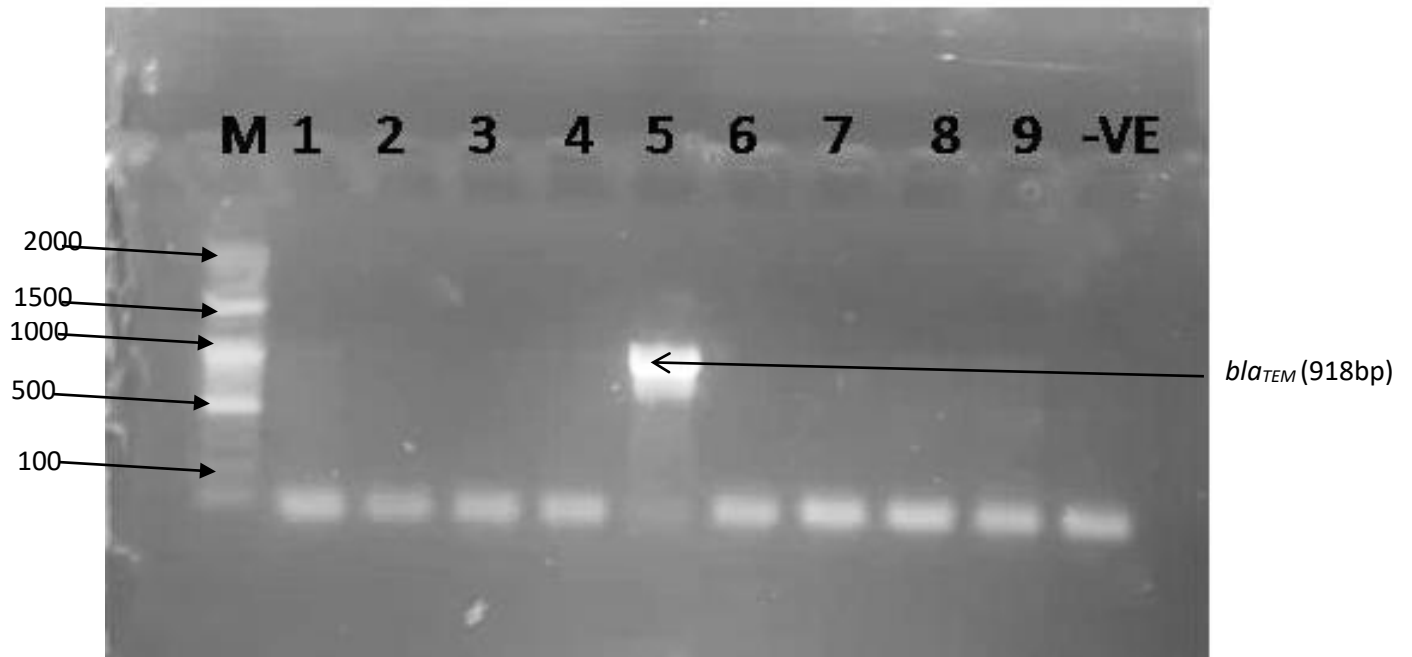


Plate 4. 1: Electrograph of Amplicon of the *bla*_{TEM} gene with a size of 918 bp.

KEY

M = 100bp molecular DNA Ladder

-ve= Negative control

1- 8D1, 2 -12C2, 3 -13B1, 4- 13B2, 5 -14A3, 6 -15A1, 7 -17B2, 8 -19A1, 9- 89B1=
Bacteria Isolates codes

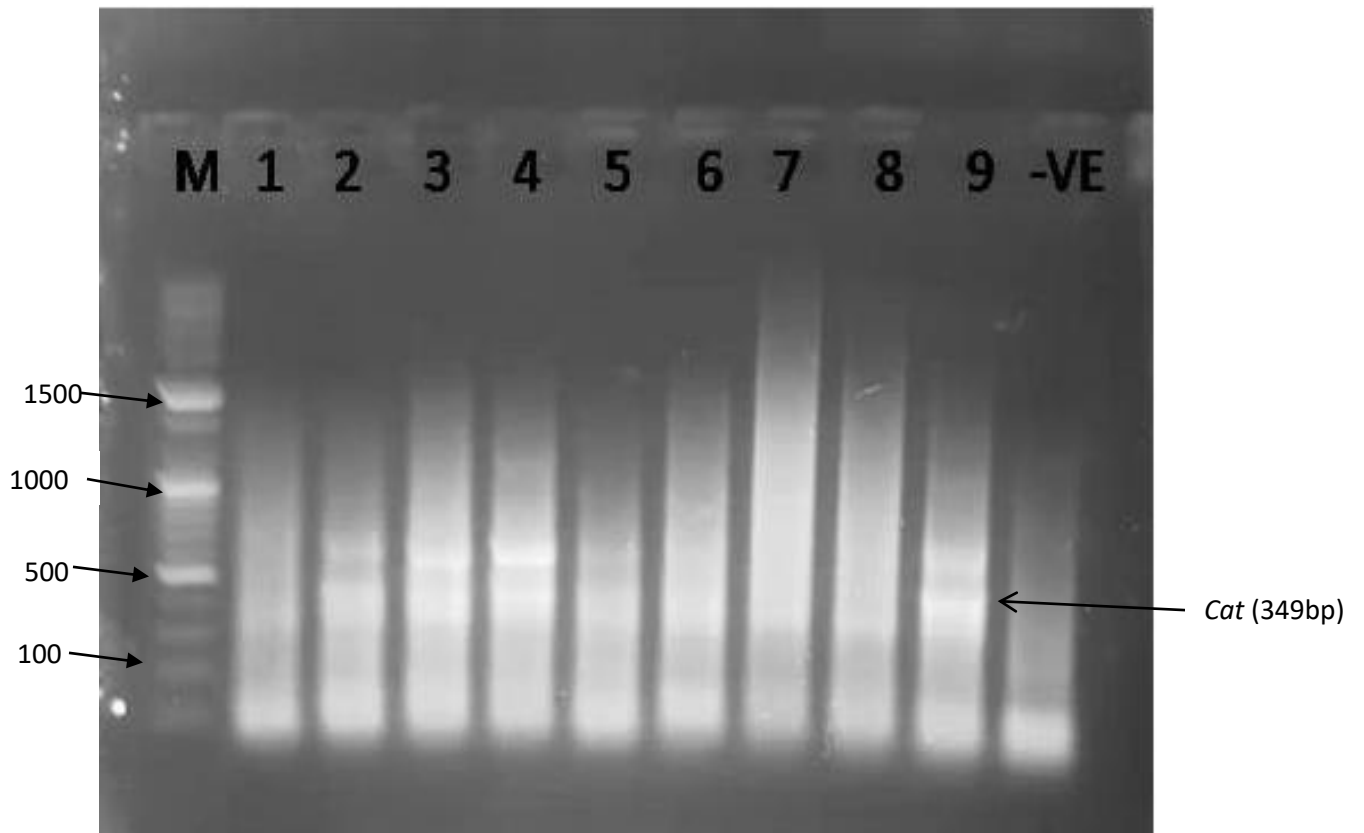


Plate 4. 2: Electrograph of Amplicons of the *Cat* gene with a size of 349 bp.

KEY

M = 100bp molecular DNA Ladder

-ve= Negative control

1- 8D1, 2 -12C2, 3 -13B1, 4- 13B2, 5 -14A3, 6 -15A1, 7 -17B2, 8 -19A1, 9- 89B1=
Bacteria Isolates codes

CHAPTER FIVE

5.0 DISCUSSION

Odontogenic infections are some of the most common diseases worldwide and the principal reason for seeking dental care (Tancawan *et al.*, 2015) with a global prevalence rate of nearly 35% (Marcenes *et al.*, 2013; WHO, 2016).

The reason for visit to the dental unit as shown in this study was periodontal disease and dental caries and this was observed in all age groups. This has also been reported to be the major oral health problem generally (Sanz *et al.* 2010; Omitola and Arigbede, 2012; Nazir, 2017).

In the present study, periodontal disease (gum disease), a condition associated with chronic inflammation of the gum, was the most frequent reason for visits with twice as many cases as dental caries. Adults are more susceptible to this condition (Umoh and Azodo, 2012) because periodontium cells in adults produce higher inflammatory mediators which affects the severity of the disease (Rajendran *et al.*, 2013). The high prevalence (65.8%) of periodontal disease in this study may be due to the fact that most of the patients were adults and are likely to seek medical consultation due to awareness of the dental services provided by the centre. The findings in this study is similar to report by Tobin and Ajayi, 2017 who reported (66%), higher than 9.1% reported by Lorenzo *et al.*, 2015, 10.1% by Carasol *et al.*, 2016 and 33.55% by Joshi *et al.*, 2016 in Uruguay, Spain, and Gujrat, India respectively but lower than the 90.8% reported in Benin by Umoh and Azodo, 2012. The CDC in collaboration with American Academy of Periodontology in 2016, reported varying level of periodontal disease among various ethnic groups; 52.79% in Mexico, 51.10% in Hawaii,

49.47% in Florida and 37.69% in Utah (Eke *et al.*, 2016). The Global prevalence of periodontal disease has also been stated to be about 11.2% (Kassebaum *et al.*, 2014a; Jin *et al.*, 2016; Tonetti *et al.*, 2017).

In this study, dental caries accounted for fewer visits when compared to periodontal condition. The prevalence 34.2% of dental carries in this study is similar with 30% obtained in Sothern Vietnam and 33 to 35.5% in Benin, Lagos, and Enugu (Nguyen *et al* 2010; Okoye and Ekwueme, 2011; Omitola and Arigbede, 2012; Oremosu and Uti, 2014; Olabisi *et al.*, 2015), but higher than 13% in Kwara, 22.6% in Port Harcourt (Omitola and Arigbede, 2012; Tobin and Ajayi, 2017) and lower than reports in Malawi (69.4%) and India (41.5-64.9%) (Agrawal *et al.*, 2015; Msyamboza *et al.*, 2016; Shah *et al.*, 2017). It is estimated that the global prevalence is between 49% - 83% and varies from country to country (Frencken *et al.*, 2017).

In this study, a decline in the number of visits was recorded from January 2015- December 2017 (587-0). Probable reason for the decline could be as a result of faulty equipment and increased cost for dental services.

More males than female visits were recorded during the study. This agrees with reports of Taiwo *et al.*, 2014 and Osunde *et al.*, 2017 in Katsina and Kano state but contrary to that of Egwari *et al.*, 2009 in Lagos. However it has been suggested that differential gene regulation such as immunosuppressive role of testosterone which may explain the high occurrence of periodontal disease in male (Shiau and Reynold, 2010; Klein and Flanagan, 2016; Roved *et al.*, 2017; Ioannidou, 2017). Reports showed that, males have poorer oral hygiene compared with females (Mamai-Homata *et al.*, 2016) and that males use preventive

medical and dental care services less often (Williams, 2003). It has also been suggested that males may also be the single earning member of a house hold and therefore pays little attention to his own need in terms of medical/dental (Iqbal *et al.*, 2015) The finding in this study is in agreement with the reports in United States, Germany, Katsina, Gujrat, Ghana, Benin City and Kano on male/female ratio (Eke *et al.*, 2012, Cachovan *et al.*, 2013, Taiwo *et al.*, 2014, Mahmoodi *et al.*, 2015, Joshi *et al.*, 2016, Nimako-Boateng *et al.*, 2016, Ojehanon and Ehizele, 2016 and Osunde *et al.*, 2017) but contradicts the reports in Burkina Faso and some parts of Nigeria where more females than males were reported (Varenne *et al.*, 2005; Egwari *et al.*, 2009; Ogbemor and Azodo, 2016).

The age distribution indicates that adults in the age bracket (26-36 and 48-58 years) were the highest. This is not unexpected since the study is conducted in a tertiary institution of learning. Similar findings was reported in Ghana by Nimako-Boateng *et al.*, (2016) who reported that approximately 70% of the cases were adults.

The treatment administered to patients was based on the diagnosis, type and severity of the disease. Patients with periodontal disease were treated using the scaling and root planning methods and deep cleaning of the teeth which is a standard procedure as recommended by America Dental Association guideline (ADA 2015), after which no further treatment is required but maintenance therapy to ensure good oral health. (American Academy of Periodontology, 2019). Surgical extraction and filling of teeth were treatment options mostly used in this study for patients with dental caries. The method usually focuses on fixing the damages caused by the disease (cavities) rather than the disease itself. These surgical treatments are a supplement to active surveillance, oral health literacy, and preventive

interventions/therapies (Slayton *et al.*, 2016). Lack of dental visit may lead to tooth loss and therefore requiring extraction.

The relatively high bacterial count from oral samples observed in this study is similar with the reports in Lagos and Ile-ife (Egwari *et al.*, 2009; Oyetola *et al.*, 2019). In Zagreb, Croatia, high oral bacteria count was also reported among smokers as a result of accumulation of plaque in the mouth (Petrusic *et al.*, 2015). High oral bacteria count if left unchecked might result in oral conditions.

Prevotella and *Bacteriodes* were the most frequently isolated bacteria in this study. High isolation rate of these bacteria was reported by Al Yahfoufi and Hadchiti (2017). Loyola-Rodrigues *et al.*, (2014) and Kalala-Kazadi *et al.*, (2018) reported high isolation rate of *Prevotella* spp while Egwari *et al.*, (2016) reported high number of *Bacteriodes* and low occurrence rate for *Prevotella*. Egwari *et al.*, (2009), however isolated *Prevotella* but it had a low occurrence rate compared to other organism. *Prevotella*, though considered a commensal that are found in healthy and periodontal patients (Brook, 2004; Nagy, 2010), under the right condition and in a sufficient quantity might cause oral infection or disease. *Bacteriodes* have the ability to invade host epithelial cell causing inflammation of the peridontium (Lazar *et al.*, 2017).

The organisms in this study showed considerably high level of resistance to metronidazole, Amoxicillin/clavulanate and Chloramphenicol which could be attributed to the misuse and abuse of antibiotics since abuse and misuse have been associated with increased resistance (Oberoi *et al.*, 2015).

High resistance to Metronidazole (100%) in this study is contrary to reports by Van Winkelhoff, *et al.*, (2005), Kulik *et al.*, (2008), Rams *et al.*, (2011), He *et al.*, (2013), Al-

ahmad *et al.*, (2014), Egwari *et al.*, (2016) and Byun *et al.*, (2019) who reported low resistance (0%-27%) to metronidazole. However, reported low resistance to metronidazole. Studies have suggested that resistance to metronidazole could be attributed to presence of *nim* gene (Brazier *et al.*, 1999; Hecht 2004). However, in this study, none of the bacteria isolates harboured the *nim* gene which may be as a result of metronidazole being used by the patient during the study period. Resistance may also be due to decreased intracellular reduction and slower uptake of the drug (Soares *et al.*, 2012). The finding from this study agrees with reports in Greece which detected no *nim* gene in metronidazole-resistant oral bacteria (Ioannidis *et al.*, 2009 and Koukos *et al.*, 2016).

Amoxicillin/clavulanic acid has been recognized as an effective antibiotic mostly used for the treatment of oral infections and as prophylaxis for systemic disease during dental therapy (Herrera *et al.*, 2000). The high bacteria resistance of 62.5% to this antibiotic observed in this study is contrary to the low resistance of 12% reported by Loyola-Rodriguez *et al.*, (2014) and Chan and Chan, (2003). The resistance of bacteria isolated in the study maybe due to the presence of *bla*_{TEM} gene identified in this study, which is an extended spectrum beta-lactam gene. Acquisition of this gene could be vertical or horizontal using a variety of methods including plasmid transfer (Zhou *et al.*, 1994; Bret *et al.*, 1997; Bush, 2018). Some studies also reported these bacteria isolates to be Extended Spectrum Beta-Lactam producers (Iwahara *et al.*, 2006; Patel 2011; Rams *et al.*, 2013; Benachinmardi *et al.*, 2014). It has been suggested that anaerobic gram negative bacteria produce zinc metallo- β -lactamases and cannot be inactivated by β -lactam/ β -lactam inhibitors (Hecht, 2004). However, inhibitor resistance TEM has also been identified to be associated with resistance of bacteria to amoxicillin/clavulanic acid which may be a

contributing factor to resistance (Bush, 2018). Resulting resistance could extend treatment period in an individual with oral condition.

High resistance of 100% was observed to chloramphenicol in this study but in contrary to low resistance 19.2% reported by He *et al.*, 2013 and 0% by Byun *et al.*, 2019. The resistance to chloramphenicol in this study could be attributed to the presence of chloramphenicol acetyl transferases (*cat*) gene in all the bacteria isolates (9). Reports of the presence *cat* gene in oral bacteria are scarce; this could be due to its limited use in dental therapy. Resistance to chloramphenicol and the presence of the *cat* gene has been reported in bacteria isolates from other conditions (Ng *et al.*, 2014; Torkan *et al.*, 2016; Dec *et al.*, 2017)

Chloramphenicol was a drug-of-choice for the treatment of serious anaerobic infections but now is used infrequently due to its considerable toxicity and serious reversible and irreversible side effects (Gajdács *et al.*, 2017).

Resistance to clindamycin was however low (25%) in this study compared to 61.6% reported by He *et al.*, 2013, 85.9% by Loyola-Rodriguez *et al.*, 2014 and 45% by Byun *et al.*, 2019 who reported high resistant pattern to clindamycin but however contrary to Kulik *et al.*, 2008 who reported a 100% susceptibility. This is an alternative antibiotic for patients that do not respond to treatment from other antibiotics or have allergy to penicillin-family drugs (Ellison, 2011; Robertson *et al.*, 2015). Clindamycin should be prescribed with caution because of potential for pseudomembranous colitis as a result of intestinal overgrowth with *Clostridium difficile* (Addy and Martin, 2005).

The quantitative method, microtitre plate (MTP) in this study showed biofilm formation in the isolates. There was no significant difference in the antibiotic resistance pattern of biofilm positive and biofilm negative bacteria isolates ($p>0.05$) which indicates that biofilm forming capacity may not be the prime factor for resistance pattern observed. However, there have been reports of biofilm involvement as a vehicle of antibiotic resistance and as a protective agent for bacteria within the matrix (Stewart 2015; Bowler 2018).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion

In this study, dental disease was observed to affect all age groups ($\leq 14-59$ years) and more visits of males than females. There was antimicrobial resistance in periodontal organisms with some of these organisms harbouring resistant genes. Most of the bacteria isolates were potential biofilm producers at varying rates with no significant difference in the antibiotic resistance pattern of biofilm producing and non-producing periodontal organism.

6.1 Recommendations

Based on the observations made in this study, the following recommendations are made:

- i) Subsequently, it is important to have a continuous antimicrobial resistance surveillance of dental organisms to detect new strains and resistant genes.
- ii) Further research in this field should consider multicenter study and extended sampling time to generate enough data which would guide policy makers and health professionals on appropriate control strategies.
- iii) Awareness on the implication of poor oral hygiene and prompt appropriate dental care seeking and interventions should be emphasized for prevention and management of dental conditions,
- iv) Dental services should be made affordable and easily accessible to the general public and well equipped with modern facilities.

6.2 Contribution to Knowledge

- (i) The study provides a foundational knowledge base for the future studies related to oral diseases in Zaria, Nigeria, in terms of:
 - Prevalence rate of oral disease, treatment patterns, age and gender variation, severity of the disease as well as the utilization of dental services.
- (ii) The study updates and adds to knowledge of bacterial etiology of periodontal disease in Zaria, Kaduna State North West, Nigeria.
- (iii) It provides evidence that these periodontal organisms are potential biofilm producers.
- (iv) It also provides a foundational knowledge base for the future studies on the occurrence of TEM, CAT and NIM genes in anaerobic Gram negative bacteria isolated from periodontal disease patient.

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APPENDICES

Appendix 1: ETHICAL CLEARANCE



DIRECTORATE OF ACADEMIC PLANNING & MONITORING AHMADU BELLO UNIVERSITY, ZARIA

Vice Chancellor: Prof. Ibrahim Garba, B.Sc. (Hons) Geology, M.Sc. (Mineral Exploration) A.B.U., Ph.D Geology (London), D.I.C., FHMGS
Director: Prof. M.F. Ishiyaku, B.Sc. (Hons) Botany (ABU), M.Sc. Plant Breeding (Unijos), Ph.D Agriculture (University of Reading, U.K.), MASN, MBSN

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e-mail: dapm@abu.edu.ng

Appl No.: ABUCUHSR/2018/ Pharmaceuticals & Pharm. Microbiology/009
Approval No: ABUCUHSR/2018/009

5th July, 2019

Dr. B.A. Tyler,
Department of Pharmaceutics and Pharmaceutical Microbiology,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

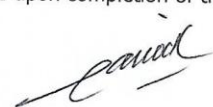
PROVISIONAL APPROVAL OF RESEARCH TITLED "ACCESSING ORAL HEALTH AND ANTIBIOTIC SUSCEPTIBLY OF BACTERIA ISOLATES FROM DENTAL INFECTION OF PATIENTS VISITING DENTAL UNIT OF A.B.U ZARIA MEDICAL CENTER (SICK BAY)"

This is to convey the provisional approval of the ABUCUHSR to you for the aforesaid study domiciled in the Department of Pharmaceutics and Pharmaceutical Microbiology of the Ahmadu Bello University. The approval is predicated on the assumption that you shall maintain and cater for the study subjects as indicated in your application.

Monitoring of the Research by spot checks, invitations, interactions with the subjects by any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCUHSR Page on the University's website. Note upon completion of the research, ethical clearance certificate will be issued.


Prof. I.H. Nock
Chairman, ABUCUHSR

Cc. Director, DAPM
" Director, IC&ICT
" Head, Department of Pharmaceutics and Pharmaceutical Microbiology
" Dr. M.K Lawan, Department of Veterinary Public Health and Preventive Medicine



Ahmadu Bello University
Zaria, Nigeria.

ABU Research Ethics Committee (ABUCRHS)

ABUCUHSF 2: Informed and Voluntary Consent Form

Date...../...../

I,....., aged.....years old, now living at the address
..... and Tel. number.....:..... hereby
express my consent to participate as a subject in the research project⁽¹⁾
entitled.....
.....
.....

In so doing, I am informed of the research project's origin and purposes; its procedural details to carry out or to be carried out; its expected benefits and risks that may occur to the subjects, including methods to prevent and handle harmful consequences; and remuneration, and expense. I thoroughly read the detailed statements in the information sheet given to the research subjects. I was also given explanations and my questions were answered by the head of the research project.

I therefore consent to participate as a subject in this research project on the condition that if I have any questions about the research procedures, or on the condition that if I suffer from an undesirable side effect from this research, I can contact (Indicate the name of the person in charge who is 24-hour ready for contact by phone or pager.).....

I am aware of my right to further information concerning benefits and risks from the participation in the research project and my right to withdraw or refrain from the participation anytime without any consequence on the service or health care I am to receive in the future.

I consent to the researchers' use of my private information obtained in this research,

I do not consent to an individual disclosure of private information

My information must be presented as part of the research results as a whole.

I thoroughly understand the statements in the information sheet for the research subjects and in this consent form. I hereby append my signature.

Name.....Sign..... Date.....

Name and Signature..... Person in Charge of Informing and Requesting a Consent/

Head of Research Project: Date.....

In case that the participant is not literate, the reader of all the statements for the participant is (Mr. /Mrs./Ms.....), who gives his/her signature as a witness.

Signature.....Witness/Date.....

Note: If the participant is a minor (under 18 years old), "I" marked with a star (*), must be replaced with the followings:

(1) I hereby express my consent to my child's participation as a subject in the research project.

(2) I therefore consent to the participation of the child under my guardianship as a subject in this

research project.

Appendix 3: Identification of Bacteria Isolates and Antibigram Study

This result showed the identity of each bacteria isolates recovered and susceptibility pattern of the tested antibiotic from suspected periodontal disease patients

CODE	ORGANISM	MTZ	A/C	C	DA
1A2	<i>Prevotella oralis</i>	R	R	R	S
1A3	<i>Prevotella intermedia</i>	R	R	R	S
1B1	<i>Prevotella intermedia</i>	R	R	R	S
1B2	<i>Prevotella intermedia</i>	R	R	R	S
3A1	<i>Prevotella oralis</i>	R	R	R	S
3A2	<i>Prevotella intermedia</i>	R	R	R	S
4A1	<i>Bacteriodes fragilis</i>	R	S	R	S
7B2	<i>Bacteriodes fragilis</i>	R	R	R	S
8D1	<i>Fusobacterium nucleatum</i>	R	R	R	R
8D2	<i>Fusobacterium nucleatum</i>	R	S	R	S
8D3	<i>Fusobacterium nucleatum</i>	R	R	R	S
8D4	<i>Bacteriodes stercoris</i>	R	R	R	S
9B1	<i>Prevotella intermedia</i>	R	R	R	R
9B2	<i>Fusobacterium varidium</i>	R	R	R	R
10A3	<i>Bacteriodes fragilis</i>	R	R	R	R
12C1	<i>Bacteriodes fragilis</i>	R	R	R	R
12C2	<i>Bacteriodes fragilis</i>	R	R	R	R
13B1	<i>Fusobacterium mortiferum</i>	R	R	R	R
13B2	<i>Fusobacterium mortiferum</i>	R	R	R	R
14A3	<i>Bacteriodes stercoris</i>	R	R	R	S
15A1	<i>Fusobacterium varidium</i>	R	R	R	R
17B2	<i>Fusobacterium varidium</i>	R	R	R	R
18B1	<i>Bacteriodes fragilis</i>	R	S	R	S
19A1	<i>Fusobacterium mortiferum</i>	R	R	R	R
19B3	<i>Bacteriodes fragilis</i>	R	S	R	S
20A1	<i>Bacteriodes fragilis</i>	R	S	R	S
23B1	<i>Fusobacterium nucleatum</i>	R	S	R	S
30C1	<i>Porphyromonas asaccharolytica</i>	R	S	R	S
30C2	<i>Bacteriodes fragilis</i>	R	S	R	S
30C3	<i>Porphyromonas</i>	R	S	R	S

	<i>asaccharolytica</i>				
75A4	<i>Prevotella oralis</i>	R	R	R	S
67D1	<i>Bacteriodes stercoris</i>	R	R	R	S
89B1	<i>Fusobacterium</i> <i>varidium</i>	R	R	R	R
37B3	<i>Prevotella intermedia</i>	R	R	R	S
49B4	<i>Prevotella intermedia</i>	R	R	R	S
36A2	<i>Fusobacterium</i> <i>nucleatum</i>	R	R	R	R
14B4	<i>Prevotella intermedia</i>	R	R	R	R
77A2	<i>Prevotella intermedia</i>	R	R	R	R
90D2	<i>Porphyromonas</i> <i>asaccharolytica</i>	R	S	R	S
50A3	<i>Prevotella oralis</i>	R	R	R	S

Keys: R- Resistant, S- Susceptibility

AMC = Amoxicillin-Clavulanate, MTZ = Metronidazole, C = Chloramphenicol, DA = Clindamycin.

Appendix 4: Minimum inhibitory concentration of identified bacterial isolates.

CODE	ORGANISM	MTZ (S≤4, R>4) (µg/ml)	AMC (S≤4,R>8) (µg/ml)	C (S≤8, R>8) (µg/ml)	DA (S≤4, R>4) (µg/ml)
1A2	<i>Prevotella oralis</i>	>256	48	24	0.75
1A3	<i>Prevotella intermedia</i>	>256	32	48	0.5
1B1	<i>Prevotella intermedia</i>	>256	64	48	1.5
1B2	<i>Prevotella intermedia</i>	>256	>256	64	0.023
3A1	<i>Prevotella oralis</i>	>256	>256	24	0.75
3A2	<i>Prevotella intermedia</i>	>256	>256	64	0.5
4A1	<i>Bacteriodes fragilis</i>	>256	0.38	>256	0.023
7B2	<i>Bacteriodes fragilis</i>	>256	>256	>256	<0.016
8D1	<i>Fusobacterium nucleatum</i>	>256	>256	>256	12
8D2	<i>Fusobacterium nucleatum</i>	>256	0.5	>256	1
8D3	<i>Fusobacterium nucleatum</i>	>256	32	64	0.38
8D4	<i>Bacteriodes stercoris</i>	>256	32	48	12
9B1	<i>Prevotella intermedia</i>	>256	>256	32	16
9B2	<i>Fusobacterium varidium</i>	>256	64	16	16
10A3	<i>Bacteriodes fragilis</i>	>256	48	24	32
12C1	<i>Bacteriodes fragilis</i>	>256	>256	32	24
12C2	<i>Bacteriodes fragilis</i>	>256	>256	>256	>256
13B1	<i>Fusobacterium mortiferum</i>	>256	>256	>256	48
13B2	<i>Fusobacterium mortiferum</i>	>256	>256	>256	64
14A3	<i>Bacteriodes stercoris</i>	>256	>256	>256	0.5
15A1	<i>Fusobacterium varidium</i>	>256	>256	>256	16
17B2	<i>Fusobacterium varidium</i>	>256	>256	>256	12
18B1	<i>Bacteriodes fragilis</i>	>256	0.032	>256	<0.016
19A1	<i>Fusobacterium</i>	>256	>256	>256	64

	<i>mortiferum</i>				
19B3	<i>Bacteriodes fragilis</i>	>256	0.38	>256	0.38
20A1	<i>Bacteriodes fragilis</i>	>256	0.032	>256 <0.016	
23B1	<i>Fusobacterium nucleatum</i>	>256	0.5	>256	1
30C1	<i>Porphyromonas asaccharolytica</i>	>256	0.023	>256	0.38
30C2	<i>Bacteriodes fragilis</i>	>256	0.38	>256	0.023
30C3	<i>Porphyromonas asaccharolytica</i>	>256	0.023	>256	0.38
75A4	<i>Prevotella oralis</i>	>256	0.5	>256	0.5
67D1	<i>Bacteriodes stercoris</i>	>256	0.032	>256	0.5
89B1	<i>Fusobacterium varidium</i>	>256	>256	>256	12
37B3	<i>Prevotella intermedia</i>	>256	0.023	64	0.75
49B4	<i>Prevotella intermedia</i>	>256	0.5	>256	0.023
36A2	<i>Fusobacterium nucleatum</i>	>256	32	48	32
14B4	<i>Prevotella intermedia</i>	>256	64	38	12
77A2	<i>Prevotella intermedia</i>	>256	32	>256	32
90D2	<i>Porphyromonas asaccharolytica</i>	>256	0.023	>256	0.38
50A3	<i>Prevotella oralis</i>	>256	0.023	>256	0.5

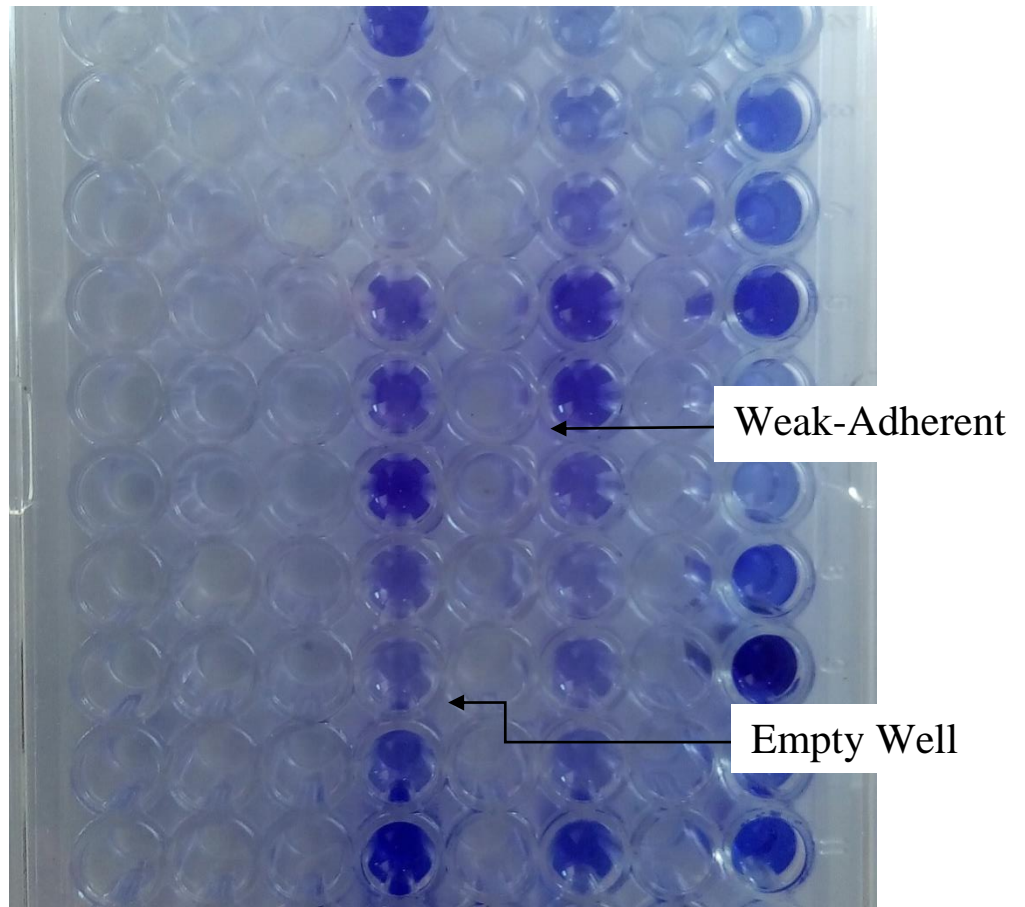
Appendix 5: Biofilm Formation of the Bacterial Isolates

Code	Biofilm
1A2	0.363nm -
1A3	0.372nm -
1B1	0.390nm +
1B2	0.497nm +
3A1	0.363nm -
3A2	0.373nm -
4A1	0.602nm +
7B2	0.352nm -
8D1	0.507nm +
8D2	0.392nm +
8D3	0.553nm +
8D4	0.413nm +
9B1	0.492nm +
9B2	0.789nm +
10A3	0.740nm +
12C1	0.740nm +
12C2	0.968nm +
13B1	0.656nm +
13B2	0.940nm +
14A3	0.443nm +
15A1	0.789nm +
17B2	1.229nm +
18B1	0.366nm -
19A1	1.123nm +
19B3	0.421nm +
20A1	0.374nm -
23B1	0.610nm +
30C1	0.401nm +
30C2	0.507nm +
30C3	0.426nm +
75A4	0.368nm -
67D1	0.413nm +
89B1	0.789nm +
37B3	0.390nm +
49B4	0.497nm +
36A2	0.507nm +
14B4	0.656nm +
77A2	0.656nm +
90D2	0.401nm +
50A3	0.366nm -

Isolates Keys: + =Biofilm formers; - =Non-biofilm formers; MTP =Microtitre plate method; nm =wavelength

Note: Cut off OD = 0.391408.

Appendix 6: Biofilm Quality and Quantity in Microtitre Plate of the Bacterial Isolates



← Moderate-Adherent

← Non-Adherent

Appendix 7: Statistical Analysis

Mean and Standard Deviation of Age

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Age	10364	2	69	35.47	.148	15.097
Valid N (listwise)	10364					

Chi Square Test for Monthly Diagnosis

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	116.066 ^a	11	.000
Likelihood Ratio	116.004	11	.000
Linear-by-Linear Association	12.353	1	.000
N of Valid Cases	10364		

Chi Square Test for Significant Difference between Male and Female

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	27.692 ^a	11	.004
Likelihood Ratio	27.657	11	.004
Linear-by-Linear Association	.718	1	.397
N of Valid Cases	10364		

Chi Square Test for Significant Difference between Periodontal Disease and Dental Caries

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	42.802 ^a	1	.000		
Continuity Correction ^b	42.525	1	.000		
Likelihood Ratio	42.588	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	42.798	1	.000		
N of Valid Cases	10364				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 1399.56.

b. Computed only for a 2x2 table