SURVEY OF PATHOGENS IMPLICATED IN DENTAL CARIES AMONG ATHLETES AND THEIR SUSCEPTIBILITIES TO SELECTED BRANDS OF ORAL WASH AND TOOTHPASTE

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BEING A DESERTATION SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, BAYERO UNIVERSITY, KANO. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN MICROBIOLOGY. (ENVIRONMENTAL MICROBIOLOGY).

SUPERVISED BY

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MARCH, 2017

DECLARATION

I hereby declare that, this work is a product of my own research effort, undertaken under the supervision of Dr. Bashir Muhammad, and it has not been presented elsewhere for the award of a degree or any certificate. All sources have been duly acknowledged.

Signature and Date

……

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CERTIFICATION

This is to certify that, this research work titled Survey of pathogens implicated in dental caries among athletes and their susceptibility to selected brands of oral wash and toothpaste, for this thesis and the subsequent preparation of it by Hussaina Bukar Ali (SPS/13/MMB/00003) were carried out under my supervision.

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APPROVAL PAGE

This is to certify that this research project titled Survey of pathogens implicated in dental caries among athletes and their susceptibilities to brands of oral wash and toothpaste. Was carried out by Hussaina Bukar Ali (SPS/13/MMB/00003), under the supervision of Dr. Bashir Muhammad and has been read and approved by the undersigned as meeting the requirement for the award of master of science Degree in environmental microbiology, Bayero University, Kano, Nigeria.

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DEDICATION

To my late mother H. Fatima and my late twin sister, may their gentle souls rest in perfect peace, amin.

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ABSTRACT

Dental caries and related oral diseases caused by various pathogens are common in both developed and developing countries. The antimicrobial activity of various tooth paste and mouth rinse to pathogens, isolated from buccal cavity of the athletes were investigated. Five different brands of toothpaste and five mouth washes were bought from Sabon Gari market and Sahad stores in Kano, and were marked A, B, C, D, and E. A total of three hundred and eighty four samples were collected randomly from volunteered athletes before and after exercise (1 hour interval), using separate sterile swab sticks, questionnaires were distributed prior to sample collection to obtain responses. The samples were processed using standard techniques to identify the microbial agents, and their frequency of occurrence based on colonial, microscopic, and biochemical characteristcs. Isolates identified comprises of *S. aureus,* 72 (37.50%)pre, and 79 (41.15%) post exercise, *S.mutans,* 61 (30.70%) pre, and 68 (35.40%) post-exercise, and *C.albican,* 22 (9.90%) pre and 24 (10.94%) post- exercise. The total number of occurrence and percentage of the pathogens for pre and post- exercise statistically shows no significant difference ($p= 0.05$) Antimicrobial susceptibility test was performed on all the isolates using Kirby Bauer disk diffusion technique. The result for mouth wash formulations A,D and E shows effectiveness against the four isolates, showing no significant difference at full strength and 1:1 dilution ($p= 0.05$). However formulations B and C were ineffective to all the four isolates. Various inhibition zones were also exhibited by the isolates by action of antimicrobial agents of different tooth paste brands where formulations A, B, C and D where equally effective against all the isolates. In case of formulation E, it was found to have significant effect on all the isolates with the exception on *S.mutans*. It can be concluded that there is slight increase in number of isolates after exercise, and that about 80% of the Isolates were sensitive to all the dentifrices evaluated.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Dental caries, also known as tooth decay or cavities, is a breakdown of teeth due to activities of bacteria. Symptoms may include pain and difficulty with eating. Complications may include inflammation of the tissue around the tooth, tooth loss, and infection (Laudenbach, 2014). Caries is Latin word for "rottenness"(Silk, 2014**).** Dental caries and related oral diseases like gingivitis and periodontitis are most common oral diseases throughout the world including both developed and developing countries, affecting people from all ages of life (Anusavice, 2002). The frequency of these oral diseases is continuously increasing with change in eating habit among people of different age group and increased consumption of sugar (Saini *et al.,* 2003).

Athletes, like other members of the population, are susceptible to dental diseases such as dental caries and dental erosion. However there is heightened susceptibility during training and performance which are often supported by carbohydrate-containing sports drinks that are taken frequently during activity (Bryant *et al.,* 2011and Lun *et al.,* 2012). Many athletes, especially endurance athletes experience long periods of hypohydration during training and competition. It is recognized that this increases the risk of dental erosion due to a decrease in salivary flow resulting in inadequate rinsing and buffering of demineralizing acids on tooth surfaces (Woltagen *et al.,* 1985). Oral health is one of the determinants of life quality, the health and welfare of athletes are paramount for their successful participation in sporting activities (Locker, 1985). Optimal performance standard of athletes can be only achieved in the presence of general health, in which dental health is included. Dental health can lead to lower performance during training and competition by interfering with concentration and confidence (Loos *et al.,* 2005).

There are many challenges to the oral health of athletes, these issues include nutritional challenges from frequent carbohydrate intake and acidic sports drinks, impairment of host responses due to dehydration, mouth drying and intensive training, poor health behaviors, oral health literacy and lack of effective health promotion (Moynihan *et al*., 2014 and Mulic *et al.,* 2012).

Dental plaque, dental caries and periodontal diseases, are primarily caused by the virulence of complex oral micro-communities (Prasanth, 2011). Dental plaque has been proved to be a paramount factor in initiation and progression of gingival and periodontal diseases through caries production (Mali, *et al.,* 2012). Poor oral hygiene is one of the reasons for accumulation of microbes and their harmful activities (Smiline *et al.,* 2012).

The customary oral hygiene method of chemical plaque control can be used as an adjunct to mechanical plaque control procedure (Mali *.et al*., 2012). Besides cleaning of teeth, the use of chemical agents with anti-plaque or antimicrobial activity, into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease by limiting the cariogenic bacteria in the oral cavity (Priya *.et al* 2012).

Dental caries was first described by miller's chemo parasitic theory in 1890 (Miller, 1993). Caries is caused by the dissolution of the teeth by acid produced by the metabolism of dietary carbohydrate by oral bacteria. It is initiated by demineralization of the enamel of teeth due to primarily the opportunistic pathogens which are mutans *streptococci* and *lactobacilli* (Gamboa *et al*, 2011). Other micro flora like *E.coli* and *Candida* are associated with active caries lesions*, Candida albicans* is the most common yeast isolated from oral cavity that shows resistance to inter-carnal medication (Often *et al,* 2010). Dental plaque is material adhering to teeth surface that has been link to gingivitis, periodontal disease or

dental caries (Jensena, 1998).It has been previously shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial tooth paste and mouth wash (Collins,1998).

Cariogenic bacteria interact by various recognized ways including co- aggregation (Kolembrander *et al* 2000), metabolic exchange, cell-cell communication (Li *et al.,* 2002), and exchange of genetic material (Roberts *et al.,* 2001). These mechanisms benefit bacterial survival and can make dental biofilms difficult therapeutic targets in dental diseases. The burden of dental caries is still a major health problem in most industrialized countries as it affect vast majority of people. This is largely due to the increasing consumption of sugar and inadequate exposure to fluoride (Petersen *et al.,* 2005). Tooth erosion is the progressive loss of dental hard tissue by acids in the process that does not involve bacteria or sugars, the intrinsic acids are from vomiting, gastro esophageal reflux and regurgitation (Schetzel, 1996). The acids are from the diets like sports beverages (Milosevic *et al.*, 1997), and Citrus product including fruit, juices, Soft drinks (Zero, 1996). Tooth erosion as a result of eating disorders (*bulimia nervosa*) (Studen *et al.,* 2001), dietary practices involving frequent intake of acidic food and beverages weaken tooth integrity (Parry *et al.,* 2001).

Mouth wash (mouth rinse, oral rinse) is a liquid which is held in the mouth passively or swilled around the mouth by the contraction of the peri-oral muscles and movement of the head, and may be gargled where the head is tilted back and the liquid bubbles at the back of the mouth (Mathews, 2003). Usually mouth washes are an antiseptic solution intended to reduce the microbial load in the oral cavity, although other mouth washes might be given for other reasons such as for their analgesic, anti-inflammatory or anti-fungal action.

Tooth paste has a history that stretches back nearly 4000years. Different abrasives, green lead, incense were used to clean stain from teeth until mid nineteenth century. In middle ages, fine sand and pumice

were the primary ingredients in the tooth cleaning formulas used by Arabs. In 1950 AD, Dr.Washington Wentworth Sheffield, a dental surgeon and chemist, invented the first toothpaste. Then, the market of the toothpaste has never been slowed down (Lee *et al.,* 2004). Modern toothpaste was invented to aid in the removal of foreign particles and food substances in addition to cleaning of tooth. During 1940-60 AD, fluoride was added which aided in protection from tooth decay. Many of the innovations were made in toothpaste after the fluoride break through which involved the addition of ingredients with special abilities to toothpaste and toothpaste packaging (Hawkins *et al.,* 2003).

1.2 Statement of the Problem

Athletes may have poor oral health, such as dental caries and dental erosion (Woltagen, 1985), and there are potential challenges to the oral health of athletes including lack of awareness and negative health behaviors. Oral health is considered as one of the determinants of life quality, and presently toothpaste and oral wash with different antimicrobial activity regarding their chemical compositions are being sold in the local markets and some of them do not meet up srequirements needed, and it is quite safe to assess the effectiveness to decelerarates the process of tooth decay among the athletes.

1.3 Justification of the study

Dental problems occur more frequently in the general population, and are associated mainly with poor oral hygiene practices. Studies have shown that athletes are more prone to such problems, because there is susceptibility during training (Ashley *et al*., 2014 and Foster *et al*., 2012). Athletes usually spend much time on exercise, during and after the strenuous exercises they produced less saliva and this cause instability in the mouth ultimately leaving mouth vulnerable to tooth decay and gum disease, because saliva contains neutralizing enzymes which helps in buffering and rinsing of demineralizing acid produced by the metabolism of carbohydrates. Consumption of excess sugary food by athletes, like sport

drinks and frequent carbohydrate diets present challenges to oral health however the welfare of athletes is paramount for their successful participation in sporting activities.. There is scarce or no data on the oral microbial status of the athletes in Kano and associated risk factors for dental caries. Hence there is need to study the population of oral microorganisms and their sensitivity to commonly sold tooth paste and mouthwash in kano, among teeming athletes.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the research is to survey for pathogens implicated in dental caries among athletes and their susceptibilities to brands of toothpaste and oral wash sold in Kano.

1.4.2 Objectives

This can be achieved though the following objectives.

1. To isolate and identify pathogens from the buccal cavity of athletes from both stadia.

2. To determine effect of exercise on the growth and population dental caries pathogens among the athletes.

3. To determine the sensitivities of dental caries pathogens to commonly sold mouthwashes and toothpastes in kano.

1.5 Hypothesis of the research

1. There is no difference in terms of microbial load before and after exercise.
2. The pathogenic microorganisms are not associated with the buccal cavity of athletes.
3. Pathogenic microorganisms are not sensitive to mouth wash and tooth pastes sold in Kano.

CHAPTER TWO

2.0 LITERATURE REVIEW

Oral diseases such as Dental caries and Periodontal diseases are caused by microorganisms belonging to the resident micro flora rather than by classic microbial pathogens (Marsh, 1994). They are caused by the ecological imbalance in oral biofilms. Oral microbial flora is dominated by gram positive microorganisms and hence dental plaque which is formed on the tooth surface contains gram positive cocci and bacilli. When plaque community equilibrium is altered, the microbial flora shifts from aerobic to anaerobic side and these anaerobic organisms cause periodontal disease (Robin *et al.,* 1992).

2.1 Dental Caries: An overview

Dental caries and related oral diseases are most common oral diseases throughout the world including both developed and developing countries, affecting people from all ages of life. The frequency of these oral diseases is continuously increasing with change in eating habit of among people of different age group, and increased consumption of sugar (Saini, 2003). Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases (Clarke, 1942). Dental caries is an infection, generally bacterial in origin, localized and transmissible, that results in the destruction of hard dental tissue. It results from accumulation of plaque on the surface of the teeth and biochemical activities of complex micro-communities. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries (Gamboa *et al* 2004). The pathogens of dental caries play a central role in fermenting carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel (Manupati, 2011). In addition, other microflora like *Escherichia coli* and *Candida* are also associated with active caries lesions. *C. albicans* is the most common yeast isolated from the oral cavity. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to inter-canal

medication (Oztan, *et al*., 2006). Poor oral hygiene is one of the reasons for growth of these microbes and their harmful activities. Dental caries is a localized and progressive decay of the teeth. Not only does it cause many people to experience a great deal of pain, it leads to continuous discomfort through inconvenient treatment. WHO's report on the Global Problem of Oral Diseases, notes that oral diseases such as dental caries (tooth decay), periodontitis (gum disease) and pharyngeal cancers are global health problem in both the industrialized and the developing countries, especially among poorer communities (Petersen, 2003). Dental caries is a major oral affliction in developing countries, affecting vast majority of adults. An estimated five billion people worldwide have experienced dental caries (Petersen, 2003), dental caries is considered to be a major public health problem (Cirino and Scantlebury, 1998).

2. 1.1 General causes of dental caries

Dental caries is a multi factorial infectious disease in which the active agent or agents are members of the indigenous oral flora. Oral cavity harbors a rich and diverse microbial flora because of its ideal humidity and temperature, the frequent passage through it of most nutrients needed by many microbial species and presence of several ecological niches. Oral microbes can adhere to surfaces throughout the oral cavity (Loesche, 1986). These include the tongue, epithelial cells lining roof of the mouth and the cheeks, and enamel of the teeth. Bacteria in a person's mouth convert glucose, fructose, and most commonly sucrose (table sugar) into acids such as lactic acid through a process called fermentation (Holloway and Moore, 1983) If left in contact with the tooth, these acids may cause demineralization, which is the dissolution of its mineral content. The process is dynamic, however, as remineralization can also occur if the acid is neutralized by saliva or mouthwash. Fluoride toothpaste or dental varnish may aid remineralization (Silverstone, 1983). If demineralization continues over time, enough mineral content may be lost so that the soft organic material left behind disintegrates, forming a cavity or hole. The I mpact such sugars have on the progress of dental caries is called cariogenicity. Sucrose, although

a bound glucose and fructose unit, is in fact more cariogenic than a mixture of equal parts of glucose and fructose. This is due to the bacteria utilizing the energy in the saccharine bond between the glucose and fructose subunits. *S.mutans* adheres to the biofilm on the tooth by converting sucrose into an extremely adhesive substance called dextran polysaccharide by the enzyme dextransucranase. The frequency with which teeth are exposed to cariogenic (acidic) environments affects the likelihood of caries development.(Anusavice, 2005). After meals or snacks, the bacteria in the mouth metabolize sugar, resulting in an acidic by-product that decreases pH. As time progresses, the pH returns to normal due to the buffering capacity of saliva and the dissolved mineral content of tooth surfaces. During every exposure to the acidic environment, portions of the inorganic mineral content at the surface of teeth dissolve and can remain dissolved for two hours. (Papas *et al*.,1995) Since teeth are vulnerable during these acidic periods, the development of dental caries relies heavily on the frequency of acid exposure.

2.1.2 Periodontal disease: General knowledge

Periodontal disease is gum disease. The word "periodontal" means "around the tooth." Periodontal disease is a chronic inflammatory disease of the gum and tissues that surround and support the teeth. If left untreated, periodontal disease can lead to tooth loss. (Amaliya*, et al*., 2007). Periodontitis is an infection of global prevalence and affects individuals of all ages, but the disease is more common in elderly individuals. The increased prevalence, extent, and severity in older age groups reflect the cumulative effect of a prolonged exposure to the established risk factors(Newman,2007 and Phlstrom, 2001). The disease is generally divided into two groups (Eberhard *et al*, 2008) these are gingivitis, which causes lesions (inflammatory) and Periodontitis, which damages the bone and connective tissue that support the teeth, is a more serious form of gum disease.

Gingivitis is an inflammation of the gingiva, or gums. It is characterized by tender, red, swollen gums that bleed easily and may cause bad breath (halitosis). Gingivitis can be treated by good dental hygiene, proper diet, and stopping smoking. Untreated gingivitis can lead to periodontitis. (Mendel *et al*., 1988).

Periodontitis occurs when the gum tissues separate from the tooth and sulcus, forming periodontal pockets. Periodontitis is characterized by gum inflammation, with redness and bleeding, deep pockets (greater than 3 mm in depth) that form between the gum and the tooth, and it is also characterized by, tooth loss, caused by loss of connective tissue structures and bone (Eberhard *et al*., 2008).

2.1.3 Causes of periodontal disease

Periodontal disease is caused by plaque, which is formed from harmful bacteria. The mouth is full of bacteria but they tend to be harmless varieties. Periodontal disease usually develops because of an increase in bacteria quantity in the oral cavity and a change in balance of bacterial types from harmless to disease-causing bacteria. These harmful bacteria increase in mass and thickness until they form a sticky film called plaque (Lamster *et al*., 2008). In healthy mouths, plaque actually provides some barrier against outside bacterial invasion. When it accumulates to excessive levels, however, bacterial plaque sticks to the surfaces of the teeth and adjacent gums and causes infection with subsequent swelling, redness, and warmth. When plaque is allowed to remain in the periodontal area, it transforms into calculus (commonly known as tartar). This material has a rock-like consistency and grabs onto the tooth surface. Tartar is much more difficult to remove than plaque, which is a soft mass. Once tartar has formed, it must be professionally removed by a dental practitioner. (Nguyen, 2008).

2.2 Common Pathogens associated with Dental caries

2.2.1 *Streptococus mutans*

Streptococcus mutans was first described by J. Kilian Clarke in 1924. In deep dentin caries lesions, he found a small, chained coccobacillus which was more oval than spherical in shape. He suggested that these microorganisms were *mutans streptococci* and called them *Streptococcus mutans* (Clarke, 1924). Clarke tried to prove the association of these *streptococci* with dental caries disease, but since other researchers did not support his hypothesis, interest in *S. mutans* waned. In the 1960s, the recently developed method of gnotobiotic animal research stimulated studies on the microbiology of dental caries disease, and *S. mutans* was convincingly connected to dental caries disease (Hamada, 1986). *Streptococcus mutans* are gram-positive cocci shaped bacteria. These facultative anaerobes are commonly found in the human oral cavity, and are a major contributor of tooth decay. The result of decay can greatly affect the overall health of the individual (Whiley *et al.,* 2013) *S. mutans* are mesophilic and grow at temperatures between 18-40 degrees Celsius. *Streptococcus mutans* is a cariogenic microorganism that breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth. The result of the conversion disintegrates the coating of the tooth then later dissolves the Calcium molecule creating a hole. *Streptococcus mutans* possesses three virulence factors these are, water insoluble glycans, acid tolerance, and production of lactic acid. (Howard, 2003) A tooth ache is the most common symptom of tooth decay. An infection or irritation of the tooth pulp usually causes the pain. Since every human has bacteria in their mouth, the only prevention is to lessen the impact of acid fermentation by practicing adequate oral hygiene.

2.2.2 Phathogenesis of *Streptococcus aureus*

Streptococcus mutans reside in the human mouth and, more specifically, in the multi-species biofilms on the surfaces of teeth (Zero *et al.,* 2009). *Streptococcus mutans* are major cariogenic organisms and the result of their ability to produce large quantities of glucans as well as acid, exceeding the salivary buffering capacities, which gives the bacteria an advantage to outcompete non-cariogenic commensal species at low pH environments (Lemos *et al*., 2013). This ability to survive in an acid environment by modulating sugar metabolic pathways coupled with irreversible binding to teeth is a key component to *S. mutans* pathogenesis. In the second stage of invasion, *S. mutans* coadhere or coaggregate with other microbial species, followed by proliferation and spread into other sites in the oral mucosa modulated by concerted action of genes and signaling molecules. In the final stage, the biofilm reaches a steady state which changes the equilibrium balance of the oral ecology, as a result, bacteria gain access into the deeper tissues and recesses in the gingival areas, ultimately causing dissolution of hydroxyapatite crystals in enamel and dentin which results in cavitations within the tooth (Isalm *et al*., 2007). If not prevented, these cavitations provide an ecological niche where microorganisms form a protected biofilm, enabling caries to progress gradually (Roubhia *et al.,* 2012). Interestingly however, although it has been accepted for decades that *S. mutans* is the etiologic agent of dental caries, recent evidence indicates high prevalence for *S. mutans* in dental biofilms where the fungal pathogen *Candida albicans* resides, suggesting that the interaction between these diverse species may mediate cariogenic development (Barbieri *et al*., 2007).

2.2.3 *Staphylococcus aureus*

Staphylococcus aureus is facultative anaerobic gram-positive cocci which occur singly, in pairs, and irregulular clusters. *S. aureus* is non motile, non- spore forming, catalase and coagulase positive.

Typical colonies are yellow to golden yellow in color, smooth, entire, slightly raised, and haemolytic on 5% sheep blood agar (Turnidge, 2008). However, many strains may appear dirty white and non haemolytic. It also gives a positive Mannitol fermentation (Turnidge, 2008).

2.2.4 Pathogenesis of *Staphylococcus aureus*

S. aureus is a major human pathogen, responsible for a number of hospital-acquired infections, initially colonizes several locations in the human body, but the mouth and hands are the main reservoirs for propagation of this pathogen in the hospital, *Staphylococcus aureus* is a putative pathogen of many oral diseases, such as oral mucositis (Gibson *et al.,* 2000), periodontitis (Passariello *et al.,* 2012), peri-implantitis (Heitz-Mayfield and Lang, 2010), endodontic infections (Poeschl *et al.,* 2011) and even dental caries (Kouidhi *et al.,* 2010). Although it is commonly detectable on skin and mucosae, the anterior nasal region is the primary ecological reservoir, followed by oro-pharynx and perineum. Persistent carriers are as many as 20% of the healthy population, while intermittent carriers are 30% carriage. *S. aureus* carriers are sources of health care associated infections (HAIs), which may also occur in dental health care settings (Petti, 2011). *S. aureus* transmission is promoted by the long-term survival, up to six months, on clinical contact surfaces (Petti *et al.,* 2012), that is, surfaces contaminated by patient materials generated during dental procedures (Kohn *et al.,* 2003), and by the passive role of dental health care workers who may act as vectors in transmitting *S. aureus* from clinical contact surfaces to receptive patients (Dancer, 2008*). S. aureus* carriers with detectable levels in dental plaque and saliva may play an important role as sources of HAIs in dental healthcare settings. Indeed*, S. aureus* is detected in saliva of patients who harbor these microorganisms in their oro-pharynx (Millar *et al.,* 2001), while dental plaque colonization generally occurs in denture-wearing elderly (Theilade *et al.,* 1983), immune-deficient subjects (Scannapieco *et al*., 1992), patients with hyposalivation (Almståhl *et al.,*1999) or with aggressive periodontal disease (Fritschi *et al.,* 2008). Oral *S. aureus* carriers may,

2.2.5 *Candida albicans*

Candida albicans yeast is a common micro flora in the oral cavity. Some factors that could change the *C. albicans* into pathogenic microbe include optimum temperature (37°C), the presence of serum, and an adequate carbon source (Berman *et al*., 2002). The yeast plays an important role in oral health; thus, its presence needs serious attention because the resulting infection can lead to oral thrush (oral candidiasis). Infection can be more severe when *C. albicans* penetrates the deeper tissues, especially for immunocompromised patients. Superficial infection may change to invasive (Invasive Fungal Infections, IFI) (Cannon *et al.,* 2009).

2.2.6 Pathogenesis of *Candida albicans*

The ability of *C. albicans* to switch its morphology between yeast and hyphal forms contributes to its pathogenesis (Calderon, 2012). In the oral cavity, *Candida albicans* is a commensal fungal species commonly colonizing human mucosal surfaces. The *Candida* species facilitate colonization and proliferation in the oral mucosa and, possibly, in periodontal pockets. These fungal organisms can co aggregate with bacteria in dental biofilm, and adhere to epithelial cells. These interactions, which are associated with their capacity to invade gingival conjunctive tissue, may be important in microbial colonization that contributes to progression of oral diseases (Järvensivu, 2004). In addition to these properties, *Candida spp* also produce enzymes, such as the collagenases and proteinases that degrade extracellular matrix proteins, and immunoglobulins (Haynes *et al.,* 2004). In the oral cavity, *Candida albicans* provide adhesion sites for *streptococci*, the *streptococci* excrete lactate that can act as a carbon source for yeast growth, which in turn reduces oxygen tension to levels preferred by *streptococci* and provide growth stimulatory factors for the bacteria (Brogden *et al*., 2008). In fact, several studies have shown that *C. albicans* enhance the adherence of *S. mutans*, indicating a possible facilitation mechanism

during their association where the yeast cells could be used by the bacteria as support for adherence. More importantly, the yeast has potentiality to induce dental caries as a consequence of its pronounced ability to produce and tolerate acids.

therefore, be responsible for environmental contamination in dental healthcare settings during dental therapy (Kimmerle *et al.,* 2012), thus increasing the chance of cross-infection.

2.3 Toothpaste and mouthwash: In perspective

Both mouthwashes and toothpastes contain active and inactive ingredients which have their own importance and will be recommended according to their different oral conditions (John *et al*., 2005), different kind of ingredients are incorporated in toothpastes and mouthwashes to keep the oral health in a perfect condition.. Modern type of toothpaste contains abrasives which help to scour off bacterial films and fluorides to harden the teeth against caries (Davies, 2010), and have thickeners that stay on the toothbrush. The role of detergents is to remove the fatty films, and water softeners to make the detergents work better. The sweeteners play the role as a non-nutritive which may help stop the attraction of bacteria. A variety of mouthwashes are available in the market according to the different oral conditions like antibacterial mouthwash, whitening mouthwash, fluoride mouthwash and bad breath mouthwash which have a strong enough flavor to hide the bad tastes of decaying bits of previous meals. The first known reference to mouth rinsing is found in Chinese medicine, around 2700 BC. Different products were used for mouth rinsing over the centuries. In the 1500s, wine or beer were used, in the late 19th century, the use of essential oils was introduced among the dental care habits. Listerine, mouthwash composed of a mixture of essentials oils, and was invented in 1987 by Dr Joseph Lawrence and pharmacist Jordan Wheat Lambert from St Louis, Missouri. Each commercial brand of mouthwash has different ingredients. The active ingredients are usually alcohol,(John *et al* 2005) chlorhexidine

gluconate, (Rosenberg *et al* 1992) cetylpyridinium chloride hexetidine, benzoic acid (acts as a buffer), methyl salicylate, triclosan, benzalkonium chloride, methylparaben, hydrogen peroxide, domiphen bromide and sometimes fluoride, enzymes, and calcium, (Levy, 2003). They can also include essential oil constituents that have some antibacterial properties, (Stoeken *et al* 2007), like phenol, thymol, eugenol, eucalyptol or menthol. Ingredients also include water, sweeteners such as sorbitol, sucralose, sodium saccharin, and xylitol (which doubles as a bacterial inhibitor) (Giertsen *et al.,* 1999)

2.3.1 Types and Ingredients of Toothpaste and Mouth wash

Mouth wash and tooth paste contain both active and inactive ingredients (John *et al*). Active ingredients are those that offer a therapeutic benefit like sodium fluoride act as anti caries. While inactive ingredients are non therapeutic and also contribute to the physicochemical properties of the dentifrice like its feel, consistency, sweetness, flavor, pH, texture, abrasiveness and appearance (Hamilton, 1990).

2.3.1.1 Fluorides

Today almost all toothpastes contain fluoride (Scheifele *et al*., 2002), in one form or the other like sodium fluoride, sodium monoflourophosphate and stannous fluoride. Toothpastes contain 1.0 to 1.5 mg fluoride per gram and based on estimates of an average ingestion of 0.5g toothpaste per use. Fluoride-containing mouthwash could contribute 0.2 to 0.4mg fluoride per use. Fluoride exerts its caries protective properties in several ways. The primary cariostatic effect of fluoride is topical which inhibits demineralization and enhances remineralization of early carious lesions (Carr *et al*., 1985). There is also biochemical incorporation of fluoride ions directly into the chemical structure of tooth which results in substitution of hydroxyl ions with the fluoride ions leading to the formation of fluoropatite. This leads to reduction in acid solubility and buffering action of fluoride released from enamel crystals during the acid formation stage in the caries process. This new enamel structure is more resistant to bacterial acid

dissolution. Fluoride rinses are not recommended for use in children under 6 years old, since young children usually have inadequate control of their swallowing reflexes.

2.3.1.2 Chlorhexidine

Chlorhexidine is considered to be the gold standard for oral antiseptics. It maintains oral health through its ability to suppress overgrowth of Gram-positive and Gram-negative bacteria, as well as yeasts, (Jones, 2010). This is beneficial as it reduces dental plaque, which leads to a reduction in dental caries, as well as preventing gingivitis and periodontitis (Addy *et al.,* 1994). Chlorhexidine is a cationic biguanide that kills bacteria by membrane damage followed by intracellular coagulation. The use of lower doses of chlorhexidine inhibits oral bacteria, including those implicated in halitosis (Al-Tannir and Goodmann, 1994), the halitosis, if arises from the oral cavity is known as oral malodor**.** CHX is more effective against Gram-positive than against Gram negative bacteria due to differences in the structural characteristics of the outer membrane (Bascones and Manso,1994).The concept of "target" used to explain the mechanism of action of antibiotics may also be applicable to CHX, however, unlike the antibiotics, it does not break down the bacterial wall (Maris, 1995). CHX, like other cationic antimicrobials known for decades as "membrane-active agents" acts on the cell membrane to increase its permeability and facilitate the release of intracytoplasmic material (Hugo, 1992; Gilbert and Moore,2005).

2.3.1.3 Xylitol

According to the studies by Makinen and Scheinen, xylitol has been widely used as a caries inhibiting sweetener and it was officially approved by the authorities in Japan (1997). Recommended as a safe food additive and incorporated in different products like chewing gum and tablets as a sugar substitute (Soderling, 2009). The use of xylitol containing dentifrice resulted in significantly lower caries

incidence compared with the use of non xylitol containing dentifrice. Xylitol, like all sweeteners, promotes mineralization also by increasing the flow rate of saliva production, but it presents a very important feature, that is, xylitol is non- fermentable by oral bacteria (Sano *et al*., 2007).

2.3.1.4 Triclosan

Triclosan (2, 4, 4′-trichloro-2′-hydroxydiphenylether) non-ionic, low-toxicity, soluble, broad spectrum antimicrobial agent that has been used for the last 25 years in soaps, cosmetics and deodorants. A more recent application as a dentifrice and mouth-rinse is to inhibit bacterial colonization, resulting in moderate but distinct positive effects on both dental bio film and marginal inflammation or gingivitis (Nogueira-Filho *et al* 2002). There is evidence indicating that the ingredients in the formula of triclosan-containing mouthwashes, including vehicle and other active substances, may influence its antimicrobial activity, and consequently its clinical efficiency (Moran *et al.,* 2000). The antibacterial activity triclosan/copolymer toothpaste is at work in controlling gingivitis but the anti-inflammatory properties provide an additional benefit against inflammatory periodontal disease (Fine *et al*., 2006).

2.3.1.5 Sodium bicarbonate

This is sometimes combined with salt to make a simple homemade mouthwash, indicated for any of the reasons that a salt water mouthwash might be used. Pre-mixed mouthwashes of 1% sodium bicarbonate and 1.5% sodium chloride in aqueous solution are marketed.Sodium bicarbonate mouthwash is sometimes used to remove viscous saliva and to aid visualization of the oral tissues during examination of the mouth (Cawson *et al,* 2002). Hot salt water mouth baths (or hot salt water mouth washes, sometimes abbreviated to "HSWMW") are also routinely used after oral surgery, to keep food debris out of healing wounds and to prevent infection. Some oral surgeons consider salt water mouthwashes the main stay of wound cleanliness after surgery (Wray *et al,* 2003). The term mouth bath implies that the

liquid is passively held in the mouth rather than vigorously swilled around, which could dislodge a blood clot. Once the blood clot has stabilized, the mouth wash can be used more vigorously. These mouthwashes tend to be advised about 6 times per day, especially after meals to remove food from the socket. Salt water mouth wash is made by dissolving 0.5-1 teaspoon of table salt into a cup of water, which is as hot as possible without causing discomfort in the mouth. Saline has a mechanical cleansing action and an antiseptic action as it is a hypertonic solution in relation to bacteria, which undergo lysis. The heat of the solution produces a therapeutic increase in blood flow (hyperemia) to the surgical site, promoting healing (Matthews, 2003) Hot salt water mouthwashes also encourage the draining of pus from dental abscesses (Clive and Paul, 2006).. Gargling with salt water is said to reduce the symptoms of a sore throat.

2.4 Athlete and oral health

The high standards of performance required of athletes can only be achieved by an individual being healthy. The time, effort and money invested in realization of this maximum level of fitness should not be jeopardized by preventable oral health problems, which occur before or during competitions (Stoler *et al*, 1994). Deterioration in oral health of athletes is believed to be related to a high intake frequency of both sugar and acid-containing products. The products are often consumed during or directly after intense exercise, resulting in breathing hard through the mouth and reduced salivary secretion rate (Millesovic *et al*., 1999). pH on the tooth surface may reach even lower levels and the duration of a low pH is prolonged during such dry mouth conditions(Sobral *et al*., 2000). The risk for dental erosion is believed to be particularly high as acidic drinks are most often consumed during this physical condition.

2.4.1 Sport Drinks vis-a- vis Dental Erosion in Athletes

Many athletes, especially endurance athletes experience long periods of hypohydration during training and competition. It is recognized that this increases the risk of dental erosion, due to a decrease in salivary flow, resulting in inadequate rinsing and buffering of demineralizing acids on tooth surfaces (Woltgens *et al*., 1985). Given the importance of dental erosion to athletes, three published studies have investigated sports drink intake and dental erosion in these individuals (Matthew *et al*., 2002). The first study was conducted in 20 cyclists and 25 swimmers. A questionnaire was used to document sports drink usage and an oral examination was performed to determine the degree of dental erosion. Salivary flow rate in response to sports drink was also established in these subjects. Recently, two studies have published data on the relationship between sports drinks consumption and dental erosion (Millesovic *et al*., 1999). A study from the University of Ohio also used a self-administered questionnaire to gather information on sports drink usage in 304 university athlete. Similar to the findings of Milosevic *et al,* although the prevalence of erosion was high (36.5%), no significant associations were found in regard to the use of sports drinks, quantity and frequency of consumption and years of usage (Matthew *et al.,* 2002).Sports drinks, especially carbonated are often consumed by the population of athletes, mainly by professional athletes and amateur sports people, with the purpose of rehydration and electrolyte replacement during highly aerobic sports (Matthew *et al*., 2002). These drinks have detrimental effect on the teeth due to the low pH and the presence of citric acid in its composition, which can be potentially erosive for tooth tissue if consumed improperly and with high frequency (Sobral *et al*., 2000).When the pH reached 5.5, the hydroxyapatite crystals begin to dissolve, so below this value the enamel is at risk of decalcification (Millesovic, 1997). Another negative effect of the low pH of carbonated sports drinks is the potential of being harmful to the properties of the composition.

CHAPTER THREE

3.0 Materials and Methods

3.1 Study population

This comprises of athletes of both sexes and of different age groups, from teenage below 20 years to adults of above 21 years. Sports engaged include football, basketball, handball, and field tracking (runners).

3.2 Sample size

This was determined by using the formula of sample size prevalence studies i.e.

$N= z^2 pq /d^2$

Where

N= sample size

Z= statistic for level of confidence (1.96) at 5%.

P = current prevalence or proportion

q =1-p

d = precision = 0.05 at 5%

From the previous prevalence of Needleman *et al, (2013*) of athlete participating in the London 2012 Olympic.

n=?

P=50%

z= 1.96

q = 1- P

d= 0.05(at 5 %)

Therefore

$N= (1.92)^2 (0.5) (0.5) / 0.05$

= 192

3.3 Sampling sites

The study was carried out at some Stadia in Kano state, ie Kano pillars stadium at Sabon Gari Kano and Sani Abacha stadium at Kofar Mata kano. These are the major centres in the state were athletes of different background attend for exercise

3.4 Sampling and Method of sample collection

3.5 Sample collection

The study involved consented volunteers from the athletes. A sterile swab stick was aseptically used to collect samples from their mouth, teeth, gum and tongue, the swab stick was labelled and washed in normal saline and immediately taken to the laboratory for microbiolocal assessment (Collee,1996).

3.6 Administration of questionnaires

A standard cosructed questionnaires were given to athlete volunteers prior to commencement of sample collection (Osazua and Azodo 2013).

3.7 Inclusion criteria

- All eligible subjects must be athletes attending or participating in a particular sporting activity.
- Include subjects between teenage and above 21 years, with minimum of 20 teeth regardless of health condition of the teeth.

3.8 Exclusion criteria

- Non-athletic subjects.
- Underage children with milk teeth.
- Individuals with dental problem attending clinic.

3.9 Macroscopic analysis

Specimens were inoculated on blood, chocolate agar, Mannitol salt and Macconkey agar and on sabouroud dextrose agar, by streak plate method (Collee 1996). All plates were incubated at 37^{o}C for 24 hours aerobically, the chocolate agar was incubated anaerobically in a candle jar. The sabouroud dextrose agar was left at 25^{o}c (room temperature) for 48 hours. Emergent colonies were identified according to standard Microbiological methods (Collee, 1996). The colonial appearances of the bacterial isolates were examined on all the plates. The features examined include, the size, shape, pigmentation, consistency, elevation, and color as described by Cheesebrough,(2000).

3.10 Microscopic investigation

3.10.1 Gram staining

This was performed according to the procedure described by Cheesebrough (2000). A clean microscopic slide containing a drop of normal saline was smeared with a bacterial colony using a sterile wire loop and was allowed to air dry and then fix by passing three times over a flame. It was placed on a staining rack then flooded with crystal violet and allowed to stand for 60 seconds. The stain was then rinsed with water and flooded with lugol iodine for 60 seconds, the iodine was poured off and washed with water. Acetone was used briefly to decolorize and washed off thoroughly with water. The slide was then flooded with neutral red and allowed to stand for 2 minutes. The slides were then finally rinsed off with water, and allowed to air dry on a staining rack. The slides were examined under microscope; immersion oil was dropped at the centre of each smear and examined at x100 objective lens of light microscope. Morphological characteristics and Gram reaction patterns were examined and recorded.

3.10.2 Coagulase test

A drop of distilled water was placed on each end of a slide. A colony of the test organism was emulsified in each of the drops and made two thick suspensions, and then a loopful of plasma was added to one of the suspensions, and mix gently. Clumping of the organisms within 10 seconds was observed. No plasma was added to the second suspension. This was used to differentiate any granular appearance of the organism from true coagulase clumping (Cheesebrough, 2000).

3.10.3 Catalase Test

A clean slide was divided into two sections and level one as control and other as test, then a drop of normal saline was placed on each section, with sterile inoculating loop two colonies were picked and emulsified , then a drop of hydrogen peroxide was placed over the test smear but not on the control, it was then observed for immediate bubbling (Cheesebrough, 2000)

3.10.4 Optochin sensitivity test

This test was used to differentiate alpha hemolytic *streptococcus pneumoniae* and alpha hemolytic *streptococcus mutans*. The specimen was first streaked onto a blood agar plate and an optochin disc was placed on the edge of the primary inoculum and Incubated at 37^{o}C for 24hours in a candle jar and examine for zones of inhibition (Murray *et al*., 1999).

3.10.5 Germ tube test

This technique is for rapid identification of *candida albicans*. Half milliliter of serum was put into a clean tube; a colony of yeast was touched and gently emulsified in the serum. It was incubated at 37^{o}C for 2- 4 hours but not longer. A drop of the serum was transferred to a clean slide for examination. A cover slip was placed on the slide and examined using x40 objective.(Murray *et al*.,1999).

3.11 Preparation of Turbidity Standard (McFarland Standard)

Solution of sulphuric acid (1%) was prepared by adding 1milliliter of H_2SO_4 into 99milliliter of water. Also 1% w/v solution of Barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride in 49.5milliliter distilled water. This was followed by combining 0.6milliliter of the prepared barium chloride with 99.4milliliter of the prepared sulphuric acid solution. The turbid solution formed was used as standard for comparison with suspension of bacterial colonies and used as inocular for sensitivity test (Cheesebrough, 2000).

From colonies of bacterial isolate, enough material was transferred into a tube containing 2ml normal saline. It was mixed until the turbidity of the bacterial suspension matched with the turbidity of the 0.5% McFarland standard that is 1% Barium sulphate. Each bacterial suspension formed was used as the standard inoculum (Cheesebrough, 2000).

3.12 Evaluation of Dentifrices

Evaluation of dentifrice solutions was made by mixing the calculated amount of the toothpaste (2g) and mouth washes (2ml) in measured volume (2ml) of distilled water to give 1:1 dilution, they were further diluted in distilled water, and four different of 1:2, 1:4, 1:8, and 1:16 were made. In addition Full Strength (FS) concentration of both dentifrices were also tested. The anti microbial assay was conducted on Mueller Hinton agar, brain heart infusion agar plates and sabouroud agar plate (Manupati, 2011).

3.13 Anti microbial susceptibility Testing / Assay

The antimicrobial activity of different concentration of the dentifrice was determined by paper disk diffusion method (Manupati, 2011). In this method, sterile filter disk were impregnated with the various dilutions of the dentifrices prepared and allowed to dry in hot air oven at 60^{o}c for 2 hours. These were placed on the surface of inoculated agar plate and incubated at 37^{o}c for 48 hours; sterile filter disk was used as negative control. Diameters of the zones of inhibition were measured in millimeters (Sadegi and Assar, 2009).

3.14 Statistical Analysis

Data on pathogenic microorganisms and their susceptibility to toothpaste and mouthwash among athletes were determined by GLM Procedure, Least Squares Means, Adjustment for multiple comparisons: Tukey. Values were considered significant when $p < 0.05$. A T- test was also used.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage occurrences of Isolates obtained from dental pathogens among Athletes

Percentage occurrence of isolates from dental pathogens among Athletes in Kano metropolis is presented in Table 1. from the result, number of occurrence of *S. mutan*, *S. aureus*, and *C. albican* (Pre and post- exercise) were observed to be 61 (31.70%), 72 (37.50%), 17 (8.90%), 22 (11.50%) and 68 (35.40%), 79 (41.15%), 19 (17.70%), 24 (10.940%) respectively. Shows that there is no significant difference in terms of occurences before and after exercise.

4.2 Diameter zones of inhibition (mm) of anti-microbial activity of toothpaste formulation against the isolates

From the results of diameter of inhibition zones (mm) of anti-microbial activity of tooth paste formulation against the isolates, shown in Table 4.3, zones of inhibition for *S.aureus* (mean± S.D) at full strength for toothpaste formulations A,B,C, D, and E were observed to be 17,33±1.52, 16.00± 1.72, 16.66 ± 1.52, 13.33 ± 1.53, and 15.33 ± 0.57 respectively. Zones of inhibition for *S.mutans* (mean ± S.D) at full strength for formulation A,B,C, D and E was found to be 13.67± 1.16, 12.00±2.00, 11.67± 0.58, 9.87 ± 1.57 respectively. In case of *C.albicans* the inhibition zones were found to be 19.67± 0.57, 17.67 ± 0.58, 19.00± 1.00, 16.00± 1.00, and 16.67± 2.89, respectively.

4.3 Diameter zones of inhibition (mm) of anti-microbial activity of mouth washes formulation against the isolates

From the result of diameter of zones of inhibition (mm) of anti-microbial activity of Mouth wash formulation against the isolates depicted in Table 4.4 Formulations A, D and E were found to be

significantly effective especially at high concentrations against the isolates while B and C were found to be ineffective. Inhibition zones for *S.aureus* (mean ± S.D) at full strength for formulations A, D, and E were found to be 14.33 ± 0.57, 15.33± 1.58, and 11.33± 1.16 respectively. Inhibition zone for *S. mutan* were 15.83 ± 1.76, 16.67 ± 1.16, and 20.00 ± 4.00. In case of inhibition zones (mean ± S.D) for *C.albicans* , they were 11.67 ±1.16, 10.33 ± 0.53, for only formulations A and E while B, C, and D were found to be ineffective for *C.albicans*. *C.albicans* was observed to have least zones of inhibition compared to zones exhibited by other isolates.

4.4 Demographic factors associated with occurrence and percentage of isolates with age and gender obtained from respondents

From the result of Table 4.5 obtained, generally there is increase in the occurrence of isolates for pre and post- exercise periods, for pre-exercise those below 20 years were recorded to have *S.mutans* occurrence at 16 (8.33%) while above 21 years were 45 (23.43%), *S.aureus* at 17(8.85%) for below 20 years while above 21 years has 55(28.6%).For *C.albicans* also 7(3.6%) for ages of 21 and 13(6.8%) for those aged above 21 years.for post- exercise *S.mutans* has 18(9.4%) isolated from age group of less than 20 years and 50(20.1%), *S.aureus* for above 21 years, 20(10.4%) while 59(30.7%) were from age of 21 and above,*C.albicans* were recorded at 8(4.2%) for those below 20 years 13(6.8%) for those above 21 years respectively. There is no significant difference between pre and post-exercise periods. Male has the highest number of isolates than female both pre and post- exercise, for pre- exercise, *S.mutans* were 49 (25.0%) while 12 (5.7%) were recorded for female. *S.aureus* at 67(34.9%) for male, while only 5 (2.6%) were for female. In case of *C.albicans* 18(9.4%) were recorded for male and only 4(2.1%) for female. After exercise *S.mutans* were 59(30.7%) for male while 9(4.7%) were for female. For *S.aueus* male has the highest number of 75(34.1%) while only 4(2.1%). *C.albicans* has 19(9.9%) for male and only

(31.6%) were from female respectively. The result shows that there is significant difference between occurrence of isolates with regard to gender of athletes for the pre and post-exercise periods.

4.5 Some risk factors associated with Dental caries obtained from athletes at Kano Pillars and Sani Abacha Stadia.

Some risk factors associated with Dental caries obtained from athletes at Kano Pillars and Sani Abacha Stadia, shown in table 4.8, include the oral problem like hole in teeth which is about 32(11.9%) for athletes below 20 years and 36(18.8%) for 21 years and above, for bleeding gum about 70% of athlete for both age groups has the problem, where 35(15.2%) were having the problem, and 64(33.3%) was recorded for above 21 years. Very few has discolored teeth recorded at only 3(1.56%) for those aged below 20 years and 31(16.1%) for those above 21 years. There is poor response of dental visit among athletes with only 50(26.1%) visiting only once a year for those aged above 21 years while 95% from both ages never visited a dental clinic. 108(56.6%) for 21 years and above always consume soft drink before exercise, very few claimed to consume occationally but none were negative. About 90% agrees that their dental problem affect their performance while only few do not complain of the problem. For those that clean their mouth once daily has 97(75.2%) for above 21 years while 35(18.2%) were recorded for less than 20 years, 20 (10.4%) for less than 20 years clean twice and 32(16.7%). Half of the respondents smoke cigarette, while almost all the athletes of below 20 years does not indulge. 119(61.97%) above 21 years noticed low flow of saliva and 48(25%) for less than 20 years, but 10(5.20%) below 20 years and 15(7.81%) were not sure. Time spent during exercise was 1-2 hours for almost all the athletes only few were able to stay more than 2 hours.

Table 4.1: Percentage occurrence of Isolates obtained from dental pathogens among Athlete

Isolates	**Occurences of Organisms**			
	Pre- exercise		**Post-exercise**	
	Frequency	**Percentage(%)**	**Frequency**	**percentage(%)**
S.mutans	61	31.70	68	35.40
S.aureus	72	37.50	79	41.15
C.albicans	22	11.50	24	12.52
No growth	37	19.30	21	10.93
Total	192	100	192	

Table 4.2: Diameter zones of inhibition (mm) of anti-microbial activity of tooth paste against the isolates

	S.aureus					*S.mutans*					*C.albican*				
Dilutions	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
FS	17.33	16.00	16.66	13.33	15.33	13.67	12.00	9.67	11.67	0.00	19.67	17.67	19.00	16.00	16.67
	±1.52	±1.72	±1.52	.±1.53	.±0.57	.±1.16	±2.00	±1.57	±0.58	±0.00	±0.57	±0.58	±1.00	±1.00	±2.89
1: 1	16.33	14.66	16.33	11.00	14.33	12.33	10.67	7.33	9.00	0.00	16.67	15.67	17.00	12.00	11.67
	±1.52	±1.53	±0.58	±1.16	±0.58	±0.58	0.58	±0.58	±1.00	±0.00	±1.16	±0.58	±1.73	±1.73	±1.57
1:2	14.00	12.67	13.67	10.67	12.67	9.67	6.67	5.67	6.67	0.00	14.33	12.67	13.67	10.67	10.67
	±2.00	±1.16	±1.16	±2.00	±0.58	±0.58	±0.58	±1.16	±0.58	±0.00	±1.16	±0.58	±1.53	±1.16	±1.16
1:4	10.67	10.00	9.67	9.33	11.67	7.67	0.00	0.00	5.33	0.00	13.33	9.33	10.00	6.67	7.67
	±1.52	±0.0	±0.58	±1.50	±1.16	±0.58	±0.00	±0.00	±0.58	±0.00	±0.58	0.58±	±1.00	±1.00	±1.16
1:8	7.33	6.00	6.67	0.00	9.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	±0.57	±0.46	±0.58	±0.00	±1.16	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
1:16	0.00	0.00	0.00	0.00	7.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	±0.00	±0.00	±0.00	±0.00	±0.83	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
NC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	±s0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00

KEY: FS: Full strength NC: Negative control (Sterile filter paper disc).

Values for zones of inhibition and ± S.D : Standard deviation.

Tooth paste formulation

A = Pepsodent B = Dabur Herbal C = Oral- B D = Macleans E = Close Up

Table 4.3: Diameters zones of inhibition (mm) of anti-microbial activity of Mouthwash formulation against the isolates

	S.aureus					*S..mutans*					*C..albicans*				
Formulation	A	B	C	D	E	A	B	C	D	E		B	C	D	E
FS	14.33	0.00	0.00	15.33	11.33	15.83	0.00	0.00	16.67	20.00	11.67	0.00	0.00	0.00	10.33
	±0.57	±0.00	±0.00	± 1..58	±1.16	±1.76	±0.00	±0.00	±1.16	±4.00	±1.16	±0.00	±0.00	±0.00	±1.16
1:1	11.67	0.00	0.00	12.67	10.33	13.00	0.00	0.00	14.33	19.00	10.33	0.00	0.00	0.00	8.00
	±1.16	±0.00	±0.00	±0.58	±1.16	±1.73	±0.00	±0.00	±0.58	±3.61	±0.53	±0.00	±0.00	±0.00	±1.00
1:2	10.00	0.00	0.00	10.00	9.33	10.67	0.00	0.00	12.00	18.67	8.67	0.00	0.00	0.00	7.33
	±2.00	±0.00	±0.00	±2.00	±1.16	±1.16	±0.00	±0.00	±0 .57	±2.31	±1.53	±0.00	±0.00	±0.00	±0.58
1:4	8.66	0.00	0.00	9.33	7.33	9.67	0.00	0.00	9.00	15.33	6.00	0.00	0.00	0.00	7.00
	±0.58	±0.00	±0.00	±0.00	±0.58	±1.16	±0.00	±0.00	±1.16	±3.10	±1.00	±0.00	±0.00	±0.00	±0.00
1:8	8.00	0.00	0.00	8.67	6.67	8.50	0.00	0.00	6.67	11.67	5.67	0.00	0.00	0.00	0.00
	±1.00	±0.00	±0.00	±1.16	±1.16	±1.50	±0.00	±0.00	±1.16	±1.16	±0.00	±0.00	±0.00	±0.00	±0.00
1:16	6.67	0.00	0.00	0.00	5.67	6.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	±1.16	±0.00	±0.00	±0.00	±0.50	±1.63	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
NC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00

KEY: F S: Full strength N C: Negative control.(Sterile filter paper disc).

Values are: mean zone of inhibition ± S.D : Standard deviation.

Mouth washFormulations

A= Colgate Plax B =Listerine original C =Brett Gold D =AM PM E = Hexedene

Table 4.4 Demographic factors associated with occurrence and percentage of isolates with age and gender obtained from respondents

Isolates	**Occurrence**	**of isolates with**	**Age**		**Occurrence**	**of isolates**	**with**	**gender**
	Pre-exercise		**Post-exercise**		**Pre-exercise**		**Post-exercise**	
	Below 20 years	**Above 21 years**	**Below 20 years**	**Above 21 years**	**Male**	**Female**	**Male**	**Female**
S.mutans	16 (8.33%)	45(23.4%)	18(9.4%)	50(20.1%0	49(25.5%)	12(6.25%)	59(30.7%)	9(4.7%)
S.aureus	17(8.85%)	55(28.6%)	20(10.4%)	59(30.7%)	67(34.9 %)	5(2.6%)	75(34.1%)	4(2.1%)
C.albicans	7(3.7%)	15(7.81%)	9(4.7 %)	15(7.8%)	18(9.4 %)	4(2.1%)	19(9.9%)	5(2.6 %)

Table 4.5: Some risk factors associated with Dental caries obtained from athletes at Kano Pillars and Sani Abacha Stadia.

Information	Athletes below 20 years No.	%	Athletes above 21 years No.	%
Oral problem				
Hole in teeth	23	11.9	36	18.8
Bleeding gum	35	18.2	64	33.3
Discolored teeth	3	1.56	31	16.1
Dental visit of respondents				
Once a year	0	0	50	26.1
Never	39	20.3	103	53.6
Soft drink consumption				
Always	51	26.6	119	61.9
Occasionally	0	0	13	6.77
Rarely	-	-	-	-
Never	-	-	-	-
Does your dental problem affect your performance				
Yes	47	24.4	125	65.10
No	11	5.72	9	4.68
How often did you clean your teeth				
Once daily	35	18.2	97	75.2
Twice daily	20	10.4	32	16.7
Not regularly	3	1.56	5	2.7
Do you smoke or take alcoholic drink				
Yes	7	3.64	86	44.8
No	51	26.6	54	28.12
Have you notice low flow of saliva during exercise				
Yes	43	22.4	124	64.6
No	10	5.20	15	7.81
Period of exercise				
1-2 hours	52	27.1	120	62.5
Above 2 hours	6	3.12	14	7.3

CHAPTER FIVE

5.0 DICUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Dental caries is one of the most common chronic infectious diseases in the world and it results from the interaction of specific bacteria with constituents of the diet within a biofilm termed "dental plaque" (Anusavice, 2005). Bacterial plaque accumulated on dental surfaces and composed of native oral flora is the primary etiologic agent of dental caries. Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases. The frequency of these oral diseases is continuously increasing with change in eating habit among people of different age group and increased consumption of sugar (Saini *et al.,* 2003), the neglect of oral hygiene is the key to the development of dental diseases.

From the results of the study, the percentage occurrences of microbial isolates for pre and post- exercise period, shows that there is slight increase in the population of organisms for post-exercise period with *S.aureus* having the highest occurrence (41.15%),(Table 4.1), however, there is no significant difference between pre and post-exercise (89.6% and 98.9%) when P = 0.05 (Appendix 4). This work corresponded with findings of Oluremi *et al*., (2010), who evaluated the anticaries activity of selected Mouth washes, at Oyo State, Ibadan, Nigeria. Where *S.aureus* isolated has the highest occurrences of 30% than *S. mutans,* with 20%, although *S.aureus* was not considered as the initiator of the disease but also among opportunistic oral microflora.

Considering the percentage of occurrences of isolates with regard to the age group of the athletes, from the data obtained there was no significant difference (p = 0.05) during pre and post-exercise period (89.6% and 98.9%), and a greater number of all the isolates were from athletes of above 21 years. This

could be attributed to the fact that athletes of above 21 years had the highest enrolment than those below 20 years, occurrence of all the isolates were observed to increase with age of the athletes showing age group of 21 years and above were at high risk of dental caries disease, this data was in line with report findings of Hand *et al*., (1988) in his 36 month work in Iowa U.S.A. on caries prevalence and incidence, he reported that incidence of caries increases proportionally with age. And also according to the data obtained from the third National Health and nutrition Examination survey of 1994-2004 reported by Dye *et al*., 2007 that the prevalence of root caries among older adults was high with 30% and 10.4% among young adults.

It could be seen from the result that for *S.mutans,* about two third of the total number isolated both for pre and post exercise period were from 21 years and above, at 45 (23.4%) and 50 (26.0%), only one third was obtained in athletes of less than 20 years, *S.mutans* as reported by Zambon and Kasprzak (1995) are bacterial organisms implicated in root caries among others. This could also be attributed to the fact that athletes are believed to oftenly consume or take soft drinks before exercise for rehydration and replacement of electrolyte due to hypohydration, as earlier reported by Matthew *et al* (2002), and it can place athletes at risk of oral disease like dental caries and dental erosion due to their cariogenic nature and erosive potential respectively, dietary practices involving frequent intake of acidic food and beverages weaken tooth integrity (Parry *et al.,* 2001).

However, from the data, there was significant relationship between frequency of soft drink consumption and occurrence of isolates. And response obtained from the data, shows that the percentage of nearly all the athletes of both age groups consume soft drinks, at 23.9% and 61.9%. Those consuming occasionally were only 13 (6.8%) for those above 21 years, this can be shown to contribute tremendously to increased number of isolates, because the oral micro-flora utilizes fermentable sugar for the growth and metabolism, especially *S.mutans* even after two hours of consumption (Lingstrom *et al.,*

2000). This can results in pronounce fall in pH, which favors the growth of aciduric bacteria . This corresponds with report findings of Lun *et al*., (2012) on dietary supplementation practices in Canadian high performance athletes. Hypo salivation can also be a contributory factor to the increased number of isolates found in older athletes, because hypo salivation can impair with protective properties of saliva (Mulic *et al.,* 2012), since saliva contains neutralizing enzymes which help in buffering the acid produced by the fermentation of sugar. From the report obtained, almost all the athletes were shown to have experienced hyposalivation with about 22.4% and 64.6% for athletes of both age groups as shown in Table 4.6, but only 10 (5.2%) out of those aged below 20, and 18 (7.81%) above 21 years were negative. This agrees with the findings of Mulic *et al*., (2012) in a report on dental erosion wear and salivary flow rate in physically active young adults in U.K.

C. albicans is the most common yeast isolated from the oral cavity. It is by far the fungal species most commonly isolated from infected root canals, showing resistance to inter-canal medication (Oztan, *et al*., 2006). *C.albicans,* was also observed to have slight increase for post- exercise period, although it was shown to have low percentage of isolation among other isolates, for pre-exercise periods at 7 (3.65%) from the age below 20 years while 11 (5.70 %) were found in age of 21 years and above, for the post-exercise period, 8(4.2%) isolates were from age group below 20 years, while 15 (7.81%) were found between the age of 21 years and above.

The time spent during exercise can also be related to the percentage increase of isolates obtained, almost all athletes aged 21 years and above and those less than 20 years spends 1-2 hours on exercise. It was believed that microorganisms can continue to grow and multiply even after 2 hours of carbohydrate consumption (Papas *et al*., 1995), and the data, shows a significant relation between number of bacterial isolates and the time spent on exercise, and this can be considered a determinant risk factor and a reason why the isolates can be found in athletes of both age groups. Increased in percentage of isolates could

also be as a result of the micro-organisms embedded in the dental plaque due to poor oral hygiene practice, as shown from the data, frequency of tooth cleaning was poor because10.4% of athletes less than 20 years and 16.7% from 21years and above, clean their teeth twice daily, this could be the reason why 20 samples showed no growth at all prior to exercise and only 4 after exercise. Over 75% of the athletes aged above 21years claim to have clean their teeth once daily recorded at 97 (75.2%), for those less than 20 years 35 (18.2%) clean their teeth only once daily and 3 (1.6%) of less than 20, and above 21, only 5 (2.7%) were not regularly cleaning their teeth which is seen as a contribution to the increased number of isolates in those aged above 21 years, where this risk factor can also contribute to the increased population of isolates in age group of above 21 years, as in the case of *S.aureus*, from the number isolated 55 (28.6%) were from the age group of 21 and above, during pre-exercise period. For post-exercise period, 20 (11.9%) isolates belong to age group below 20 years while 59 (30.7%) were obtained from athletes of 21 years and above. In addition more than half of athletes of above 21 years, were found to indulge in cigarette smoking at 86 (44.8%).

The percentage of those involved in smoking was found to be in 21years and above while those not indulge were 51 (26.6%). This behavior can be seen as a risk factor that can lead to any of the dental problem, especially gum bleeding, and might cause the increase number of isolates more especially in those of older age who agrees on smoking of cigarette, this report of the data was in consensus with the findings of Ringelberg *et al*., (1996), in Florida U.S.A. on root caries and root defects in urban and rural adults, and that of Brown *et al.,* (1996) on dental caries restoration and tooth conditions in U.S adults. *Escherichia coli* and *Candida* are also associated with active caries lesions (Oztan, *et al*., 2006). this could be related to the fact that athletes were found to experience one or two of the supposed dental problems, since 20.3% aged below 20 years, and more than half of those above 21 years (53.6%) have

never visited a dental clinic, and only 26.04% from the response claimed to attend dental clinic only once a year, this may contribute to isolation of the pathogens in athletes.

In the study however, 24.4% of athletes below 20 years and 65.1% from 21 years and above were of the opinion that their dental problem affect their performance during competition but 5.72% for age below 20 years, and only 4.7% above 21 years do not experience, which signifies relationship of athletes to the percentage of dental problems observed from the study. The findings correspond with recent findings of Osazuwa and Azodo (2013) on dental conditions among competitive University athletes in Nigeria who reported that two third of the respondents (66.7%) admits that dental problems affect their performance. In terms of percentage occurrence in relation to gender of athletes, highest number which is about 90% of the isolated pathogens were from male athletes, generally showing that there is statistically highly significant difference between percentages of isolates from both male and female, when $p = 0.05$ (Appendix 6).

The highest number of isolates from male athletes could be due to the fact that the male outnumbered the female in both stadia, moreover Ringelberg *et al*., (1996) had demonstrated that male athletes are more prone to root caries and root defects in a study conducted on root caries and root defects in urban and rural adults in Florida, U.S.A. and also Chi *et al*., (2013) stated that other factors such as financial hardship and insurance status may modify the effect of gender on root caries risk, although Joshi *et al.,* (1994) indicated no significant relationship.

Among the investigated mouth washes, there was high significant difference ($p< 0.05$) on the test organism in relation to their sensitivity to different formulations, From the result of zones of inhibition exhibited by the oral formulations, formulation A shows effectiveness to all the isolates even at higher dilution of 1:16 is notable , and showed no significant difference between full-strength and dilutions of

1:1, 1:2 and also between lower dilutions of 1:4 and 1:8. This might be apparently due to the presence of sodium fluoride as active ingredients, and this result agrees with findings of Grant *et al*., (1979), who stated that fluorides are abundant in use in many oral health care products including mouth wash and tooth paste as they help in caries prevention, when formulated correctly and used as directed, fluorinated formulations will help to safely and effectively prevent decay. And also with the previous study by Jenkins *et al,* (1985), who stated that fluoride products such as both tooth paste and mouth rinses were shown to reduce caries from 30% to 70% compared with no fluoride therapy. The effectiveness of fluoride formulations are concentration dependant (Fererskov *et al.,* 2003).

Formulation D contains triclosan and sodium fluoride. Formulation D was found to show effective zones of inhibition against *S.aureus, and S. mutans*, but shows a maximal inhibition zones for only *E. coli* and significantly ineffective against *C. albicans*. This was in consensus with the work of Tiwari *et al* (2008) where in their study the formulations containing triclosan were found to be very effective against *S.mutans*, and also in line with the work of Manupati (2011), where formulation containing triclosan was found to be effective even at higher dilution of 1:16. This was also in line with the work of Okpalugo *et al* (2009) who reported that dentifrice containing two antimicrobial sodium fluoride and triclosan had a 20% more reduction in oral bacterial flora than non-triclosan containing dental formula.

Formulation E was found to have effective antimicrobial activity against *S. aureus* and shows no significant difference at lower concentrations of 1:4, 1:8 and 1:16. In this study formulation E was found to be more efficacious against *S.mutan* than all other isolates, and shows no statistical difference at full strength, 1:1, and 1:2 at ($p< 0.05$). This may be due to the presence of chlorhexidine as the major ingredient in the formulation. The work corresponded with recent research carried out by Aneja *et al*., (2010), where mouth wash containing chlorhexidine as ingredient shows maximum effectiveness against *S.aureus* followed by *S.mutans* and *C.albicans.* Chlorhexidine gluconate is a cationic biguanide with

broad spectrum anti microbial action, whose effectiveness in decreasing the formation of dental biofilm and gingivitis have been demonstrated in several clinical studies (Lorenz *et al*., 2006). This work was also in line with work of Gehlen *et al.,* (2000) whose study was oneffect of 0.2% chlohexidine mouth rinse on plaque re- growth. Mouth wash B and C, contain the same major constituents that is thymol (deodorant) and ethanol (antibacterial agent). They were potentially ineffective against all the four isolates tested. This is a similar finding with previous work of Oluremi *et al.,* 2010. Also with the findings of Gabraiel *et al*., (2014) in Ghaziabad India.

The antimicrobial agents in tested toothpastes include sodium lauryl sulfate (SLS), sodium fluoride (SF) and sodium monofluorophosphate (SMPF) for only formulation B which also contain herbal extract. Although SLS exist in the toothpastes as a detergent, it is also known to have antibacterial and plaque inhibitory activities (Moran *et al.,* 1988). Formulation A was effective on all the isolates with no significant difference between full strength and 1:1 dilution, and also between 1:1 and1:2 dilutions in all the isolates, with *C.albicans* showing maximal inhibition zones (19.67±0.58). Formulation B was found to be effective on all the four isolates more especially *C.albicans* (17.67±0.58) followed by *S.aureus* (16.00±1.72) and shows that there is no significant difference between 1:1 and 1:2 dilutions. Formulations C, D and E showed effectiveness against the isolates. The result of this study is in line with a 3 year trial in the UK which shows that dental decay was reduced by as much as one third by regular use of Colgate containing fluoride. Fluoridated tooth paste is associated on average with a 24% reduction in tooth decay as reported by Marinho *et al.,* (2011), *S.mutans* isolates were found to resist active ingredients in formulation E. The data of inhibition zones exhibited by the test organisms against the evaluated formulations, generally shows that there is highly significant difference $p< 0.05$ (Appendix 7) when comparing the efficacy of the antimicrobials against the isolates, this is because the different

formulations have different potentiality against the isolates. Moreover, for the comparison of the organisms with the different dilutions, shows that there is highly significant difference when $p< 0.05$.

5.2 Conclusion

From result of this study it can be concluded that *S. aureus* had the highest incidence among the other isolates from the buccal cavity of athletes in Kano, and that there was slight increase in number of isolates for the post- exercise period. Male athletes had the highest number of enrolment than their female counterparts, and the isolates were 70% sensitive to all mouth wash and toothpastes evaluated in the study.

5.3 Recommendations

The following recommendations were made from the finding of this work, these include:

1. Athletes are advised to visit dental clinic regularly, for medical check-up at least twice a year, in order to find early signs of both dental caries and dental erosion.
2. Athletes are advised to appropriately brush, rinse and floss your teeth at least twice a day and always within an hour of starting and completing an exercise. Brushing and flossing before exercise helps reduce the bacterial numbers already in the mouth, and doing so again afterwards helps to remove the bacteria that may have fed and reproduced during workout.
3. When not in training, eliminate as many forms of refined sugar, simple carbohydrates and acid from the diet in order to help reduce risk of decay.

REFERENCES

Addy, M.(1990). Chemical plaque control. In: *Kieser B J, editor. Periodontics*: a practical approach. London: Wright Publishing Company; pp. 527–34.

Almaståhl, A. and Wikström M. (1999). Oral microflora in subjects with reduced salivary secretion. *J. Dent. Res.* 78, 1410-1416.

Al-Tannir, M.A., and Goodman, H.S. (1994). A review of clorhexidine and its use in special populations. *Special Care in Dentistry*. 14:116-122.

Amaliya, K., Timmerman, M.F., Abbas, F., Loos, B.G., Vander Weijden, G.A., and Van Winkelhoff, A.J. (2007) Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. *J Clin Periodontol*. 34(4):299-304.

Aneja, K.R., Joshi, R., and Sharma, C. (2010).The antimicrobial potential of ten often used mouthwashes against four dental caries pathogens. Jundishapur *J Microbiol*; 3(1): 15-27.

Anusavice, K.J. (2005). Present and future approaches for the control of caries. *J Dent Educ*. 69:538–554.

Barbieri, D., Vicente, V.A., Fraiz, F.C., Lavoranti, O.J. and Svidzinski, T.I.E. (2007). Analysis of the in vitro adherence of Streprococcus mutans *and* Candida albicans. *Braz J Microbiol* 38: 624–663

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disc Method. *The American Journal of Clinical Pathology,* 45:4:493.

Berman, J. and Sudbery, P.E. (2002). Candida albicans: A molecular revolution built on lession from budding yeast. *Nature Review Genetic*. 3: 918-930.

Bascones, A. and Manso, F.J. (1994). Clorhexidina en Odontoestomatología*: Conceptos actuales y revisión de la literatura. Avances en Odontoestomatología.* 10:685-708

Brambilla, E. (2001). Fluoride -is it capable of fighting old and new dental diseases. An overview of existing fluoride compounds and their clinical applications.*Caries Res, 35 Suppl 1*: p. 6-9.

Bryant, S., McLaughlin, K. and Morgaine, K. (2011). Elite athletes and oral health. *Int J Sports Med*; **32**: 720–724.

Cannon, R.D., Lamping, E., Holmes, A.R., Niimi, K., Baret, P.V., Keniya, M.V., Tanabe, K., Niimi, M., Goffeau, A. and Monk, B.C. (2009). Efflux- Mediated Antifungal Drug Resistance. Clinical Microbiology Review; 22: 291-321.

Cheesbrough M. (1984) Medical Laboratory Manual for Tropical Countries. Butterworth and Co. (Publishers) Ltd.; Vol. 2.

Carr, L.M., Craig, G.C.,Machennan, R.,.Martin, T.J., Stacey, N.H. and Woods, R.(1985). Report of working party on fluorides in the control of dental caries. *Aust Dent J*;30: 433.

Cheesbrough M. (2000). Laboratory Diagnostic for Tropical countries (part 2) Cambridge University press.

Chi, D.L. and Tucker-Seeley, R.(2013). Gender-stratified models to examine the relationship between financial hardship and self-reported oral health for older US men and women. *Am J Public Health*103:1507–1515.

Clarke, J.K. (1942). The bacterial factor in the etiology of dental caries. *British Journal of Expert Pathology;* 5: 141-7.

Collee, J.G., Fraser, A.G., Marmion, B.P.and Simmons, E. (1996). A Mackie and McCartney Practical Medical Microbiology. 14th edition. Singapore: Longman Singapore Publishers (Pre) Limited.

Collins, W.J, and Walsh T.F. (1998). Handbook for dental hygienists. pp 272-273.

Dancer, S..J. (2008). Importance of the environment in methicillin-resistant Staphylococcus aureus acquisition: the case for hospital cleaning. Lancet Infect. Dis. 8, 101-113.

Davies, R. Scully, C. Preston, A.J. (2010). Dentifrices-an update. *Med Oral Patol Oral Cir Bucal*;15:976-982

Eberhard, J., Jepsen, S., Jervøe-Storm, P.M., Needleman, I. and Worthington, H.V. (2008). Full-mouth disinfection for the treatment of adult chronic periodontitis. *Cochrane Database Syst Rev*. (1):CD00462

Eberhard, J., Jervøe-Storm, P.M., Needleman, I., Worthington, H. and Jepsen, S. (2008). Full-mouth treatment concepts for chronic periodontitis: a systematic review. *J Clin Periodontol*. 35(7):591-604.

Fejerskov, O, and Kidd E, (2003). Dental Caries; the Disease and its Clinical Management. Oxford: Blackwell Munksgaard; **179-188.**

Fine, D.H. (1988). Mouthrinses as adjuncts for plaque and gingivitis management. A status report for the American Journal of Dentistry. *American Journal of Dentistry* **1:259-263**.

Fine, D.H., Furgang, D. and Bonta, Y. (1998). Efficacy of a triclosan/NaF dentifrice in the control of plaque and gingivitis and concurrent oral microflora monitoring. *American Journal of Dentistry*; **11:259 70**.

Fritschi, B.Z., Albert-kiszely, A, and Persson, G.R. (2008). *Staphylococcus aureus* and other bacteria in untreated periodontitis. *J. Dent. Res*. **87, 589-593.**

Gamboa, F., Estupinan, M. and Galindo, A. (2011). Universitas Scientiarum 2004; 9(2):23-7.

Gehlen, I., Netuschil, L., Berg, R., Reich, E. and Katsaro C. (2000) The influence of a 0.2% chlohexidine mouth rinse on plaque regrowth in orthodontic patient. A randomised progressive study. Part I: Clinical parameters. *J.Orofac orthop*; 61 : 54-62.

Gilbert, P. and Moore, L.E. (2005). Cationic antiseptics: diversity of action under a common epithet. *Journal of Applied Microbiology*. **99:703–715**.

Gordon, J.M., Lamster, I.B. and Seiger, M.C. (1985). Efficacy of Listerine antiseptic in reduction of existing plaque and gingivitis. *Clinical Preventive Dentistry* **5:12-16**.

Grant, D.A., Stern, I.B., Everett, F.G.(1979). Periodontics in the Tradition of Orban and Gottlieb. 5th ed. Louis: Mosby;

Hamada, Y., Igarashi, K., Nishimaki, H., Sakurai, S.and Kamiyama, K. (1987). The acidogenic potential of plaque from sound enamel, white spot lesions, and cavities in children. *Pediatr Dent*; **9: 212-215**

Hamilton, I. R. and Bowden G. H.. (1982). Response of freshly isolated strains of Streptococcus mutans and Streptococcus mitior to change in pH in the presence and absence of fluoride during growth in continuous culture. *Infect. Irnmun*. **36:255-262**.

Hand, J.S., Hunt, R.J. and Beck, J.D.(1988). Coronal and root caries in older Iowans: 36-month incidence. *Gerodontics*;**4:136–139**.

Hawkins, R., Locker, D.,Noble, J. and Kay, E.J. (2003). Professionally applied topical fluorides for caries prevention. *British Dental Journal*;**6:313-7.3**

Heitz-Mayfield, L.J. and Jang, N.P. (2010). Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. *Periodontol. 2000*. **53,** 167- 181.

Hefti, A.F. and Huber, B. (1987). The effect on early plaque formation, gingivitis and salivary bacterial counts of mouthwashes containing hexetidine/zinc, aminefluoride/tin or chlorhexidine. *J Clin Periodontol*.;**14(9):**515–8.

Holloway, P.J. and Moore, W.J. (1983). "The role of sugar in the etiology of dental caries". *J Dent* **11** (3): 189–213.

Holt, J.G., Williams, S.T. and Sharpe, M.E. Bergey's manual of systematic bacteriology, vol.2 4th ed. Baltimore, Md: Williams and Williams.

Hugo, W.B. (1992). Disinfection mechanisms. In: Russell AD, Hugo WB, Ayliffe GAJ, eds. Principles and Practice of Disinfection, Preservation and Sterilization. Oxford: Blackwell; 1992:187-210.

Howard, K. (2003). "Virulence factors of Mutans Streptococci: Role of Molecular genetics." Critical Reviews in Oral Biology and Medicine. "Dental Cavities." *The New York Times.*

Isalm, B. K.S. and Khan A.U. (2007) .Dental caries: from infection to prevention. *Med Sci Monit* 13: RA196–203

Järvensivu, A., Hietanen, J., Rautemaa, R., Sorsa, T. and Richardson, M. (2004). Candida yeasts in chronic periodontitis tissues and subgingival microbial biofilms in vivo. *Oral Dis* 10, 106-112.

Jensena, J.L. and Barkvoll, P. (1998). Clinical Implications of the Dry Mouth: Oral Mucosal Diseases. Annals of the New York Academy of Sci.; 842:1, 156–162.

Jones, R.D., Jampani, H.B., Newman, J.L, and Lee A.S. (2000) Triclosan: a review of effectiveness and safety in health care settings. *Am J Infect Control*.;28(2):184–96.

John, T., Darrell, K., Kathleen, V. and Cleef, T. (2005).Chemical composition of everyday products. Green wood publishing group, Inc. USA 48.

Kimmerle, H., Wiedmann, A.L., Ahmad, M., Pelz. K., Wittmer, A., Hellwig, E. and Al-Ahmad, A. (2012). Airborne microbes in different dental environments in comparison to a public area. Arch. *Oral Biol*. 57, 689-696

Kolenbrander, PE. (2000). Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol; 54: 413-437.

Kouidhi, B., Zmantar, T., Hentati, H. and Bakhrouf, A. (2010). Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in Staphylococcus aureus associated to dental caries. *Microb. Pathog*. 49, 14-22..

Lamster, I.B., Alfano, M.C., Seiger, M.C. and Gordon, J.M. (1983). The effect of Listerine on the reduction of existing plaque and gingivitis. *Clinical Preventive Dentistry5*:12-16.

Lamster, I.B., DePaola, D.P., Oppermann, R.V., Papapanou, P.N. and Wilder R.S. (2008). The relationship of periodontal disease to diseases and disorders at distant sites: communication to health care professionals and patients. *J Am Dent Assoc* 139(10):1389-97.

Laudenbach, J.M.and Simon, Z. (November 2014). "Common Dental and Periodontal Diseases: Evaluation and Management.". The Medical clinics of North America **98** (6): 1239–1260.

Li, Y. P. and Caufield .W. (2005). "Mode of Delivery and Other Maternal Factors Influence the Acquisition of *Streptococcus mutans* in Infants." *Journal of Dental Research* 84(9): 806-811.

Lemos, J.A., Quivey, R.G., Koo, H. and Abranches, J. (2013). *Streptococcus mutans*: a new Gram-positive paradigm? Microbiology 159: 436–45 [PMC free article] [PubMed

Levy, S.B., Darout, I.A.and skaug, N. (2001). chewing sticks: timeless natural tooth brushes for oral cleansing. *j periodontal res* 36:275-84 [

Locker, D. (1988). Measuring oral health: a conceptual framework. *Community Dent Health* **5**: 5–13.

Loesche, W.J. (1996). Microbiology of dental decay and periodontal disease. In: Baron's Medical Microbiology. 4th Ed. University of Texas Medical Branch.

Loos, B. G. and Tjoa, S. (2005). Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? *Periodontol* **39**: 53–72.

Lorenz, K., Bruhn, G., Heumann, C., Netuschil, L., Brecx, M. and Hoffmann, T. (2006). Effect of two new chlorhexidine mouthrinses on the development of dental plaque, gingivitis and discoloration. A randomized, investigator-blind, placebo-controlled, 3 week experimental gingivitis study. *J Clin Periodontol;* 33: 561-7.

Lun, V., Erdman, K. A. and Fung T. S. (2012). Dietary supplementation practices in Canadian high-performance athletes. *Int J Sport Nutr Exerc Metab*; **22**: 31–37.

Mali, A.M., Behal, R. and Gilda, S.S. (2012). Comparative evaluation of 0.1% turmeric mouthwash with 0.2% chlorhexidine gluconate in prevention of plaque and gingivitis: A clinical and microbiological study. *J Indian Soc Periodontol*.;16(3):386-91.

Mandel, I.D. (1988). Chemotherapeutic agents for controlling Plaque and gingivitis. *J Clinical Periodontol*.;8:488−498.

Manupati, P. (2011). Antimicrobial Efficacy of Different Toothpastes and Mouthrinses: An In Vitro Study. *Dent Res J* (Isfahan).; 8(2):85-94.

Marinho, V.C. (2003). Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev, (1): p. CD002278

Maris, P. (1995). Modes of action of disinfectants. Revue Scentifique et Technique (International Office of Epizootics). 1995;14:47-55.

Marwin, R.M. (1949). Relative incidence of Candida Albicans on the skins of persons with and without disease *J.Invest.Derm*.12;229-241.

Mathew, T., Casamassimo, P.S. and Hayes, J.R. (2002). Relationship between sports drinks and dental erosion in 304 University Athletes in Columbus, Ohio, USA. *Caries Res.* 36:281-7

Millar, M.R,. Walsh, T.R., Linton, C.J., Zhang, S., Leeming, J.P., Bennett, P.M. and Alspac Study Team. (2001). Avon longitudinal study of pregnancy and childhood Carriage of antibiotic-resistant bacteria by healthy children. *J. Antimicrob. Chemother*. 47, 605-610.

Miller,W.D. (1993). The micro-organism of the human mouth. KG ed. Basel,Switzerland:skarger.

Milosevic, A. (1997). Sports drinks hazard to teeth. *BrJ Sports Med*.;31:28-30.

Moran, J., Addy, M., Newcombe, R.G. and Marlow, I. (2000). A study to assess the plaque inhibitory activity of a new triclosan mouthrinse formulation. *J Clin Periodontol*. 27(11):806.

Moore, L.,and Goodwin, A.C.(1991). Antimicrobial Susceptibility Testing Protocol, CRC Press.

Moynihan, P. J. and Kelly, S. A. (2014). Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines. *J Dent Res*; **93**: 8–18.

Mulic, A. Tveit. A. and Songe, D. (2012). Dental erosive wear and salivary flow rate in physically active young adults. *BMC Oral Health*; **12**: 8

Needleman, I., Ashley, P., Fine, P., Haddad, F., Loosemore, M., De medici, A., Donos, N., Newton, T., Van Somren, K., Moazzes, R., jaques, R., Hunter, G., khan, K., Shimma, M. and Brewer, J. (2014). Elite sport performance, *Br J sport med*; sep 28, pii: bjsports-2014-093804., doi: 10.1136/bjsports- 2014093804

Needleman, I., Ashley, P., Petrie, A., Fortune, F., Tuner, W., Jones, J., Niggli, J. and Engebrestsen, L. (2012*)*. Health and impact on performance of athletes participating in the London olympic games: a cross-sectional study. *Br j sports med* 2013; 47(16): 1054

Newman, M.G. (2007). Classification and epidemiology of periodontal diseases. In: Newman, M.G., Takei, H., Carranza, F.A. editors. Carranza's Clinical Periodontology. 10th ed. Philadelphia: W.B Saunders Company; pp. 100–29.

Nguyen, D.H. and Martin, J.T. (2008). Common dental infections in the primary care setting. *Am Fam Physician*. Mar 15;77(6):797-802.

Nogueira-Filho, G.R., Duarte, P.M., Toledo, S., Tabchoury, C.P.and Cury, J.A. (2002). Effect of triclosan dentifrices on mouth volatile sulphur compounds and dental plaque trypsin-like activity during experimental gingivitis development. *J Clin Periodontol*.;29(12):1059–64.

Often, M.P., Busscher, H.J. and Van der Mei, H.C. (2010). Retention of antimicrobial activity in plaque and saliva following mouthrinse use in vivo. Caries Res.; 44:459-464.

Okpalugo, J., Ibrahim, K. and Inyang, U.S. (2009).Toothpaste formulation efficacy in reducing oral flora. *J. Pharm Res*.; 8(1):71-77.

Oluremi, B. B., Osungunna, M. O., Idowu, O. A. and Adebolu O.O. (2010). Evaluation of Anticaries Activity of Selected Mouthwash Marketed in Nigeria *Tropical Journal of Pharmaceutical Research,* Vol. 9, No. 6. pp. 581-586.

Osazua, O. and Azodo C.C. (2013). Dental conditions among competitive University athletes in Nigeria. *Tropical dental journal.*

Oztan, M.D., Kiyan, M. and Gerceker, D. (2006). Microbiology of dental decay and periodontal disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 102(3): 410-35.

Parry, J., Shaw, L., Amoud, M.J. and Smith A. J. (2001). investigation of mineral water & soft drinks in relation to dental erosion. *J oral rehabil*;28:766-72 medline.

Passariello, C., Puttini, M., Jebba, V., Pera, P. and Gigola, P. (2012). Influence of oral conditions on colonization by highly toxigenic Staphylococcus aureus strains. *Oral Dis.* 18, 402-409.

Papas, A.S., Joshi, A., Palmer, C.A., Giunta, J.L.and Dwyer, J.T. (1995). Relationship of diet to root caries. *Am J Clin Nutr*. 1995; 61:423S–429S.

Petti, S. and Polimeni, A. (2011). Risk of methicillin-resistant Staphylococcus aureus transmission in the dental healthcare setting: a narrative review. Infect. Control Hosp. *Epidemiol. 32*, 1109-1115.

Petersen, P.E., Bourgeois, D., Ogawa, H., Estupinan, S. and Ndiaye, C. (2005). The global burden of oral diseases and risks to oral health. *Bull World Health Organ*.;83(9):661–9.

Petti, S., Polimeni, A. and Dancer, S.J. (2013). Effect of disposable barriers, disinfection, and cleaning on controlling methicillin-resistant Staphylococcus aureus environmental contamination. *Am. J. Infect. Control*. 41, 836-840.

Pihlstrom, B. (2001) Periodontal risk assessment, diagnosis and treatment planning. J Periodontol.;25:37–58.

Poeschl, P.W., Crepaz, V., Russmueller, G., Seemann, R., Hirschl, A.M. and Ewers, R. (2011). Endodontic pathogens causing deep neck space infections: clinical impact of different sampling techniques and antibiotic susceptibility. *J. Endod*. 37, 1201- 1205.

Priya, S., Uma, E. and Reddy, M.K.R. (2012). Effect of different types of tea on *Streptococcus mutants*: An in vitro study. Indian *J Dent Res*.;23(1):43-48.

Ringelberg, M.L., Gilbert, G.H. and Antonson, D.E. (1996). Root caries and root defects in urban and rural adults: the Florida Dental Care Study. *J Am Dent Assoc*.;127:885–891.

Robin, A., Seymour, D., Peter, A. and Heasman, A. (1992). Overview Drugs, Disease and Periodontium, *Open Mycol J7*: 27–32.

Rouabhia, M. and Chmielewski, W. (2012). Diseases associated with oral polymicrobial biofilms. *Open Mycol J* 6: 27–32.

Sadeghi, M. and Assar, S. (2009). An in Vitro Antimicrobial Activity of Ten Iranian Made Toothpastes. *Dent Res J* (Isfahan). Autumn; 6(2): 87–92

Saini, S. Gupta, N. Mahajan ,A. Arora, D.R.(2003) Indian J Med Microbiol;21:111-114.

Sano, H., Nakashima, S., Songpaisan, Y. and Phantumvanit, P. (2007) Effect of xylitol and fluoride containing toothpaste on the remineralization of human enamel in vitro. Journal of *Oral Science*, Vol 49, No. 1, 67-73.

Soderling, E.M. (2009). Xylitol, Mutans Streptococci, and Dental Plaque. Adv Dent Res 21:74-78 .

Scannapieco, F.A., Stewart, E.M. and Mylotte, J.M. (1992). Colonization of dental plaque by respiratory pathogens in medical intensive care patients. Crit. Care Med. 20, 740-745..

Schentzel, P. (1996). etiology of dental erosion-intrinsic factors *eur J.oral sci*,104:178-90.

Scheifele, E., Studen, P. D. and Markovic, N. (2002) Practitioner's guide to fluoride. *Dent ClinNorthAm*.;46:831-846.

Silverstone, L.M. (1983). "Remineralization and enamel caries: new concepts". Dent Update **10** (4): 261–73.

Silk, H. (2014). "Diseases of the mouth.". Primary care ***41*** *(1): 75–90.*

Sobral, M.P., Luz, M.C., Teixeira, A. and Garone, N. (2000). Influência da dieta líquida ácida no desenvolvimento de erosão dental. *Pesqui Odontol Bras*.;14:406-10.

Studen-parlovich, D. and Elliot, M.A. (2001). Eating disorder in women's oral health. Dent *clim north Am*;45:491-5 medline.

Tiwari K.B., Shrestha,U.T., Acharya, A., Subedi, B. and Paudyal, B. (2008). Antibacterial activities locally usedtoothpastes against dental pathogens. JIOM; 30:2 15-218.

Whiley, R.A. and Beighton, D.(2013) "Streptococci and Oral Streptococci." Bite-Sized Tutorials.N.p..Web. 23 Jul.

Wolff, L. F. (1985). Chemotheraputic agents in the prevention and treatment of periodontal disease. Northwest Dentistry 64:15-24.

Woltgens, J.H., Vingerling, P., De Blieck-Hogervorst, J.M. and Bervoets, D.J. (1985). Enamel erosion and saliva. *Clin Prev Dent;* 7: 8-10.

World Health Organization. (WHO) (2008). Appropriate use of fluoride for human health. 1986; Geneva. *www.intechopen.com www.jiom.com.np Journal of Institute of Medicine,* August, 30:2

Zero, P.D., Martinez-Mier, E.A., Ferrera-Zandona, A., Ando, M. and Gonzalez, C. (2009). The biology, prevention, diagnosis and treatment of dental caries. *JADA* 140: 25S–34S

Zero, P.D (1996).Etiology of dental erosion-extensive factors .*eur j.oral sci*,104:162-77.

APPENDIX 1

Ingredients of Mouth wash formulations tested for anti Bacterial potential.

Name	Manufacturer	Ingredients as listed on packages
Colgate Plax (A)	Colgate Palmolive Guildford. Dublin Ireland	Aqua, Glycerin glycol.Sobitol, Polaxar 407, Cetyl pyridinium Chloride, Potassium Sorbate,Sodium Flouride,Sodium Saccharine,

		Cl42051, Cl19140.
Listerine Original (B)	Johnson & Johnson Limited, Maiden Head, Uk,SL63UG.	Aqua, Alcohol, Benzoic acid, Polaxamer 407,Eucalyptol, Methyl Salicylate, Thymol, Menthol, Sodium Benzoate, Caramel.
Brett Gold (C)	Pharma Deko Plc Agbara Industrial Estate, Agbara Ogun State.	Thymol B.P 0.063%, Ethanol 25.40%.
AM PM (D)	Elder Pharmaceutical Ltd. D.220,TTC Industrial Land Navi Mumbai India-400706	Triclosan USP, Sodium, Flouride BP, Sodium Benzoate B.P, Aqeous base, Brilliant blue FCF and Tartrazine.
Hexedene (E)	Pharma Deko Plc Agbara Industrial Estate, Agbara Ogun state	Hexedine 100mg, Excipients, water 100ml

APPENDIX 2

Ingredients of Toothpaste Formulations tested for Antimicrobial Potential.

Name	**Manufacturer**	**Ingredients as listed on package**
Pepsodent (A).	Unilever Nigeria Plc. Billingsway, Oregun Lagos State Nigeria.	Sorbitol, Hydrated silica, Sodium Lauryl sulphate, PEG-30,Sodium Flouride, Sodium Sccharine, Calcium Gluconate, Cl 19140, Cl 4208.
Dabur Herbal (B).	African Consumer Care Ltd.	Basit oil, Herbal extract, Calcium

	Odofin Ind, Area, Festac town. Lagos Nig.	Carbonate, Sodium Lauryl sulphate, Peppermint, Spearmint, sodium silicate, Sodium mono flouridephosphate, Sodium pyrophosphate.
Oral-B (C).	Procter and Gamble Green Street Ltd. 6200 Bryan Park Road Brown Summit, North Carolina 27214, USA.	Sorbitol, Aqua,Hydrated silica, Sodium Lauryl sulphate, Trisodium phosphate, Sdium saccharine, SodiumFlouride, Carbomer, Polyethylene, Cl77891,Cl42090.
Macleans (D)	Glaxo Smithkline Consumer Nigeria Plc, Igbesa road ,Agbara, Ogun, Nigeria.	Sodium fluoride, Aqua, Hydrated silica,Sorbitol, Glycerin, PEG-6, Sodium Lauryl sulphate, Flavour, Xanthan gum, Sodium saccharine, Cl 73360,Cl 174160.
Close Up (E).	Unilever Nigeria Plc. Billingsway, Oregun, Lagos Nigeria.	Aqua, Sodium lauryl sulphate, PEG-32, Cellulose gum, Sodium saccharine, Sodiumflouride, Zinc sulphate, Sodium hydroxide, Glycerine, Cl 16035, Cl 12490.

APPENDIX 3

Biochemical Tests on Isolates

Isolates	***Staphylococcus aureus***	***Streptococcus mutans***	***Candida albicans***

Coagulase	+	-		-
Catalase	+	-		-
Oxidase	+	-		-
Citrate	-	-		-
Urease	-	-		-
TSA	-	-		-
Motility	-	-		-
Germ-	-	-		+
tube			-	
Optochin	-	+		-
			-	

APPENDIX 4

(Questionnaire for the Athletes)

DEPARTMENT OF MICROBIOLOGY

BAYERO UNIVERSITY KANO

I am a Masters student from department of Micro biology conducting a research titled: Survey of pathogens implicated in Dental Caries among Athletes and their susceptibility to brands of Oral wash and toothpaste**.**

This questionnaire is specially design to aid in survey of Pathogens implicated in dental caries among athletes and their susceptibility to brands oral wash and toothpaste.

Your cooperation would be highly required and all information provided will be used for research purpose only, and will be treated as confidential.

a. Personal information

1. Age 10-20 ☐ 20 and above ☐
2. Sex male ☐ female ☐
3. Station……
4. Sport involved…….
5. Level of Education ……..

b. questions

Instruction: please tick in the space provided or comment where necessary.

1. How long have you been in the sport involved 1 year ☐ 2 years ☐ 3 ☐ and above.
2. How many hours do you usually spend in the field …….
3. duration of exercise (a) morning (b)afternoon (c) evening (d) a and b
4. Does your dental problem affect your performance during competition

Yes ☐ No ☐

5. Have you ever visited any dental clinic, if yes on what problem

6. How often did you clean your teeth

Once daily ☐ twice daily ☐ not regularly ☐

7. Do you use any oral wash or toothpaste, if yes which brand. ……

8. Do you normally take any soft drink during the period of exercise

Yes ☐ No ☐

9. Do you smoke cigarette yes ☐ No ☐

10.Have you ever notice any change in saliva flow during exercise

Yes ☐ No ☐

Thank you.

APPENDIX 5

Occurences of organisms for pre and post exercise

Paired T test is given by:

$$tp = \frac{X1 - X2}{SE}$$

Where tp = paired T test

X1= mean of the 1st test population

X2 = mean of the 2nd test population

SE = standard error

$$SE = \sqrt{\frac{SS}{n(n-1)}}$$

$$SS = Css - Cf$$

$$Css = \Sigma d^2$$

$$Cf = \frac{(\Sigma d)^2}{n}$$

Where n = number of observation

Pre and post-exercise occurrence of the organisms

Organisms	1	2	3	4	Σx	$\overline{X}$
Post (A)	68	79	19	24	190	47.5
Pre (B)	61	72	17	22	172	43

A – B	7	7	2	2	**Σd**	**18**
$(A – B)^2$	49	49	4	4	$\mathbf{(\Sigma d)^2}$	**106**

tp =X1- X1 /SE

Applying formulae above, first calculate the SE

$$SE= \sqrt{\frac{SS}{n(n-1)}}$$

SS = Css – Cf

Css = Σd^2 = 106 (calculated in the table)

Cf = Cf $= \frac{\Sigma d^2}{n} = 18^2/4 = 324/4 = 81$

SS = Css – Cf = 106 – 81 = 25

Therefore;

$$\sqrt{\frac{25}{4(4-1)}} = 1.44$$

tp =X1-X2/SE

47.5 -43/1.44

40/1.44 =3.125

The calculated value (3.12) is not upto table value (3.183),the result is not significant and therefore there is no difference between pre and post-exercise period

APPENDIX 6

Occurrence of organisms with age of athletes

Organisms	1	2	3	4	Σx	$\overline{X}$
Above 21 years (A)	45	144	22	30	261	65.25
Below 20 years (B)	34	37	14	16	101	25.25
A – B	61	77	8	14	**Σd**	**160**
$(A - B)^2$	3721	5929	64	196	$\mathbf{(\Sigma d)^2}$	**9910**

tp = X1-X2 /SE

Applying formulae above, first calculate the SE

$$SE = \sqrt{\frac{SS}{n(n-1)}}$$

SS = Css – Cf

Css = Σd^2 = 9910 (calculated in the table)

Cf = Cf $= \frac{\Sigma d^2}{n} = 160^2/4 = 262/4 = 6561$

SS = Css – Cf = 9910 – 6561 =3349

Therefore;

$$SE = \sqrt{\frac{3349}{4(4-1)}}$$

=16.705

tp = X1-X2/SE

tp = 65.25 – 25.25 /16.705

=2.395

The table value is higher than the calculated value, therefore there is no significant difference between organisms isolated from different age groups at 5% degree of freedom

APPENDIX 7

Occurrence of organisms in association with gender of athletes

Organisms	1	2	3	4	Σx	$\overline{X}$
Male (A)	108	142	30	37	317	79.25
Female (B)	21	9	6	9	45	11.25
A – B	87	133	24	28	**Σd**	**272**
$(A - B)^2$	7569	17689	576	784	$\mathbf{(\Sigma d)^2}$	**26618**

tp = X1-X2 /SE

Applying formulae above, first calculate the SE

$$SE = \sqrt{\frac{SS}{n(n-1)}}$$

SS = Css – Cf

Css = Σd^2 = 26618 (calculated in the table)

Cf = Cf $= \frac{\Sigma d^2}{n} = 272^2/4 = 73984/4 = 18496$

SS = Css – Cf = 26618 – 18496 = 8122

Therefore;

$$SE = \sqrt{\frac{8122}{4(4-1)}}$$

=26.016

tp = X1-X2/SE

tp = 99.25 – 11.25 /26.016

=3.382

The table value (3.12) is less than the calculated value (3.382),therefore there is significant difference between the isolates obtained from athletes of both sexes

APPENDIX 8

Mouth wash

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
Rep	3	1 2 3
Organism	3	*C.albicans S.aureus S.mutans*
Formulation	5	A B C D E
Dilution	7	1:1 1:2 1:4 1:8 1:16 FS NC

Number of Observations Read	420
Number of Observations Used	420

Dependent Variable: ZOI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	141	20372.51786	144.48594	27.57	<.0001
Error	278	1456.94405	5.24081		

Corrected Total	419	21829.46190			

R-Square	Coeff Var	Root MSE	ZOI Mean
0.933258	41.55133	2.289281	5.509524

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	9.722619	4.861310	0.93	0.3967
Organism	3	2733.533333	911.177778	173.86	<.0001
Formulation	4	8315.795238	2078.948810	396.68	<.0001
Organism*Formulation	12	2013.347619	167.778968	32.01	<.0001
Dilution	6	3217.536905	536.256151	102.32	<.0001
Organism*Dilution	18	324.991667	18.055093	3.45	<.0001
Formulation*Dilution	24	2717.921429	113.246726	21.61	<.0001
Organi*Formul*Diluti	72	1039.669048	14.439848	2.76	<.0001

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Organism	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
C.albicans	2.14285714	0.22341092	<.0001	1
S.aureus	4.72380952	0.22341092	<.0001	3
S.mutans	5.93333333	0.22341092	<.0001	4

Least Squares Means for effect Organism
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ZOI

i/j	1	2	3	4
1		<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001
3	<.0001	<.0001		0.0009
4	<.0001	<.0001	0.0009	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Formulation	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	9.69047619	0.24978100	<.0001	1

B	0.00000000	0.24978100	1.0000	2
C	0.23809524	0.24978100	0.3413	3

Least Squares Means for effect Formulation
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ZOI

i/j	1	2	3	4	5
1		<.0001	<.0001	<.0001	1.0000
2	<.0001		0.9619	<.0001	<.0001
3	<.0001	0.9619		<.0001	<.0001
4	<.0001	<.0001	<.0001		<.0001
5	1.0000	<.0001	<.0001	<.0001	

D	7.91666667	0.24978100	<.0001	4
E	9.70238095	0.24978100	<.0001	5

Least Squares Means for effect Organism
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ZOI

i/j	1	2	3	4	5
1		<.0001	<.0001	<.0001	1.0000
2	<.0001		0.9619	<.0001	<.0001
3	<.0001	0.9619		<.0001	<.0001
4	<.0001	<.0001	<.0001		<.0001
5	1.0000	<.0001	<.0001	<.0001	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Formulation	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	9.69047619	0.24978100	<.0001	1
B	0.00000000	0.24978100	1.0000	2
C	0.23809524	0.24978100	0.3413	3
D	7.91666667	0.24978100	<.0001	4
E	9.70238095	0.24978100	<.0001	5

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Dilution	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1_1	7.96666667	0.29554486	<.0001	1
1_16	2.38333333	0.29554486	<.0001	2

1_2	7.05000000	0.29554486	<.0001	3
1_4	6.20000000	0.29554486	<.0001	4
1_8	5.17500000	0.29554486	<.0001	5
FS	9.02500000	0.29554486	<.0001	6
NC	0.76666667	0.29554486	0.0100	7

Least Squares Means for effect Dilution Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: ZOI							
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1		<.0001	0.3025	0.0006	<.0001	0.1519	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	0.0026
3	0.3025	<.0001		0.3958	0.0002	<.0001	<.0001
4	0.0006	<.0001	0.3958		0.1812	<.0001	<.0001
5	<.0001	<.0001	0.0002	0.1812		<.0001	<.0001
6	0.1519	<.0001	<.0001	<.0001	<.0001		<.0001
7	<.0001	0.0026	<.0001	<.0001	<.0001	<.0001	

APPENDIX 9

Tooth paste

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
Rep	3	1 2 3
Organism	4	*C.albicans S.aureus S.mutans*
Formulation	5	A B C D E
Dilution	7	1_1 1_16 1_2 1_4 1_8 FS NC

Number of Observations Read	420
Number of Observations Used	420

Dependent Variable: ZOI

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	141	16592.55238	117.67768	135.42	<.0001
Error	278	241.58095	0.86900		

Corrected Total	419	16834.13333			

R-Square	Coeff Var	Root MSE	ZOI Mean
0.985649	14.87553	0.932200	6.266667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	2.41905	1.20952	1.39	0.2503
Organism	3	1553.58095	517.86032	595.93	<.0001
Formulation	4	311.10952	77.77738	89.50	<.0001
Organism*Formulation	12	624.30000	52.02500	59.87	<.0001
Dilution	6	11951.30000	1991.88333	2292.17	<.0001
Organism*Dilution	18	1054.45238	58.58069	67.41	<.0001
Formulation*Dilution	24	441.89048	18.41210	21.19	<.0001
Organi*Formul*Diluti	72	653.50000	9.07639	10.44	<.0001

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Organism	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
Calbicals	7.65714286	0.09097337	<.0001	1
Ecoli	5.05714286	0.09097337	<.0001	2
Saureus	8.56190476	0.09097337	<.0001	3
Smutans	3.79047619	0.09097337	<.0001	4

Least Squares Means for effect Organism Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: ZOI				
i/j	**1**	**2**	**3**	**4**
1		<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001
3	<.0001	<.0001		<.0001
4	<.0001	<.0001	<.0001	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Formulation	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	7.94047619	0.10171132	<.0001	1
B	5.86904762	0.10171132	<.0001	2
C	6.00000000	0.10171132	<.0001	3
D	6.04761905	0.10171132	<.0001	4
E	5.47619048	0.10171132	<.0001	5

Least Squares Means for effect Formulation Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: ZOI					
i/j	**1**	**2**	**3**	**4**	**5**
1		<.0001	<.0001	<.0001	<.0001
2	<.0001		0.8928	0.7271	0.0520
3	<.0001	0.8928		0.9974	0.0030
4	<.0001	0.7271	0.9974		0.0009
5	<.0001	0.0520	0.0030	0.0009	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Dilution	ZOI LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1_1	12.0666667	0.1203465	<.0001	1
1_16	0.3666667	0.1203465	0.0025	2
1_2	9.6500000	0.1203465	<.0001	3
1_4	6.1000000	0.1203465	<.0001	4
1_8	1.7166667	0.1203465	<.0001	5
FS	13.9666667	0.1203465	<.0001	6
NC	-0.0000000	0.1203465	1.0000	7

Least Squares Means for effect Dilution Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: ZOI							
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	0.3241
3	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
7	<.0001	0.3241	<.0001	<.0001	<.0001	<.0001	