PREVELANCE OF DRUG-RESISTANT BACTERIA FROM ORAL-SWAB OF CALEB UNIVERSITY STUDENTS

BY

ERHABOR THELMA EBI,

MATRIC NO 19/6083

A PROJECT REPORT TO BE SUBMITTED TO THE DEPARTMENT BIOLOGICAL SCIENCE AND BIOTECHNOLOGY , CALEB UNIVERSITY, IMOTA LAGOS STATE, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN MICROBIOLOGY AND INDUSTRIAL BIOTECHNOLOGY FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, CALEB UNIVERSITY, IMOTA, LAGOS STATE

JULY, 2022

DECLARATION

I ERHABOR THELMA EBI,with matriculation mumber, 19/6083 hereby declare that this project report titled “PREVELANCE OF DRUG-RESISTANT BACTERIA FRO ORAL-SWAB OF CALEB UNIVERSITY STUDENTS, IKORODU, LAGOS STATE" submitted to the department of biological sciences and biotechnology was solely completed and carried out by me, under the supervision of DR(MRS) CHINYERE EZEANYA BAKPA, Head of Department of Biological Sciences and biotechnology. Results embodied in this project report have not been submitted to any other university for the award of any type of diploma or degree.

_____ _____

ERHABOR THELMA EBI **DATE**

CERTIFICATION

This is to certify that the project titled **"**PREVELANCE OF DRUG-RRESISTANT BACTERIA IN ORAL SWAB OF CALEB UNIVERSITY STUDENTS" was fully accomplished and completed by ERHABOR THELMA EBI with matric number: 19/6083 in the Department of Biological sciences and Biotechnology, College of Pure and applied sciences, Lagos state, Nigeria.

_____- _____

DR. (MRS) C.C EZEANYA-BAKPA DATE
(PROJECT SUPERVISOR)

_____. _____

DR(MRS) C.C EZEANYA-BAKPA DATE
(HEAD OF DEPARTMENT)

_____ _____

(EXTERNAL SUPERVISOR) DATE

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor Dr (Mrs) Chinyere Ezeanya-Bakpa for giving me this wonderful project topic and for support and putting me through on how to to successfully carry out and write this report, I sincerely express my gratitude for the time spent on the corrections of my mistakes.

I would also like to acknowledge my able lecturers.I would also like to acknowledge with much appreciation the role of the laboratory technician in charge of the microbiology Laboratory, who gave me the permission to use the laboratory equipment and tools.

DEDICATION

I would like to dedicate this project to God Almighty the giver of life, my pillar, and inspiration, for giving me wisdom and understanding. I also dedicate this report to my dear mother Engr. Mrs Maria Prefa-Erhabor for whom has always showed her love, care and support through out my life, always being there for me and has made sure I never lack anything. I also thank my beloved Grandmother Mrs Grace Prefa for her encouragement and words of wisdom in my life, I thank my Aunty Barr Mrs Pat Sini-Ototo, for her support and emboldement through out this program, and my younger brother Master Jaden E. Erhabor whom has affected and motivated me in every good way in fulfillment of this quest. My love for all of you can never be compromised, thank you so much, God bless you.

TABLE OF CONTENT

ABSTRACT

Over the past years, changes in world technology have occurred which have accommodated the fast transport of people, food, and goods but, antibiotic residues and antibiotic-resistant bacteria have been carried along as well. In this review, the main mechanisms of resistance to the important antibiotics used for treatment of disease caused by oral/respiratory bacteria-including tetracycline etc are talked on in full detail.

Oral diseases, such as dental caries and periodontal disease are connected with the ability of bacteria to form biofilm. The development and formation of dental caries involve acidogenic and aciduric Gram-positive bacteria colonizing the supra gingival biofilm (Streptococcus, Lactobacillus and Actinomycetes). Periodontal diseases have been linked to anaerobic Gram-negative bacteria forming a plaque (Porphyromonas gingivalis, Actinobacillus, Prevotella and Fusobacterium). Cells embedded in biofilm are up to 1000-fold more resistant to antibiotics compared to their planctonic ones. Given the increased bacterial resistance to antibiotics currently used in dentistry, a great importance is given to natural compounds for the prevention of oral bacterial growth, adhesion and colonization. The oral swab samples were collected using sterile, unused swab sticks which were used to swab the surface of the inner cheeks in the oral cavity, theses samples were collected without the swab stick touching the tongue as it might give inaccurate results as the tongue contains diverse microorganisms. This study 4 microorganisms were isolated from the oral cavity *Staphylococcus aureus* of which 6 antibiotics were susceptible ot of the 11 antibiotics used for the

susceptibility test *Streptococcus mutans* of which 6 antibiotics were resistant and 5 susceptible, *Klebsiella pneumonia* of which 5 antibiotics were resistant and there was 6 in total number of susceptible microorganisms, *Escherichia coli* of which 6 antibiotics showed resistance and 5 showed susceptibility. The most organism identified was *Staphylococcus aerus* about 40%, *Klebsiella pneumonia* which had a percentage of 25%, *Escherichia coli* with a percentage of about 20%, *Streptococcus mutans* with percentage of 15%.. Most of the organisms were catalase positive, coagulase positive and non motile organisms.

CHAPTER ONE

INTRODUCTION

1.1BACKGROUND OF STUDY

Human oral is cavity is one of the most common habitats for various species where they undergo intense interspecies competition to form multispecies biofilm structure. Various species of genus streptococcus,lactobacillus, *Lactococcus*, S*taphylococcus*, Corynebacterium, veillonella and bacteriods are the most prominent bacteria commonly found in the oral cavity (Bush k. improving known classes of Antibiotics: An Optimistic approach for the future. Among the oral bacteria streptococcus and Enterococcus are two important members because they can shift their lifestyle from beneficial microflora on the surface of oral cavity to destructive pathogens when they gain access into the oral tissue and blood stream. Among the disease caused by oral bacteria include dental caries, periodontitis, endocarditis, phargyngitis, pneumonia, meningitis etc. The Role of Human Oral microbiome in Dental Biofilm Formation (wirginia krzysciak Anna Jurczak and Jakub Piatkowski et al 2015)

Most of the oral streptococcus are gram positive facultative anaerobes showing efficient survival strategies such as the means to stick with hard and soft tissues, cell communication, biofilm formation and to adapt with fastly changing oral habitats (soto SM. Role off efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virukence. 2013).

(Bush K. Improving Known Classes Antibiotics: An Optimistic approach for the Futu re) Among the oral bacteria, *Streptococcus* and *Enterococcus* are 2 very important

members because they capable of shifting their mode of living from being beneficial in the oral cavity to highly pathogenic organisms when they have acess into the bloodstream or oral tissues through the aid of the epithelial cells.

Bacterial include dental caries,periodontitis, endocarditis, pharyngitis, pneumonia, meningitis etc. The Role of Human Oral Microbiome in Dental Biofilm Formation (Wirginia,Krzyściak, AnnaJurczak and Jakub Piątkowski *et al* 2015). Most of the oral Streptococcus are gram positive facultative anaerobes showing efficient survival strategies such as the means to stick or adhere with hard and soft tissues, cell to cell communication, biofilm formation and to adapt with the fastly changing oral habitats . (Soto SM. Role of efflux pumps in the resistance of bacteria embedded in a biofilm. Virulence.2013) .

A bacterium has to challenge other bacteria to colonize oral cavity. Therefore, they un dergo a series of extensive, invasive intra-species and inter-species communication.

Production of bacteriocin as a toxin is important in challenging other bacteria in this different environment. Most gram positive bacteria produce bacteriocins which will act as toxins against other bacteria, while the producer bacterial strain is highly immune to its own bacteriocin due to some of the immunity factors, since the oral cavity is highly competitive, the bacterial species isolated from such a habitat will produce inhibiting substances against other bacteria. Oral swab is a non invasive procedure used in detecting for bacterial, viral strains and even fungi. The oral cavity is an ecosystem which accommodates diversity of microorganisms like fungi, virus and bacteria. Some of these microorganisms are involved in causing

multiple infections. Oral flora changes continuously due to connection with the external environment and produces bacteriocin toxin against each other to compete for nutrient in this ecosystem. (Sharma et al., 2018, Albandar et al., 1999, Lim et al., 2020, Costalonga and Herzberg, 2014,)

1.2 STATEMENT OF THE PROBLEM

Bad oral breath is one of the biggest results of accumulated harmful oral bacteria. If it is not inhibited or destroyed properly, they can damage an individual's dentition for life in terms of functions and sometimes morphology which will lead to serious dental health issues.

1.3 JUSTIFICATION OF THE STUDY

The justification is on the prevalence of this study is to provide useful and very acurate information on antimicrobial resistant bacteria and isolation and susceptibility test of students on the campus, understanding the whole, major concept and idea of antibiotic/drug-resistant bacteria and to find out antibiotics that would actually inhibit bacterial growth.

Lack of clean and purified water, inadequate prevention and control promotes the spre ad of microbes,some are resistant to antimicrobial treatment.

1.4 AIM AND OBJECTIVES

The aim and objective of this project study of this is to give detailed tests on the oral-swab samples of individuals within the university, isolation, characterization and differentiation of the oral samples isolates with different biochemical tests.

a. To seek proper information and insight on individuals who often use antibiotics.

b. To identify and isolate drug resistant bacteria in the oral swab sample of individuals

c. To detect the antibiotic susceptibility of oral swab samples.

1.5 OBJECTIVES

The main goal of this study was to carry out more profound information and details in the to determination of the occurrence and prevalence of drug resistant bacteria in oral swab sample of students of Caleb University, Ikorodu, Lagos state.

CHAPTER TWO

LITERATURE REVEIW

Rising harder to treat as the antibiotics used to treat them less effective. As of Decemb er 2019, approximately 41 new antibiotics with the potential to take care of very severe infections.Antibiotics, also called antibacterial/antimicrobial drugs, are used in the tre atment of infections caused by strains of bacteria by inhibiting the growth of these ba cteria while body's natural defenses work in concert to eliminate the infection.

Antibiotics are powerful medicines which fights infections caused by microorganisms such as fungi and bacteria and can save lives when used properly, in the right way. they Antibiotics stop and inhibit the process of reproduction in bacteria reproduction, before bacteria can multiply and cause symptoms, immune system can inhibit them. (Church DL, BryantRD, Sim V., Lishley EJ (1996).

Antibiotics are used to treat or prevent bacterial infections. They are not effective agai nst viral infections. Antibiotics should only be prescribed to treat health problems and not for minor issues as Influenza and others which will run their own course without any treatment. Drug resistant bacteria are caused by; selective pressure, gene transfer, inappropriate drug usage, inadequate diagnosis, hospital usage, agricultural usage, phenotypic changes, wrong diagnosis (Murell and Macintosh et al 2018).

2.1.1 TYPES OF DRUG-RESISTANT BACTERIA

2.1.2 METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS (MRSA)*

Methicillin-resistant *Staphylococcus aureus* is a broad group of Gram-positive cocci shaped bacteria that are genetically-diverse or different from other strains of the disease.

Staphylococcus aureus. (Adrian PV,Klugman KP (1997). MRSA is responsible for sev eral very difficult-to-treat infections in humans. The average 30-day mortality rate for MRSA bloodstream infections is 30 percent. (Barton E, MacGowan A.Watkins and Holubar *et al* 2019).

2.1.3 MODE OF TRANSMISSION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS (MRSA)*

MRSA can be transmitted orally through kissing, sharing of unwashed eating utensil. The disease is usually spread in the community by close contact with infected individuals it can also be spread through objects carrying the disease (direct contact), objects carrying the bacteria. This includes through contact with contaminated open wound surfaces or by sharing personal and intimate items, such as towels or razors, needles that have touched infected skin.Several people with active infections are treat ed, and no longer have MRSA. Sometimes MRSA leaves after treatment, whenever MRSA is transmitted orally it spreads and enters the bloodstream through the oral cavity.

Oral antibiotics treatments and curing a can often be used in the treatments of MRSA, because it doesn't respond to several and common types of drugs such as;

methicillin,amoxicillin, penicillin, oxacillin, cephalorsporins, which are administered eintravenously with Vancomycin if very severe.(Davis et al 2021).

2.1. CLINICAL SYMPTOMS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

The following are the symptoms of MRSA:

- General body pain and malaise
- Redness, and swelling around the affected tooth
- Sensitivity to temperature or pressure
- Swelling of cheeks or face
- Sour taste or bad smell in oral cavity
- Fever (Lights et al 2019).

2.2 PATHOPHYSIOLOGY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AERUS*

Staphylococcus aureus is a commensal organism and pathogen. The anterior nares are the main ecological niche for Staphylococcus aureus. Approximately 20% of individuals are persistently nasally colonized with Staphylococcus aureus, and 30% ar e intermittently colonized. However numerous other organisms colonize the oral cavity, Colonization increases the risk of reoccurring infections in living organisms,

It can also allow the bacterial disease Staphylococcus aureus to be transmitted among individuals in both health care and community settings.The basis for the disease colon

introduced or induced when the host's defenses are breached by different causes such as; shaving, aspiration, insertion of an object into body or in the case of immunocompromised patients e.g HIV, diabetes, or the aged. Colonization increases the risk of reoccurent infection.s in living organisms.

Colonization can also allow *Staphylococcus aureus* to be transmitted among individuals in both health care and community settings.The basis for the disease colon y iis not understood but it appears to involve the host's contact with the bacterial infection(e.g.,othercarriers). *Staphylococcus aureus* adheres to host cells and to evade the immune response, the bacteria adhere to the host cell through the epithelial cells. However, the role of the different virulence factors in the formation of staphylococcal infections is misunderstood Some clonal types are well equipped to cause disease across the globe, while others are facileat causing disease among comm unity members(Schumann and Nicholas *et al* 2019).

2.2.1 CLASSIFICATION OF METHICILLIN RESISTANT

STAPHYLOCOCCUS AERUS

It is a Latin word which means golden clusters, because of its yellow-golden colour.

Taxon Name

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Specie: *aerus* (Suebert *et al* 2009)

2.2.2. MULTI-DRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS

The bacteria that can cause *Tuberculosis* (TB) can also cause and form resistance to the antimicrobial/ antibiotics drugs used to cure the disease . Multi drug-resistant T*uberculosis* bacterial diseases are diseases which do not at all respond to isoniazid and rifampicin antibiotic,the 2 most powerful anti-*Tuberculosis* d rugs, if the spread of disease can be controlled through tnot overcrowding to avoid direct contact. The two main reasons why multi drug resistance continues to emerge and spread are the mismanagement of T*uberculosis* treatment and direct contact or they are person-to-person transmission which is the most common form of transmission in organisms of this nature

Most people with *Tuberculosis* are cured by a strictly followed, 6-month drug and treatments and cures that is provided to patients with the support and supervision *Tuberculosis*(MDRTB) is caused by *Mycobacterium tuberculosis* which is resistant against the two first-line drugs rifampicin and isoniazid.

(Sueng *et al.,* 2015) (Kehahavi *etal., 2015) (*Rich *et al 2015).*

2.2.3 CLASSIFICATION OF *MYCOBACTERIUM TUBERCULOSIS*

Domain: Bacteria

Phylum: Actinomycoceta

Order: Corynebacteriales

Family: Mycobacteriaceae

Genus: *Mycobacterium*

Specie: *tuberculosis (*World Health Organisation *et al 2015)*

2.2.4 CELL CYCLE OF *MYCOBACTERIUM TUBERCULOSIS*

The infection happens in 4 different stages: the initial and first macrophage response, the growth stage, the immune control stage, and the lung cavitation stage. These four stages happen over roughly a short period one month.(Sueng *et al.,* 2015) (Kehahavi *etal., 2015) (*Rich *et al 2015).*

2.2.5 SYMPTOMS OF *MYCOBACTERIUM TUBERCULOSIS*

Cough for longer than three weeks either dry, green and bloody mucus,Weight Loss, Fatigue, Shortness of Breath, Fever

2.2.6 VIRULENCE FACTORS OF *MYCOBACTERIUM TUBERCULOSIS*

For most intracellular pathogens, the phagosome is the place of interactions that shape the outcome of infection. Phagosomal membrane damage is proposed to benefit

invading pathogen. A wide range of genes were identified with enhanced expression in retaliation to the mutant genes.

Expression of the later component was inherent to TLR2 activation, dependent upon endosomal uptake, and enhanced by phagosome acidification. Canonical MDRTB virulence factors that contribute and added to phagosomal membrane-damage blunted phagosome acidification and undermined the endosome-specific response. Profiling cell survival and bacterial growth in macrophages demonstrated that the attenuation of these mutants is partially dependent upon TLR2. Further, TLR2 contributed to the attenuated phenotype of one of these mutants in a murine model of infection. These results demonstrate two distinct components of the TLR2 response and identify a component dependent upon endosomal uptake as a point where pathogenic bacteria interfere with the generation of effective inflammation. This interference promotes tuberculosis (TB) pathogenesis in both macrophage and murine infection models. (Amelia E Hinman et al. Elife. 2021)

2.3. CARBAPENEM-RESISTANT ENTEROBACTERIACEAE (CRE) GUT BACTERIA.

Enterobacteriaceae are a family of bacteria that usually live in the bowel or intestinal tract without causing any illness. Carbapenems are powerful antibiotics used to treat very severe and serious infections, which are fatal. Some Enterobacteriaceae have recently and suddenly become resistant to these antibiotics which means they do not cure any infections that develop. They are referred to as carbapenem-resistant Enterob

acteriaceae (CRE).Sometimes these types of bacteria can spread outside of the bowels in the digestive system also known as the alimentary canal and cause various gastrointestine-infection, for example a urinary tract infection(UTI) ,wound infection or pneumonia (Schulman and Sisson *et al* 2020).

2.3.1 SYMPTOMS OF CRE

The symptoms that develop with CRE infection are very common with any other bacterial infection such as; Fever, Feeling generally unwell, Rapid pulse rate, Redness, swelling, pain or heat at a specific site of inflammation (Schulman and Sisson *et al* 2020)

2.3.2 VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE)

Vancomycin-resistant *Enterococcus*, or vancomycin-resistant Enterococci, are a group of bacterial strains of the genus *Enterococcus* that are resistant to the antibiotics initially identified in the mid-1980s,vancomycin-resistant *Enterococci* (VRE) spread f astly and has became a very major and main cause of most problem in many countries both in Europe and the United States. Since VRE have intrinsic or spontaneous resistance to most of the commonly and mostly used antibiotics and the ability to acquire resistance to most of the current available antibiotics, either by gene mutation as they have a selective and non-generalized advantage and usefulness over other microorganisms in the intestinal flora possess a major therapeutic challeng-e.The most essential capabilities and possibilities of the successful moving and

transfer of vancomycin resistance genes to other gram-positive microorganisms ganisms show very outstanding and very distinguished concerns on the emergence of vancomycin-resistant *Stapphylococcus aureus* including their development, life cycle history, mechanisms of resistance, disease epidemiology, control measures, and treatment.(Balli et al Eleni et al Venetis et al, Chris A.Miyakis et al, Spiros et al 2014).

2.3.3 MECHANISMS OF RESISTANCE OF VRE

Full resistance to β-lactams is a very obvious and spontaneous characteristic feature of the genus Enterococcus. *Enterococcus faecalis* is typically more less susceptible to penicillin than most streptococci, while *Enterococcus faecium* is less susceptible than *Enterococcus faecalis* which belongs to the Enterobacteriaceae family of

the species *Enterococcus faecium* and *Enterococcus faecalis* are very different among enterococci and recognized as a very important reservoir of glycopeptide and multidrug-resistance (MDR) . A study and meta-analysis of which involved Iran and a few European countries showed a near rate of prevalence between countries of VRE infections in humans and ranges between 8–13%, largely from *Enterococcus faecalis* carriers.

CHAPTER THREE

METHODOLOGY

3.0. MATERIALS AND METHODS

The materials used were; Glass slides, petri dish,incubators, autoclaves, syringe, cotto n wool, foil paper,gram stain, Agars used were; ,Nutrient Agar, Eosin Methylene Blue Agar(EMB) and Mannitol Salt Agar (MSA), Mueller Hinton Agar for the antibiotic susceptibility testing, culture and growth. The oral swab sample were collected using a sterile and unused swab stick which was carefully and hygienically with heat swabbed and dipped into 500µl phosphate buffered saline(PBS) buffer (0.12M NaCl, 0.01M Na2HPO4, 5mMKH2PO4 [pH 7.5]) under hygienic conditions.

3.1 STUDY SETTING/AREA

The area of this study was taken place at Caleb University, Ikorodu Lagos State,where the oral swab samples were taken from 36 male students and 40 female students The practical aspect of this project study was carried out successfully in The Department of Biotechnology and Biological Science, College of Pure And Applied Sciences under hygienic conditions. The Identification and Isolation of bacteria was carried out alongside the culture methods, using different types of culture medium, the various biochemical tests e.g catalase test, coagulase test, gram stain test, citrate tests, indole tests, antimicrobial susceptibility tests were carried out to know the organisms susceptible and resistant to different species of bacteria.

This study involves seventy- six (76) consenting students in total, 38 male and 40 female students will be tested for drug-resistance bacteria in oral swab sample.

3.4 SAMPLE COLLECTION

Samples were then collected/taken with the aid of a sterile and unused swab stick inserted into the oral/buccal cavity it was swabbed on the surfaces of the inner cheeks for at least 5 times on all ends of the swab stick, the patient is advised to not eat anything before the swab test is carried out so as to avoid misinterpretation of the results. Before collecting oral samples patients are asked to fill a consent form patient will be given a questionnaire to fill in,immediately after sample is collected it will be labelled containing patient's personal data was obtained such as; name, age, level etc. The sample collection will be done inside the inner cheek of the oral cavity, samples were collected under aseptic and hygienic conditions to avoid contamination. The sterile swab stick was placed in the mouth and rubbed the side of the inner cheek for about 6 times, when the sample was taken. The swab stick was then taken out of the oral cavity and placed the swab tip into the opened tube containing freshly prepared peptone water.

3.4.2 BIOCHEMICAL TEST

Isolated bacterial strains were subjected to different biochemical tests specialized and specified for bacteria of the oral/buccal cavity.

These tests will fasten the identification and characterization of the e isolated strains.

Biochemical tests that will be used in this study are:Gram staining, catalase test, coag ulase tests, urease test, citrate test.

3.4.3 ANTIBIOTIC SUSCEPTIBILITY TEST

Each of the isolated strains were tested for sensitivity,against different antimicrobial a gents using agar disc-diffusion method, disc diffusion method can be considered the best method for sucesptibilty test since it gives proper. Overnight grown bacteria were tuned to 0.5 McFarland turbidity standards (~1.0×108colony forming units or cfu ml-1) the McFarland turbidity standard is the most standard turbidity used in antibiotic susceptibility testing, the samples were then put into the Mueller-Hinton agar with a sterile cotton swab. Plates were left at room temperature for 15 to 20 minutes. The culture (100µl) was then spread-over the Mueller-Hinton agar plate. Antibiotic disks was then ingested on the seeded culture and incubated in micro aerophillic conditions overnight, plates were then incubated at 35°C for 18-24hours. The antimicrobial drugs that were used for the test includes: Ciprofloxacin(5µg), Tetracycline(30µg),Gentamicin(10µg),Cefoxitin(30µg),Ceftazidime(30µg), Cotrimoxazole(50µg), Erythromycin(15µg), Augmentin(30µg), Ceftriaxone(30µg), Cefotaxime(30µg) and Imipenem(10µg).

3.4.4 ISOLATION OF DRUG-RESISTANT BACTERIA FROM ORAL SWAB

Isolation of drug-resistant bacteria from oral swab samples was done using Mannitol

Salt Agar (MSA), Blood agar, Eosin Methylene Blue (EMB) and Using a sterile inoculating wire loop, the samples were taken with sterile oral swab

and preserved in prepared peptone water for at least 12 hours at 37°c to facilitate the growth process after it is streaked in the agar plates and inoculated into Mannitol Salt Agar plates, Blood Agar plates and Eosin Methylene Blue Agar plates by streaking.

3.4.5 BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF THE ISOLATES.

The isolates were subjected to morphological examination and biological test and their identity were confirmed using the characteristics. The test carried out include Catalase Test, Gram staining, Citrate Test, Urease Test, Motility Test, Indole Test, hemolytic test.

3.5 CATALASE TEST

Colonies of the isolates were picked with a sterile inoculating loop and dropped on a clean free microscopic slides, a drop of Hydrogen Peroxide was added to the colony. Effervescence of bubbles indicated a positive reaction while absence of bubble formation indicated a negative reaction for catalase production.

3.5.1 GRAM STAINING

The isolates were smeared on a clean, free, microscopic slide and allowed to dry. Crystal violet was added for a minute and rinsed with distilled water, Iodine was

added for a minute and rinsed with distilled water, then decolorized with acetone for 15 seconds and rinsed with distilled water, and Safranin was then added for a minute, rinsed with distilled water and finally allowed to air dry.

A drop of immersion oil was placed on the glass slide and microscopy evaluation was carried out under the X100 objective lens light microscope. The Gram reaction and cell shape was determined with pink or red color which indicated Gram negative organisms.

3.5.2 CITRATE TEST

Simmons citrate agar was prepared and dispensed into cryovial bottles and kept slant. The isolates were pierced by the top with the help of a needle. Incubated at 35 degree Celsius for 18-24hours.

Color changed to blue indicates a positive result. No color change indicates a negative result.

3.5.3 UREASE TEST

Urease base agar and pure urea was prepared and dispensed in cryovial bottles in a slant manner to solidify and inoculated on the slants aseptically. Incubated aerobically at 37 degree Celsius for 24hours or more. A pink coloring of the agar indicated positive test while yellow color indicated negative results.

3.5.4 MOTILITY TEST

It is a differential test medium used to determine whether an organism has flagella. Half strength nutrient agar (2.1g for 150ml), was sterilized and was dipped into cryovial bottles and stabbed with the bacteria colonies. It was then incubated at 37 degree Celsius for 24hours.

The results of motility agar are difficult to interpret generally, if the entire tube is turbid, this is an indication that the bacteria moved away from the stab mark (motile), and if the stab mark is clearly visible and rest of the tube is not turbid, the organism is likely non motile.

3.5.5 INDOLE TEST

This test is a biochemical test that is performed to determine the ability of bacterial species tryptophan. After which the isolates were inoculated into the peptone water and incubated, a drop of Kovacs reagent was then added into peptone water with the isolate. Color appearance at the top indicated positive results. No color appearance indicated negative result.

CHAPTER FOUR

RESULTS

This chapter is to show and properly interpret the data information and results of the tests based on the questionnaires, biochemical test results, antibiotics susceptibility test results, percentages of the disk Synergy tests and all data presentation Firstly on the analysis of the questionnaire at (Table 4.1) the questionnaire gave proper descriptive analysis on the students based on their name, age, level of study, family size and sex. The students were between the ages of 15-19 years of age. A cumulative of 40% male students used antibiotics only once in a while, same as the female students, a majority of only 40% of the female students practiced oral hygiene twice daily(50%), 50% of female students also chewed gums and sweets often. In the cultural characterization and morphology at (Table 4.3) showed the morphological characteristics and appearances of the different organisms that were Isolated. Most of the isolates were gram positive cocci, Catalase positive, coagulase positive, indole negative, oxidase negative, non motile microorganisms and so on.

4.1 Data collection of activities from the questionnaire given to students

Table 4.1

		Female	Male
Age	15 – 19	40%	40%
	20 – 24	40%	35%
	25 - 29	20%	25%
Family	1-3	20%	20%
	4-6	35%	35%
	7-9	35%	35%
	9 and above	10%	10%
Often usage of antibiotics	Sometimes	30%	30%
	Very often	30%	30%
	Once in aa while	40%	40%
Oral hygiene twice daily	Sometimes	20%	20%
	Very often	20%	20%
	Never	40%	40%

		Female	Male
Chewing of gums, sweets	Sometimes	10%	9%
	Very often	50%	51%
	Never	30%	40%
Washing of hands before eating	Sometimes	20	15%
	Very often	50	35%
	Never	30	30%

4.2 BIOCHEMICAL TESTS RESULTS

Table 4.2

Sample code	Citrate test	Urase	coagulase	Gram stain	insole	Oxidase	Catalase	Motility
FO1	-	+	+	+	-	-	+	-
FO2	-	+	+	+	-	-	+	-
FO3	-	+	+	+	-	-	+	-
FO4	-	+	+	+	-	-	+	-
FO5	-	-	+	+	-	-	+	-
FO6	+	+	-	-	+	-	-	-
FO7	-	-	+	+	+	+	+	-
FO8	-	-	+	+	-	-	+	-
FO9	-	-	+	+	-	-	+	-
FO10	-	-	+	+	-	-	+	-
FO11	-	-	+	+	-	-	+	-
FO12	-	-	+	+	-	-	+	-
FO13	-	-	+	+	-	-	+	-
FO14	-	-	+	+	-	-	+	-
FO15	-	-	+	+	-	-	+	-
FO16	+	+	-	-	-	+	-	-
FO17	-	+	+	+	-	-	+	-
FO18	-	-	+	+	-	-	+	-

FO19	-	-	-	+	-	-	+	+
FO20	-	-	+	+	-	-	+	-
FO21	-	-	+	+	-	-	+	-
FO22	+	-	+	-	+	-	-	+
FO23	-	+	+	+	_	-	+	-
FO24	-	_	+	+	_	-	+	-
FO25	-	+	+	+	-	-	+	-
FO26	-	-	+	+	-	-	+	-
FO27	-	-	+	+	-	-	-	-
FO28	+	+	-	-	+	-	-	+
FO29	-	_	+	+	-	-	+	-
FO30	+	+	_	-	+	-	+	-
FO31	-	_	+	_	+	-	+	-
FO32	+	+	_	+	-	+	-	-
FO33	-	_	+	+	-	-	+	-
FO34	-	_	_	+	-	-	+	-
FO35	-	_	+	+	-	-	+	-
FO36	-	_	-	+	-	-	+	-
FO37	+	+	-	-	+	+	-	-
FO38	-	-	-	+	-	-	+	+
FO39	-	_	+	+	-	-	-	-
FO40	+	+		-	+	-	+	+

MO1	-	-	+	+	_	-	+	-
MO2	-	-	+	+	_	-	+	-
MO3	-	-	+	+	_	-	+	-
MO4	+	+	-	-	+	-	+	-
MO5	-	_	-	+	_	-	-	+
MO6	-	_	-	+	_	-	+	-
MO7	+	_	-	+	_	-	+	-
MO8	+	+	+	-	+	-	+	-
MO9	-	_	+	+	_	-	+	-
MO10	-	_	_	+	-	-	_	-
MO11	-	_	-	+	-	-	+	+
MO12	-	_	+	+	-	-	+	-
MO13	-	_	-	+	-	-	+	-
MO14	-	_	+	+	-	-	+	-
MO15	-	_	-	+	-	-	+	-
MO16	--	_	-	+	-	-	+	+
MO17	+	_	-	+	-	-	+	-
MO18	+	_	-	+	-	-	+	-
MO19	+	-	+	+	-	-	+	-
MO20	_	-	-	-	+	-	+	-
MO21	_	-	-	+	_	-	+	-
Mo22	-	-	-	-	+	-	+	-

MO23	-	-	-	+	_	-	+	-
MO24	-	-	-	+	_	-	+	-
MO25	-	-	-	+	_	-	+	-
MO26	+	-	-	-	+	-	+	-
MO27	+	-	-	+	_	-	+	-
MO28	_	-	-	-	_	-	+	-
MO29	+	-	+	+	_	-	+	-
MO30	_	-	+	+	_	-	+	-
MO31	+	-	-	-	_	-	+	+
MO32	+	-	+	+	_	-	+	-
MO33	+	_	-	+	-	-	+	-
MO34	_	_	-	-	+	-	+	+
MO35	_	_	+	+	-	-	+	-
MO36	_	_	+	+	-	-	+	-

4.3 ANTIBIOTIC SUSCEPTIBILITY TEST RESULTS FOR EACH SPECIE OF ISOLATES

Table 4.3

S/N	Ce	Ge	Te	Cip	Im	Am	Ct	Cef	Ery	Cer	Clo	Probable organisms
FO2	S	R	S	R	S	R	S	S	S	R	R	*Staphylococcus aureus*
FO3	S	R	S	R	S	R	S	S	S	R	R	*Staphylococcus aureus*
FO4	S	R	S	R	S	R	S	S	R	R	R	*Escherichia coli*
FO6	S	S	R	S	R	R	R	S	R	S	R	
MO5	S	S	R	S	R	R	R	S	R	S	R	*Escherichia coli*
MO15	S	S	R	S	R	R	R	S	R	S	R	*Klebsiella pneumoniae*
M28	S	S	R	S	R	R	S	R	R	S	S	*Staphylococcus aureus*
FO13	R	S	S	R	S	S	S	R	S	R	R	*Streptococcus mutans*

MO6	R	S	S	R	S	S	S	R	S	R	R	*Streptococcus mutans*
FO12	S	S	R	S	R	R	R	S	R	S	R	*Klebsiella pneumoniae*

Code name for antibiotics used: Cefoxitin (Cef), Gentamicin (Gen), Tetracycline(Tet), Ciproflaxin(cip), Imepenem(Ime), Amoxicillin (Amo), Cefotaxime (Cefo), Ceftizidime(Ceft), Erythromycin (Ery), Ceriftaxone (Cer) Coltrimazole(col

4.2: CULTURAL CHARACTERIZATION AND MORPHOLOGY OF ISOLATES

Table 4.3

Sample code	Blood Agar	Mathinol Salt Agar	Eosin Methylene Blue Agar	Probable oraganisms
FO1	Yellow-golden, circular colonies	Yellow round colonies.	Purple, small colonies	*Stapphylococcus aureus*
FO2	Yellow-golden, circular colonies	Yellow round colonies	Purple, small colonies	*Stapphylococcus aureus*
FO3	Yellow-golden circular colonies.	Yellow to Orange round colonies moist formed,	Purple, small round, moist colonies	*Stapphylococcus aureus*
FO4	Whitish-grey, circular colonies formed.	No growth occured.	Slightly colorless colonies formed	*Stapphylococcus aureus*
FO5	Whitish-grey, circular colonies formed.	No colonies formed/ No growth occured.	Purple, small, round, individual colonies formed.	*Stapphylococcus aureus*
FO6	Whitish-grey circular colonies formed	No colonies formed/ No growth occured.	Greenish, thin branched colonies formed	*Escherichia coli*

FO13	Yellow-golden circular, dry colonies formed.	Yellow to orange round, moist colonies formed.	Purple, small, round colonies formed.	*Streptococcus mutans*
FO14	Brownish-grey, circular, moist colonies formed.	Reddish to pink colonies formed with almost no growth.	Purple, small colonies formed.	*Streptococcus mutans*
FO15	Yellow to golden, circular colonies formed.	Yellow to orange round, moist colonies formed.	Purple, small, circular colonies formed.	*Streptococcus mutans*
FO16	Whitish-grey circular colonies formed	No growth formed.	Purple, small, circular, colonies formed.	*Klebsiella pneumonia*
FO17	,Yellow to golden circular colonies	Yellow to orange, round circular colonies formed.	Purple, round individual colonies formed.	*Staphylococcus aureus*
FO18	Brownish to grey, moist colonies formed	Reddish to pink colonies formed with almost no growth.	Purple small colonies formed.	*Streptococcus mutans*

FO19	Whitish-grey circular colonies formed	No growth formed	Greenish thin-branched colonies formed.	*Escherichia Coli*
FO20	Yellow to golden circular colonies	Yellow to orange, circular colonies	Purple,circular colonies formed.	*Staphylococcus aureus*
FO21	Brownish to grey, moist colonies formed	Reddish to pink colonies formed with almost no growth	Purple smal colonies formed	*Streptococcus mutans*
FO22	Whitish to grey circular colonies formed	No growth occcured	Purple circular colonies formed	*Escherichia coli*
FO23	Yellow to Golden, circular colonies	Yellw to orange circular colonies	Purple circular colonies	*Staphylococcus aureus*
FO24	Brownish to grey moist colonies formed	Reddish to pink colonies formed with almost no growth	purple small colonies formed	*Streptococcus mutans*
FO25	Yellow to Golden circular colonies formed	Yellow to orange circular colonies	Purple, small colonies formed	*Staphylococcus aureus*

FO33	Whitish-grey colonies formed	Reddish- pink colonies formed	Purple, small colonies formed	*Streptococcus mutans*
FO34	Yellow to golden circular colonies formed	Yellow to orange circular colonies	Purple, small colonies formed	*Stapphylococcus aureus*
Fo35	Brownish to grey colonies formed	Reddish to pink colonies formed	Purpe, small colonies formed	*Streptococcus mutans*
FO36	Brownish to grey colonies formed	Reddish to pink colonies formed	Purple, small colonies formed	*Streptococcus mutans*
FO37	Whitish- grey colonies formed	No growth formed	Purple, circular colonies formed	Klebsiella pneumonia
FO38	Whitish-grey circular, moist colonies formed	No growth formed	Greenish thin-branched metallic colonies f	*Escherichia coli*
FO39	Yellow to Golden, moist, circular colonies	Yellow to orange circular colonies formed	Purple, moist, round colonies	*Stapphylococcus aureus*
FO40	Whitish-grey circular colonies formed	No colonies formed at all	Greenish thin-branched colonies formed	*Escherichia coli*

MO8	Yellow to golden circular colonies formed	Yellow to orange circular colonies	No growth	*Stapphylococcus aureus*
MO9	,Yellow to golden circular colonies	Yellow to orange, round circular colonies formed.	No growth	*Stapphylococcus aureus*
M010	Brownish to grey, moist colonies formed	Reddish to pink colonies formed with almost no growth.	Purple small colonies formed.	*Streptococcus mutans*
MO11	Whitish-grey circular colonies formed	No growth formed	Greenish thin-branched colonies formed.	*Escherichia Coli*
MO12	Yellow to golden circular colonies	Yellow to orange, circular colonies	No growth	*Stapphylococcus aureus*
MO13	Brownish to grey, moist colonies formed	Reddish to pink colonies formed with almost no growth	Purple smal colonies formed	*Streptococcus mutans*
MO14	Yellow to Golden circular colonies formed	Yellow to orange, circular colonies	No growth	*Stapphylococcus aureus*

MO32	Yellow to golden circular colonies	Yellow to orange, circular colonies	Purple,circular colonies formed.	*Stapphylococcus aerus*
MO36	Brownish to grey moist colonies formed	Reddish to pink colonies formed with almost no growth	purple small colonies formed	*Streptococcus mutans*
MO15	Whitish- grey colonies formed	No growth growth formed.	Dark pink moist colonies	*Klebsiella pneumonia*
MO16	Whitish- grey colonies formed	No growth occured	Green thin branched colonies	*Escherichia coli*
MO17	Brownish to grey moist colonies formed	Reddish to pink colonies formed	Purple, small colonies formed	*Streptococcus mutans*
MO18	Whitish-grey colonies formed	Reddish- pink colonies formed	Purple, small colonies formed	*Streptococcus mutans*
MO19	Yellow to golden circular colonies formed	Yellow to orange circular colonies	No growth	*Stapphylococcus aureus*

MO20	Whitish-grey circular, colonies formed.	No growth occured.	dark pink, moist colonies	*Klebsiella pneumonia*
MO21	Yellow-golden circular moist, colonies formed.	Reddish to pink, small moist colonies formed	Purple, small, round colonies formed	S*treptococcus mutans*
MO22	Whitish-grey circular, dry colonies formed.	No growth occured.	Dark pink moist colonies	*Klebsiella pneumonia*
MO22	Whitish-grey circular, dry colonies formed.	No growth occured.	Dark pink moist colonies	*Klebsiella pneumonia*
MO23	Brownish-grey, moist colonies formed.	Red, small colonies formed, with almost no growth	Purple, small, round colonies formed.	*Streptococcus mutans*
MO24	Whitish-grey, circular, colonies, formed.	No growth occured	Dark pink moist colonies	*Klebsiella pneumonia*
MO25	Yellow-golden circular, dry colonies formed.	Yellow to orange round, moist colonies formed.	Purple, small, round colonies formed.	*Streptococcus mutans*

MO26	Brownish-grey, circular, moist colonies formed.	Reddish to pink colonies formed with almost no growth.	Purple, small colonies formed.	*Streptococcus mutans*
MO27	Yellow to golden, circular colonies formed.	Yellow to orange round, moist colonies formed.	Purple, small, circular colonies formed.	*Streptococcus mutans*
MO28	Whitish-grey circular colonies formed	No growth formed.	Dark pink, moist colonies	*Klebsiella pneumonia*
MO29	,Yellow to golden circular colonies	Yellow to orange, round circular colonies formed.	Purple, round individual colonies formed.	*Stapphylococcus aureus*
MO30	Brownish to grey, moist colonies formed	Reddish to pink colonies formed with almost no growth.	Purple small colonies formed.	*Streptococcus mutans*
MO31	Whitish-grey circular colonies formed	No growth formed	Greenish thin-branched colonies formed.	*Escherichia Coli*

MO32	Yellow to golden circular colonies	Yellow to orange, circular colonies	Purple,circular colonies formed.	*Stapphylococcus aerus*

4..4 PERCENTAGE OF DOUBLE DISK SYNERGY TEST

Table 4.4

Probable organisms	AUGM	CAZ	CFX
Staphylococcus aureus	13.3	29.4	35.8
Klebsiella pneumonia	31.3	33.5	36.2
Streptococcus mutans	30.9	42.2	22.7
Escherichia coli	51.2	56.3	45.4

4..5 RATIO OF PROBABLE ORGANISMS SUSPECTED IN BOTH FEMALE AND MALE SAMPLE

Table 4.5

Bacteria Isolates	Percentage
Staphylococcus aureus	40%
Klebsiella pneumonia	25%
Escherichia coli	20%
Streptococcus mutans	15%

CHAPTER FIVE

DISCUSSION

Antibiotic resistance has emerged and is now known as worldwide issue in bacterial pathogens leading to extreme fails in most of the treatments in human diseases. Resistance against antibiotics by bacteria is a main and concern in the anti-infective therapy of both humans and animals. Bacteria are able to adapt fastly to new and complex environmental conditions such as the presence of antimicrobial drugs and, as the result, resistance may add more with the exposure to antimicrobial drugs. Very Critical concerns about bacterial antimicrobial resistance from community-acquired and food-borne pathogens have been increasing for a number of years, and have been raised at both national and international levels. (Aeastrup *et al* 2009).

Bacteria resistance of biocides (including disinfectants, antiseptics, preservatives and sterilants) has been studied and characterized as a leading world wide problem Only few scientific evidence is available to effectively weigh the risks of options antimicrobial resistance induced by the resistance to biocides. The inappropriate use of antibiotic plays a major duty in the resistance generation.Therefore, major efforts are urgently required by using phenotypic and genetic analysis of bacterial strains against antibiotics to increase the characterizing and identifying of mutant resistant strains and finding new processes to alleviate the spread of antimicrobial resistance infections.

Furthermore, research indicates that biocides and antimicrobial drugs may have some similar and common interactions and target sites with bacteria, which might express shared resistance mechanisms to both antimicrobials(Battah *et al* 2021).

CONCLUSION

Most of the oral swab of Caleb University students were positive of bacterial pathogens mainly of *Staphylococcus aureus* and the rest *Klebsiella pneumonia, Streptococcus mutans, Escherichia coli.* The total number of species of organisms isolated were four in number. I observed that most of the male students were positive of *Staphylococcus aureus* in the oral cavity than that of the female students. Antimicrobial resistance is rapidly becoming the major problem globally right now, it is best it is prevented by following good practices and proper use of antibiotics among individuals.

References

Al-Nawas, B., and Karbach, J. (2017). *S3 Leitlinie: Odontogene Infektionen Online verfügbar*at.

A.-P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hinndler *et al*. 2011 *Multidrug-resistant, extensively drug-resistance and pandrug-resistant bacteria...Clinical Microbiology and Infecti*

Dabour, Rajaa; Meirson, Tomer; Samson, Abraham *et al* 2016). "Global antibiotic resistance is mostly periodic". *Global antibiotic resistance is mostly periodic*.

Daniel Murell and James Macintosh *et al October* 2018 *Medical News today.*

Elizabeth Peterson *et al* 2018 *Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens.*

Gerber JS, Ross RK, Bryan M, Localio AR, Szymczak JE, Wasserman R, et al. (December 2017). *"Association of Broad- vs Narrow-Spectrum Antibiotics With Treatment Failure, Adverse Events, and Quality of Life in Children With Acute Respiratory Tract Infections".*

Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI (July 2011). "*Selection of resistant bacteria at very low antibiotic concentrations"*

Massimo Costalonga, Mark C Herzberg *et al* 2014 *Immmunology letters 162 (2), 22-38, 2014.*

Richard R Watkins *Antimicrobial resistance in methicillin- resistant Staphylococcus aureus to newer antimicrobial agents.*

Rudolf, J. D., Bigelow, L., Chang, C., Cuff, M. E., Lohman, J. R., Chang, C. Y., *et al* 2015. *Crystal structure of the zorbamycin-binding protein ZbmA, the primary self-resistance element in Streptomyces flavoviridis.*

Stephens *et al* 2019 An*tibiotic drug resistance* 2019.

Massimo Costalonga, Mark C Herzberg *et al* 2014. *Immmunology letters 162 (2), 22-38, 2014.*

Wirginia Krzyściak, Anna Jurczak and Jakub Piątkowski *et al* 2015 *Thhe role of human micro biome in dental cavity.*

Yohei Doi, MD, PhD, Jun-ichi Wachino, PhD, and Yoshichika Arakawa, MD, PhD *et al* 2016. *Infect Dis Clin North Am. Author manuscript.*