ISOLATION, CHARACTERIZATION AND HEMOLYSIN PRODUCTION OF BACTERIA ISOLATES FROM PALMS OF UNDERGRADUATE STUDENTS IN UNIVERSITY OF BENIN, BENIN CITY

BY

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CERTIFICATION

This is to certify that **Omoluwabi Uzezi Precious** with Mat. No. **LSC1605525** of the Department of Microbiology carried out this project.

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APPROVAL

This project was carried out by **Omoluwabi Uzezi Precious** (Miss) under supervision in partial fulfilment of the Award of a Bachelor of Science (B.Sc) degree in Microbiology.

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PROF. S. E. OMONIGHO DATE
(Head of Department)

DEDICATION

This work is dedicated to my family members; my mum, brothers, sisters and in memory of my dad for their loving support.

ACKNOWLEDGEMENT

My appreciation goes to God Almighty for all He has done for me and for giving me the grace to carry out this work successfully

My profound gratitude goes to my supervisor Prof. (Mrs) O.I. Enabulele for her time, contributions and support during this work.

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ABSTRACT

Human hands usually harbor microorganisms both as part of normal flora and microorganisms contacted from the environment. A prospective study was carried out in the University of Benin, Benin City, Nigeria. This study was aimed at identifying the types of bacteria associated with hands of undergraduate students in the University of Benin and to isolate and determine the hemolysin production of bacteria isolates present on the hands of the students. A total of 6 samples were obtained. The samples were analyzed using cultural, morphological and biochemical characteristics (citrate, indole, oxidase, catalase, motility, triple sugar ion and coagulase). The bacteria isolates recovered were *S. aureus* 3(25%), *C. jeikeium* 1(8.33), *S. epidermidis* 4(33.33), *S. hominids* 2(16.67) and *Escherichia coli* 2(16.67). All isolates of *S. aureus*, *E. coli* and *S. hominis* showed complete hemolysis (Beta hemolysis) while *S. epidemidis* and *C. jeikeium* were non hemolytic (Gamma hemolysis). All Gram positive isolates were resistant to amoxicillin, rifampin and septrin. Gram negative bacteria isolates were highly resistant to nalixidic acid, gentamicin, ciprofloxacin and ampicillin.

CHAPTER ONE

INTRODUCTION

The hand is the main body organ by which we work and maneuver through our everyday activities. Hands are the primary modes of transmission of many infectious diseases, particularly among those living and working in close proximity to one another such as class rooms, dormitories, camps and barracks. Close environments, doorknobs and other inanimate objects serve as resting vehicles for transmission and they all contribute to increased infection rates among these groups.

Human hands usually harbour microorganisms both as part of normal microflora and microorganisms contacted from the environment. These normal microflora such as *Staphylococcus aureus* resident in the human skin can therefore be passed from one individual to another (WHO, 2009). An easy way by which microbes that are not resident in the hands are contacted is by contact with surfaces such as doorknobs or handles, toilet handles and taps in restrooms. Microbes carried on the human skin are of two types i.e. resident and transient (WHO, 2009).

Pathogens that may be present on the hand as transient types includes *Salmonella* spp, *Shigella* spp and *Escherichia coli* (Nobel and Pitcher, 2013). Since human hands usually harbor microorganisms both as residents and transient, it is conceivable that transfer of pathogens could occur between people who access the same area or surfaces. The hands can also transfer intestinal tract infection and respiratory tract infections such as the corona virus. Appropriate hand hygiene practices can potentially result in minimal transmission of disease agents.

Most hand hygiene compliance studies have focused and documented this practice in hospital environment while few studies have focused on schools. In Nigeria, the need for such studies in schools is necessitated by the observation of National Hospital Discharge Survey (NDHS) outbreak of diseases and absence of enabling environment and facilities for the practice of hand hygiene (National Population Commission, 2015).

According to the US Centre for Disease Control and Prevention and the Association for Professionals in Infection Control and Epidemiology, simple hand washing is the single most important and effective method of preventing the spread of transmissible disease (Bloomfield *et al*., 2007).

AIM AND OBJECTIVES

AIM

This study therefore seeks to identify the types of bacteria associated with contaminated hands of undergraduate students in the University of Benin, to isolate and also characterize bacteria present on the hands of students.

OBJECTIVES

I. To isolate bacteria from palms of undergraduates in the university of Benin, Ugbowo campus.

II. To determine the antibacterial susceptibility patterns of isolates.

III. To determine hemolysin production by the isolates.

CHAPTER TWO

LITERATURE REVIEW

The human hand (palm)

The palm (volar), which is the central region of the anterior part of the hand is located superficially to the metacarpus and are the primary mode of transmission of many infectious diseases.

Handshaking is a known vector for bacterial transmission between individual because a number of infectious diseases can be spread from one person to another by contaminated hands. These diseases includes gastrointestinal infections such as Salmonellosis, and respiratory infections such as influenza, colds and corona virus (COVID-19).

Feces (poop) from people or animals is an important source of germs like *Salmonella, E.coli* 0157 and norovirus that cause diarrhea, and it can spread some respiratory infections like adenovirus and hand-foot-mouth disease. These kinds of germs can get onto hands after people use the toilet or change a diaper, but also in less obvious ways, like after handling raw meats that have invisible amounts of animal poop on them. A single gram of human feces which is about the weight of a paper clip can contain one trillion germs (Frank et al., 1998). Germs can also get onto hands if people touch any object that has germs on it because someone coughed or sneezed on it or was touched by some other contaminated object. When these germs get onto hands and are not washed off, they can be passed from person to person and make people sick (Aiello et al., 2008).

Microorganisms isolated from the palms

According to a study done on the Study of Hand Contamination and Hand Washing Practices among Medical Students in Egypt (Wilson, 2005), 10 different bacterial species were identified. Namely: *Staphylococcus aureus, Staphylococcus haemolyticus Staphylococcus capitis, Staphylococcus hominis, Staphylococcus epidermidis, Kocuria rhizophila,* and *Arthrobacter aurescens*. *Staphylococcus aureus* was isolated from the hands of 21 students (18 males and 3 females) after toilet use of which 15 belonged to the group that did not use soap to wash hands. Before toilet use, *Staphylococcus aureus* was isolated from the hands of only three students (Wilson, 2005).

Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Micrococcus spp., and *Escherichia coli* were identified from the hands and mobile of University students in Jordan (Waleed *et al*., 2019).

Research done on isolation and identification of bacteria associated with the palms of primary school pupils in Wukari, North East, Nigeria indicated that the following bacteria isolates where obtained; *staphylococcus aureus, staphylococcus epidermidis*, *enterococcus faecalis, Esherichia coli*, and *shigella dysenteriae* (Kenneth *et al*., 2018).

A study carried out on the comparison of the bacteria found on the hands of homemakers and neonatal intensive care unit nurses in New York City shows that the five most prevalent species of bacteria found on the hands of homemakers were: *Pseudomonas fluorescens/putida* , *Staphylococcus Warner, Klebseilla pneumonia, S. aureus* and *Enterobacter cloacae*. The five most prevalent species of bacteria found on the hands of the nurses were: *S. epidermidis*, *S. Warner, Enterococcus faecalis*, *S. hominis* and *Enterobacter agglomerans (Aiello et al., 2003).*

Sources of hand contamination

Hand washing with soap and water is a universally accepted practice for reducing the transmission of potentially pathogenic microorganisms. However, liquid soap can become contaminated with bacteria and poses a recognized health risk in health care settings. In particular, bulk-soap-refillable dispensers (ones in which new soap is poured into a dispenser) are prone to bacterial contamination, and several outbreaks linked to the use of contaminated soap in health care settings have been reported (Weber *et al*., 2007).

Bulk-soap-refillable dispensers are the predominant dispenser type in community settings, such as public restrooms. However, few studies have been conducted to evaluate the occurrence of microbial soap contamination in community settings. One study, conducted in Japan, examined bacterial contamination of hand washing soaps obtained from restrooms of various public use facilities. The authors found 17 different species of bacteria, many of which were opportunistic pathogens, including *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* species, and *Pseudomonas* species. Recent studies conducted in the United States demonstrated that 25% of bulk-soap-refillable dispensers in public restrooms were excessively contaminated (Chatman *et al*., 2011). Bacterial loads averaged more than 106CFU/ml of soap, and 16% of the samples contained coliform bacteria. Interestingly, of the 15 different species isolated in this study, 7 were identical to those found in the Japanese study, including both *K. pneumoniae* and *S. marcescens*. Both *S. marcescens* and *K. pneumoniae* are opportunistic pathogens known to transmit via the hands (Reybrouck, 1983).

Washroom door handles are a focal point for the accumulation of harmful fecal based bacteria, causing spread of infections. The hands can also be contaminated by touching contaminated surfaces, raw meats and vegetables etc.

Staphylococcus aureus

S. aureus is a facultative aerobic, Gram-positive coccal (round) bacterium also known as "golden staph". *S. aureus* is nonmotile and does not form spores. *S. aureus* is one of the most common causes of bacteremia and infective endocarditis.

S. aureus infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person, and contact with objects used by an infected person

such as towels, sheets, clothing, or athletic equipment. Joint replacement put a person at particular risk of septic arthritis, staphylococcal endocarditis (infection of the heart valves), and pneumonia (Kuehnert *et al*., 2005).

S. aureus can cause various skin and soft tissue infections (Tony *et al*., 2015), particularly when skin or mucosal barriers have been breached that is when there is a cut or wound.

staphylococcus epidermidis

S. epidermidis is a very hardy microorganism, consisting of nonmotile,Gram positive cocci, arranged in grape-like clusters. It forms white, raised, cohesive colonies about 1–2 mm in diameter after overnight incubation, and is not hemolytic on blood agar(Salyers and Whitt, 2002). It is a catalase -positive, coagulase-negative, facultative anaerobes that can grow by aerobic respiration or by fermentation. Some strains may not ferment.

Staphylococcus hominis

Staphylococcus hominis is a coagulase-negative member of the bacterial genus staphylococcus , consisting of Gram positive, spherical cells in clusters. It occurs very commonly as a harmless commensal on human and animal skin and is known for producing thioalcohol compounds that contribute to body odor (Rudden *et al*.,2020). Like many other coagulase-negative staphylococci, *S. hominis* may occasionally cause infection in patients whose immune system are compromised, for example by chemotherapy or predisposing illness.

Escherichia coli

Escherichia is a genus of Gram negative ,non-spore-forming,facultative anaerobes, rod-shaped bacteria from the family Enterobactericeae (Tenaillon *et al*., 2010).

Escherichia coli O157:H7 is a serotype of the bacterial species Escherichia coli and is one of the ships like toxins producing type of *E. coli*. It is a cause of disease, typically foodborne disease, through consumption of contaminated and raw food (Gally and Stevens, 2017). Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhea , and to kidney failure; these have been reported to cause the deaths of children younger than five years of age, of elderly patients, and of patients whose immune systems are otherwise compromised.(Karch *et al*.,2005).

E.coli infection is developed by ingesting certain strains of *E.coli* bacteria. The bacterial travels down to the digestive tract, releases a toxin called Shiga toxin. This toxin damages the lining of the small intestine and causes an infection.

Corynebacterium jeikeium

Corynebacterium jeikeium is commonly found in the human skin flora. Patients who are diagnosed with *C. jeikeium* show signs of normal bacteria infection such as fever. The predicted virulence factor of *C. jeikeium* are mainly involved in ensuring the availability of Exogenous fatty acids by damaging the host tissues. Studies done on the presence of plasmids in *C. jeikeium* have shown that instead of extra Chromosomal DNA, it is the bacteria chromosome that encodes most of the multiresistance phenotype. Because of its broad spectrum resistance to antimicrobial agents, the susceptibility of *C. jeikeium* strains is studied to a wide range of antibiotics. The strains were separated into two groups depending on the susceptibility to erythromycin, with one group representing resistant organism and the other representing susceptible organism (Wang *et al*., 2001).

Antibiotics susceptibility pattern

Studies carried out by Waleed *et al* (2019) on hand isolates reported that resistance rates of the isolates to the antibiotics tested were quite variable. *S. aureus* were most resistance clindamycin (35.7%) and least to trimethoprim/sulfamethoxaxole and cefepime (14.2%) respectively. *S. epidermidis* isolates were not resistant to penicillin (43.2%) and least to ciprofloxacin, chloramphenicol and amoxicillin /clavulanic acid. *S. pyogenes* was susceptible to most of the antibiotics tested and *S. pneumoniae* (25%) was resistant erythromycin.

In a research carried out by Mnyambwa *et al,* (2021) on antibiotic resistance pattern of bacteria isolates. In this study Gram negative bacteria were prevalent although both Gram negative and Gram positive organism demonstrated a high level of resistance to widely used antibiotics tested. *E.coli* isolates were highly resistant to ampicillin (100%), amoxicillin-clavulanic acid (75%), gentamicin (70.2%), tetracycline (70.2%) and ciprofloxacin (42.6%) and least resistant to ceftriaxone, meropenem, and nalixidic acid. *Klebsiella spp* demonstrated resistance to ampicillin (100%), amoxicillin-clavulanic acid (90.9%), gentamicin (64.7%), and ceftriaxone (55.6%), ciprofloxacin (52.6). *S. aureus* was highly resistant to erythromycin (76.3%), gentamicin (54%),ciprofloxacin (40%) and clindamycin (34.9%) and was resistant to cefoxitin (66.7%).

Preventive measures to minimize hand contamination

Hand hygiene is the act of cleaning one's hands for the purpose of removing soil, dirt and microorganisms. It is a general term that applies to hand washing, antiseptic hand washing, alcohol based hand rub or surgical hygiene/antiseptic (Lax and Smith, 2014). Hand washing which is the easiest and commonest among these hand hygiene practices refers to washing hands with plain soap and running water and remains the most sensible and affordable strategy for hand hygiene among the general population (WHO, 2012). School children can be encouraged to wash their hands with water and a small amount of wood ash if soap is not available and water points should be close to the classrooms as much as possible (Aremu, 2012). International agencies and governments because of the obvious benefits of hand hygiene in infectious disease reduction have been mounting interventions to improve the adoption of hand washing as a standard practice among community members. In Nigeria, hand washing was introduced as one of the strategies for hygiene promotion in the Federal government of Nigeria/UNICEF/water sanitation and hygiene (WASH) programme in 2004, it was also relaunched on 20th May 2008 as one of the programmers designed to mark the international year of sanitation declared by the united nations general assembly (Luby *et al*., 2005).

CHAPTER THREE

MATERIALS AND METHOD

MATERIALS
MEDIA

Nutrient agar (TM Media, India)

Lab-Lemco powder	1.0g
Yeast extract	2.0g
peptone	5.0g
Sodium chloride	5.0g
Agar	15.0g
PH adjusted to	7.4
Distilled water	1000ml

Preparation of nutrient agar

Nutrient agar was used as nutrient agar slant to store isolate for further test. This was prepared by dissolving 28.0g, of the nutrient agar powder in one litre of distilled water and then boiled to dissolve after which it was autoclaved at 121°c for 15 minutes to sterilize it. It was then cooled at 45°c and then aseptically dispensed into sterilize slant bottle and slanted to allow cooling and to solidification.

MacConkey Agar (TM Media, India)

Peptone	20.0g
Lactose	10.0g
Bile salt	5.0g
Sodium chloride.	5.0g

Neutral red	0.075g
Agar	12.0g
pH	7.4

Preparation

The MacConkey agar medium (Mac), which was used as the primary culture medium, was prepared by dissolving 52,9g of the commercially available Mac powder in 1000m1 of distilled water. It was followed by cooling to 45°c and then aseptically dispensed into petri dishes for solidification before inoculating with the organisms by making use of a swab stuck containing the samples from palms of students. The plates were incubated at 37°C for 24hours.

Mueller Hinton Agar (TM Media, India)

Beef extract	2.0g
Acid Hydrolysate of casein	17.50g
Starch	1.50g
Agar	17.00g
Distilled	1000ml

Preparation

Mueller Hinton agar is used for antimicrobial Susceptibility testing. 38g of Mueller Hinton agar is suspended in 1000ml of water and autoclaved at 121°C for 15minutes. This was allowed to cool to room temperature which was dispensed into sterile Petri dishes and allowed to solidify before inoculating and aseptically placing antibiotics disc.

Stains and reagent

I. Crystal violet solution

Crystal violet	2.0g
Ammonium oxalate	9.0g
Ethanol (absolute)	20.0ml
Distilled water	80.0ml

II. **Lugol's iodine**

Iodine 1.0g

Potassium iodide 2.0g

Distilled water. 300ml

III. **Decolorized acetone**

IV. **Safranin**

Defranin 0.5g

Distilled water. 100ml

Simmons citrate agar (TM Media, India)

Sodium chloride 5.0g

Ammonium dihydrogen phosphate. 1.0g

Magnesium sulphate 0.2g

Sodium citrate 2.50g

Bromothymol blue 0.018g

Dipotassium phosphate 1.0g

pH 7.0g

Distilled water 1000ml

Preparation

24.28g of Simmons citrate agar was suspended in 1000ml of water. It was properly mixed and distributed into tubes sterilized by autoclaving at 121°C for 15minutes. The tubes were cooled in a slant position before inoculation of a 24 hours culture.

SIM medium (HiMedia, India)

HM peptone 3.0g

Peptone 30.0g

Peptonized iron 0.2g

Sodium thiosulfate 0.025g

Agar 3.0g

pH 7.3

Preparation
Suspend 36.23g of SIM medium in 1000ml of distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and autoclave at 121°C for 15 minutes. Allow tubes to cool in an upright position.

Kovacs's reagent (HiMedia, India)

p-dimethylamino benzaldehyde 5.000

Amy alcohol 75.000

Concentrated HCl 25.000

Triple sugar ion agar (HKM, Ireland)
Beef extract 3.0g

Yeast extract 3.0g

Peptone 20.0g

Glucose 1.0g

Lactose 10.0g

Sucrose 10.0g

Ferrous sulphate or ferrous ammonium sulphate 0.2g

NaCl 5.0g

Sodium thiosulfate 0.3g

Phenol red 0.024g

Agar 13.0g

Distilled water 1000ml

Preparation
Suspend 64.2g of TSI agar medium in 1000ml of distilled water. Heat to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 121°C for 15 minutes. Cool in a slanted position.

Gram positive disc (Maxi disc)
CN- Cephalexin 10µg
APX- Ampiclox 30µg
Z- Zinnacef 20µg
AM- Amoxicillin 30µg
R- Rifampin 25µg
CPX- Ciprofloxacin. 10µg
S- Streptomycin 30µg
SXT- Septrin 30µg
E- Erythromycin 10µg
PEF- Perfloxacin 10µg

Gram negative disc (Maxi disc)
CEP-Ceporex 30µg
OFX- Ofloxacin 10µg
NA- Nalidixic acid 30µg
PEF- pefloxacin 10µg
CN- Gentamicin 30µg
AU- Augmentin 10µg
CPX- ciprofloxacin 30µg
SXT- Sulfamethoxazole 30µg
S- Streptomycin 30µg
PN- ampicillin 10µg

METHODS

Collection of samples
A total of 6 samples was collected randomly from the palms of undergraduates with a sterile cotton swab sticks. The swab sticks were transferred immediately to the laboratory to prevent dryness.
Following collection of samples, nutrient agar was prepared and sterilized by autoclaving at 121°C for 15 minutes. The media was poured into Petri dishes before before getting solidified. The swab sticks were immediately streaked on the plates of solidified nutrient agar and incubated at 37°C for 24 hours.

Isolation of pure culture
Samples were cultured on sterile petri-dishes of Nutrient agar and incubated at 37°c for 24 hours. Distinct colonies were purified by sub-culturing into nutrient agar plates and incubated again overnight at 37°C. Pure cultures were stored in nutrient agar slants.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATES
Standard bacteriological techniques were used to isolates, characterize and identify bacteria from the respective samples. Criteria included; cultural and morphological characteristics as well as biochemical test

BIOCHEMICAL TEST

1ndole test
A sterilized tube containing 4ml of SIM medium was taken. This was inoculated aseptically by taking the growth from a 18-24hours culture and incubated at 37°C for 24 hours. After which 0.5ml of Kovacs's reagent was added to the sterilized tube containing the SIM medium. Formation of pink to red color in the reagent layer on top of the medium within seconds of adding the Kovacs's reagent indicated a positive reaction.

Citrate utilization test
An isolated colony from the nutrient agar as picked with a wire loop, then inoculated into a sterilized tube containing citrate agar and inoculated at 37°C and examined daily. A positive test was indicated by a change from green to blue.

Motility test
Sulfide indole motility (SIM) mediums used for the detection of motility in bacteria. In carrying out this test, a bacteria culture is inoculated into the sterilized test tube containing SIM medium with the aid of a sterile needle/wire. The bacteria culture was stabbed two-third of the way into the media and this is inoculated at 37°C for 24 hours. Visible stab line, with cloudiness of the agar or other presence of turbidity as a result of organism migrating from the stab line to diffuse into the medium indicates a positive result. There is no visible clear line and the agar media is clear for a negative result.

Oxidase test
This test is done to determine the ability of the organisms to produce the cytochrome oxidase enzyme.
Filter paper was taken and soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloridd. After which the paper was moistened with sterile distilled water. The colony to be tested was transferred with a sterile loop and smeared on the filter paper. The inoculated area of the paper was observed for a color change to deep blue or purple within

10-30seconds.

Catalase test

With the use of a sterile inoculating loop, a small amount of organism was collected from a well isolated 18-24hours colony and placed on a microscopic slide. Be careful not to pick any agar. Using a dropper or Pasteur pipette, add one drop of 3% H_2O_2 to the organism on the microscopic slide without mixing. Formation of bubbles means the result is positive.

Triple sugar ion test (TSI)

Triple sugar ion test was done to differentiate among the different groups or genera of the Enterobacteriacae based on the ability to reduce sulfur and ferments carbohydrates. Triple sugar ion agar slants were prepared in the test tubes by autoclaving at 121°C for 15minutes. A small amount of the experimental bacteria from 24 hours old pure culture was inoculated into the tubes by means of a stab streak inoculation method with an inoculating needle. The screw caps were not fully tightened and the tubes were incubated for 24hours at 37°C. Fermentation is indicated by yellowing of the butt and the slant of Triple Sugar Iron (TSI) agar media. If gas was formed during the fermentation, it is shown in the butt either by the formation of bubbles or cracking.

Coagulase test

The test was carried out because of the presence of positive cluster cocci. A drop of normal saline was placed on a slide; colony of the test organism was emulsified in the saline. A drop of human plasma was added and mixed gently. After about 10seconds, it was observed for blood clotting. The presence of Agglutination means a positive result, no Agglutination means a negative results.

Hemolysis test

Blood agar is enriched medium used to culture fastidious bacterial. Hemolysis test is carried out by suspending 28g of nutrient agar powder in 1litrd of distilled water an autoclaved at 121°C for 15minutes. After autoclaving the nutrient agar is allowed to cool but not solidified. 5% of sterile defribrenated blood is added to the sterilized nutrient agar and mixed gently avoiding bubbles. The mixture of Nutrient agar and blood is dispensed into sterile plates. With the use of a sterile loop, an isolated colony was inoculated into the blood agar. Gamma hemolysis is indicated by lack of hemolysis, beta hemolysis indicates a clear zone while alpha hemolysis caused a green and brown discoloration in the medium.

Antibiotic Susceptibility test

The antibiotic sensitivity pattern of the isolates was determined using the diffusion method. Mueller Hinton agar plates was prepared and appropriately labeled. The plates were inoculated with the standardized bacterial cultures by spread plate technique. The inoculated plates were left to dry for 15minutes. Commercially available antibiotics disc containing varying concentration of various types of antibiotics were placed at adequate distances off each of the seeded agar plates with the aid of sterile forceps under aseptic conditions. The antibiotics disc was; ceftaizidime(caz) (30g), cefuroxime(crx) (30g), gentamicin(gen) (10g), cefixime(cxm) (5g), ofloxacin (ofl) (5g), augmentum (Aug) (30g), nitrofurantoin (Nit) (300g), ciprofloxacin (cpr) (5g). The plates were incubated for 24hours. The resultant

visible zones of inhibition were measured and interpreted using the criteria published by the clinical and laboratory standard institute.

CHAPTER FOUR
RESULTS

Five (5) bacteria species were isolated from the palms of undergraduate students in the University of Benin (Table 1). *Staphyloccocus epidermidis* were the most common isolates (33.3%). Females had the highest occurrence of isolates 3(75%) and male 1(25%). *Staphylococcus aureus* were isolated from 25% of samples. *Corynebacterium jeikeium* were isolated from 8.33% of samples while *Escherichia coli* and *staphylococcus hominis* were both isolated from 16.67% of the sample. Table 2 shows the cultural, morphological, and biochemical characteristics of bacteria isolates recovered from the palm. Table 3 shows the hemolysin production of the isolates. All isolates of *S. aureus, E. coli* and *S. hominis* showed complete hemolysis (Beta hemolysis) while *S. epidermidis* and *C. jeikeium* were non hemolytic (Gamma hemolysis). Table 4 shows the antibacterial resistance pattern of Gram positive bacteria isolates. *S. aureus* were highly resistant to erythromycin (100%), rifampin (100%), amoxicillin (100%), septrin (66.67%), and Cephalexin (66.60%) and least resistant to Ampiclox (33.3%), zinnacef (33.3%), ciprofloxacin (33.3%), and perfloxacin (33.3%). *S. epidermidis* were highly resistant to amoxicillin (100%), septrin (100%), erythromycin (100%) and Cephalexin (75%) and least resistant to Ampiclox (25%), rifampin (25%), ciprofloxacin (50%) and Streptomycin (25%). *S. hominis* were resistant to amoxicillin (100%), rifampin (50%), ciprofloxacin (100%), septrin (50%), erythromycin (50%) and perfloxacin (100%). *C. jeikeium* were resistant to Cephalexin, amoxicillin, rifampin, streptomycin, septrin and perfloxacin (100%). Table 5 shows the antibacterial resistant pattern of Gram negative bacteria isolates. *Escherichia coli* were highly resistant to nalidixic acid, gentamicin, ciprofloxacin, and ampicillin (100%) and least resistant to ceporex, augmentin, sulfamethoxazole and Streptomycin (50%).

Table.1 Bacteria isolates recovered from palms of undergraduate students

Bacteria isolates	Number of isolates	Male no of isolates (%)	Female no of isolates (%)
S. aureus	3(25)	2(66.67)	1(33.3)
E. coli	2(16.67)	0	2(100)
S. hominis	1(8.33)	0	1(100)
S. epidermidis	4(33.33)	3(75)	1(25)
S. hominis	2(16.67)	1(50)	1(50)

N=12

Table.2 Cultural, morphological and biochemical characteristics of bacteria isolates from the palm

Characteristics	1	2	3	4	5
Cultural					
Size	2mm	1mm	1mm	3mm	Pinpoint
Shape	Round	Round	Round	Round	Clublike
Elevation	Raised	Raised	Raised	Raised	Raised
Margin	Smooth	Smooth	Smooth	Smooth	Rough
Color	Yellow	White	White	Grayish white	Grayish white
Morphological					
Gram stain	+	+	+	-	+
Cell types	Cocci	Cocci	Cocci	Rod	Short rod
Cell arrangement	Cluster	Cluster	Cluster	Singly	Cluster
Biochemical					
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Indole	-	+	+	+	+
Mannitol	+	+	-	+	-
Coagulase	+	-	-	-	-
Motility	-	-	-	+	-
Citrate	+	-	-	-	+
TSI test					
H_2S	-	+	+	-	-
Gas	-	+	+	+	+
Sucrose	+	+	+	+	-
Lactose	+	+	+	+	-
Glucose	+	+	+	+	-
Probable isolates	*S. aureus*	*S. epidermidis*	*S. hominis*	*E. coli*	*C. jeikeium*

Table.3 Hemolysis test of bacteria isolated from the palm

Bacteria isolates	Number of isolates	Beta hemolysis	Gamma hemolysis
S. aureus	3(25.00)	3(100)	0(0)
C. jeikeium	1(8.33)	0(0)	1(100)
E. coli	2(16.67)	2(100)	0(0)
S. epidermidis	4(33.33)	0(0)	4(100)
S. hominis	2(16.67)	2(100)	0(0)

n=12

Table.4 Antibacterial resistance patterns of Gram positive bacteria isolates

Bacteria isolates	No of isolates	CN	APX	Z	AM	R	CPX	S	SXT	E	PEF
S. aureus	3(25)	2(66.67)	1(33.3)	1(33.3)	3(100)	3(100)	1(33.3)	0(0)	2(66.68)	3(100)	1(33.3)
S. epidermidis	4(33.3)	3(75)	1(25)	0(0)	4(100)	1(25)	2(50)	1(25)	4(100)	4(100)	0(0)
S. hominis	2(16.67)	0(0)	0(0)	0(0)	2(100)	1(50)	2(100)	0(0)	1(50)	1(50)	2(100)
C. jeikeium	1(8.33)	1(100)	0(0)	0(0)	1(100)	1(00)	0(0)	1(100)	1(100)	0(0)	1(100)

KEY
O.O- No visible inhibition
CN- Cephalexin
APX- Ampiclox
Z- Zinnacef
AM- Amoxicillin
R- Rifampin
CPX- Ciprofloxacin
S- Streptomycin
SXT- Septrin
E- Erythromycin
PEF- Perfloxacin

Table.5 Antibacterial resistance pattern of Gram negative bacteria isolates

Bacteria isolates	No of isolates	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
E. coli	2(16.6)	1(50)	0(0)	2(100)	0(0)	2(100)	1(50)	2(100)	1(50)	1(50)	2(100)

KEY
O.O- No visible inhibition
CEP-Ceporex
OFX- Ofloxacin
NA- Nalidixic acid
PEF- pefloxacin
CN- Gentamicin
AU- Augmentin
CPX- ciprofloxacin
SXT- Sulfamethoxazole
S- Streptomycin
PN- ampicilin

CHAPTER FIVE
DISCUSSION, RECOMMENDATION AND CONCLUSION

Discussion

Human hands usually habor microorganisms both as part of normal micro flora and microorganisms contacted from the environment. These normal micro flora such as *Staphylococcus aureus* resident in the human skin can therefore be passed from one individual to another (WHO, 2009).

In this study, the overall prevalence of *Staphylococcus epidermidis* among the students was 33.3%. *Staphylococcus epidermidis* was reported as one of the most common organism found on the palms of the students. This assessment of hand samples revealed that the hand harboreds various bacteria. The bacteria species isolated were four, which are *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hominis and Escherichia coli. Staphylococcus aureus* and *Staphylococcus epidermidis* were the most occurring isolates present in the samples of both male and female students. This is similar to the research work by Grice and colleagues in the year 2008, where *Staphylococcus aureus* and *Staphylococcus epidermidis* were thought from a cultural based research to be dominant (Grice *et al.*, 2008). *Staphylococcus aureus* is a normal flora of the skin and mucous membrane and is a common etiological agent of septic arthritis in human (Kolawole *et al*., 2007). It is an opportunistic pathogen and has been implicated in nosocomial infections (Anaukwu *et al*., 2015). *Staphylococcus epidermidis* is a major inhabitant of the skin, and in some areas it makes up more than 90% of the resident aerobic flora.

According to a work done by Imarenezor *et al*., (2008), to isolate and identify bacteria associated with palms, the results showed that *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most frequently isolated pathogens.

Some S. aureus strains can cause diarrhea, while others can be opportunistic pathogens as wound infections. Various kinds of microorganisms are easily contacted from the environment and the hands can be the most important means of which pathogenic microorganisms are transmitted. Some pathogenic organisms are spread by contaminated hands (Mathieu *et al*., 2013).

The result of the study shows that well over 95% of the students was contaminated with one bacterial pathogen or the other, and this is an indication of poor personal hygiene particularly hand hygiene, this agreed with the report of world health organization in 2017. This has been attributed to lack of appropriate hand washing facilities or poor location of these facilities in schools (Strina *et al*., 2002). Unfortunately, most schools do not provide appropriate hand washing facilities and where these facilities are available, they may be poorly located, have insufficient hand washing materials, be improperly used and most times be inaccessible to students.

Ofonime *et al*., (2018),in their assessment of bacteria carriage in the hands of school children in Calaber municipality, Nigeria recorded 80.0% of prevalence of bacteria hand carriage among school children. *Staphylococcus aureus* was the most common isolates (68.9%). Males had the highest occurrence of isolates 82(62.1%) than female 50 (37.8%).
In this study, *S. epidermidis* were resistant to erythromycin and amoxicillin and this is similar to a work done by Waleed *et al,* (2019) on antibiotics susceptibility of bacteria isolates recovered from the hands of University students in Jordan, 8 bacteria species were isolated and identified *S. epidermidis* were the most frequently isolated bacteria. *S. epidermidis* isolates were highly resistant to most antibiotics such as penicillin (43.2%), erythromycin (16.2%), clindamycin (13.5%), trimethoprim/sulfamethoxazole (13.5%) and norfloxacin (8.1%).
Hemolysis are thought to be responsible for many events in host cells. For example, iron may be a limiting factor in the growth of various pathogenic bacteria. Sine free iron may generate damaging radicals, free iron is typically maintained at low concentrations within the body. Red blood cells are rich in iron-containing heme. Lysis of these cells releases heme into the surroundings, allowing bacteria to take up the free iron (Sritharan, 2006).
According to a research done Moraveji *et al,* (2014) on the characterization of hemolysis of *Staphylococcus* Strains isolated from human and bovine in Southern Iran, most of the *Staphylococcus* strains isolated from bovines showed delta hemolysis while most of the human isolates produced alpha hemolysin.
As reported by WHO hand contamination is a leading cause of Nosocomial infection and the spread of multi-drug resistant bacteria leads to a significant contribution to outbreaks of infectious diseases.

Recommendation

It is recommended that regular hand washing is necessary even when the hands are not visibly dirty, before eating and after using the rest room. Hand hygiene is equally advocated after using the rest room in cases of diarrhea and after blowing the nose in case of an upper respiratory tract infection.

Conclusion

Various kinds of microorganisms are easily contacted from the environment and the hands can be the most important means by which pathogenic microorganisms are transmitted. Appropriate hygiene and public enlightenment of the hands in the spread of diseases should be advocated. Functional sinks should be placed in or around lecture theatres with appropriate soaps or sanitizer, as this would encourage students to wash their hands often. The use of hand sanitizer should be encouraged among the student after using the restroom and school campaigns should be organized to create awareness on the consequences of the use of contaminated hands.

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APPENDIX
MATERIALS

Sterile cotton swab, incubator, test tubes, test tubes racks, Petri dishes, wire loop, saline water, autoclave, forceps, labeling tape, marking pen, sterile niddle and Bunsen burner.

COMPOSITION AND PREPARATION OF MEDIA USED

Nutrient agar (TM Media, India)

Lab-Lemco powder	1.0g
Yeast extract	2.0g
peptone	5.0g
Sodium chloride	5.0g
Agar	15.0g
PH adjusted to	7.4
Distilled water	1000ml

Preparation of nutrient agar

Nutrient agar was used as nutrient agar slant to store isolate for further test. This was prepared by dissolving 28.0g, of the nutrient agar powder in one litre of distilled water and then boiled to dissolve after which it was autoclaved at 121°c for 15 minutes to sterilize it. It was then cooled at 45°c and then aseptically dispensed into sterilize slant bottle and slanted to allow cooling and to solidification.

BLOOD AGAR

5ml of sterilized blood was added into the nutrient agar mixture after autoclaving at 121°C for 15minutes , allowed to cool and dispensed aseptically into the Petri dishes.

MacConkey Agar (TM Media, India)

Peptone	20.0g
Lactose	10.0g
Bile salt	5.0g
Sodium chloride.	5.0g
Neutral red	0.075g
Agar	12.0g
pH	7.4

Preparation
The MacConkey agar medium (Mac), which was used as the primary culture medium, was prepared by dissolving 52,9g of the commercially available Mac powder in 1000m1 of distilled water. It was followed by cooling to 45°c and then aseptically dispensed into petri dishes for solidification before inoculating with the organisms by making use of a swab stuck containing the samples from palms of students. The plates were incubated at 37°C for 24hours.

Mueller Hinton Agar (TM Media, India)

Beef extract	2.0g
Acid Hydrolysate of casein	17.50g
Starch	1.50g
Agar	17.00g
Distilled	1000ml

Preparation
Mueller Hinton agar is used for antimicrobial Susceptibility testing. 38g of Mueller Hinton agar is suspended in 1000ml of water and autoclaved at 121°C for 15minutes. This was allowed to cool to room temperature which was dispensed into sterile Petri dishes and allowed to solidify before inoculating and aseptically placing antibiotics disc.

Stains and reagent

I. **Crystal violet solution**

Crystal violet 2.0g

Ammonium oxalate 9.0g

Ethanol (absolute) 20.0ml

Distilled water 80.0ml

II. Lugol's iodine

Iodine 1.0g

Potassium iodide 2.0g

Distilled water. 300ml

III. Decolorized acetone

IV. Safranin

Defranin 0.5g

Distilled water. 100ml

Simmons citrate agar (TM Media, India)

Sodium chloride 5.0g

Ammonium dihydrogen phosphate. 1.0g

Magnesium sulphate 0.2g

Sodium citrate 2.50g

Bromothymol blue 0.018g

Dipotassium phosphate 1.0g

pH 7.0g

Distilled water 1000ml

Preparation

24.28g of Simmons citrate agar was suspended in 1000ml of water. It was properly mixed and distributed into tubes sterilized by autoclaving at 121°C for 15minutes. The tubes were cooled in a slant position before inoculation of a 24 hours culture.

SIM medium (HiMedia, India)

HM peptone 3.0g

Peptone 30.0g

Peptonized iron 0.2g

Sodium thiosulfate 0.025g

Agar 3.0g

pH 7.3

Preparation
Suspend 36.23g of SIM medium in 1000ml of distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and autoclave at 121°C for 15 minutes. Allow tubes to cool in an upright position.

Kovacs's reagent (HiMedia, India)

p-dimethylamino benzaldehyde 5.000

Amy alcohol 75.000

Concentrated HCl 25.000

Triple sugar ion agar (HKM, Ireland)

Beef extract 3.0g

Yeast extract 3.0g

Peptone 20.0g

Glucose 1.0g

Lactose 10.0g

Sucrose 10.0g

Ferrous sulphate or ferrous ammonium sulphate 0.2g

NaCl 5.0g

Sodium thiosulfate	0.3g
Phenol red	0.024g
Agar	13.0g
Distilled water	1000ml

Preparation
Suspend 64.2g of TSI agar medium in 1000ml of distilled water. Heat to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 121°C for 15 minutes. Cool in a slanted position.

Gram positive disc (Maxi disc)

CN- Cephalexin	10µg
APX- Ampiclox	30µg
Z- Zinnacef	20µg
AM- Amoxicillin	30µg
R- Rifampin	25µg
CPX- Ciprofloxacin.	10µg
S- Streptomycin	30µg
SXT- Septrin	30µg
E- Erythromycin	10µg
PEF- Perfloxacin	10µg

Gram negative disc (Maxi disc)

CEP-Ceporex	30µg
OFX- Ofloxacin	10µg
NA- Nalidixic acid	30µg
PEF- pefloxacin	10µg

CN- Gentamicin	30µg
AU- Augmentin	10µg
CPX- ciprofloxacin	30µg
SXT- Sulfamethoxazole	30µg
S- Streptomycin	30µg
PN- ampicillin	10µg