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# A STUDY OF BACTERIAL ISOLATES AMONG HEALTHY SUBJECTS IN UNIVERSITY ENVIRONMENTS: A CASE STUDY OF ADEKUNLE AJASIN UNIVERSITY, AKUNGBA-AKOKO, ONDO STATE

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**Abstract**
Bacteria among other microorganisms viz fungi, viruses, protozoans and even some metazoans are ubiquitous and as well survive in different environments. Despite the fact that some microorganisms are beneficial while others are not, yet the diversity in habitat occupied by bacteria makes it easier for them to cause infections especially whenever the environments is conducive as in the case of opportunistic infections. The study was undertaken to identify the bacteria associated with hand palms and armpits of healthy subjects and also to evaluate the antibiotics sensitivity patterns of the bacteria. The study population consisted of seven males and four females healthy students of Adekunle Ajasin University Akungba-Akoko, Ondo State Nigeria between the age of eighteen (18) and thirty (30) years old. All the bacteria encountered were cultured on tryptone soya agar and eosin methylene blue agar, while only Gram-positive cocci were cultured on mannitol salt agar. The bacterial isolates were identified based on cultural, morphological and biochemical characteristics. Out of the sixteen bacteria obtained, 4(25%) were *Staphylococcus aureus*, while *Streptococcus* species and *Staphylococcus* species were 3(18.75%) each. Other bacteria identified were 2(12.5%) of each *Corynebacterium* species and *Klebsiella* species. In addition, *Clostridium* species and *Bacillus* species constituted 1(6.25%) each. Antibiotics susceptibility patterns of the bacteria were determined by disc diffusion method. All the bacterial isolates tested showed multiple antibiotics resistance.

**Keywords:** Hand palm, armpits, bacterial isolates, resistance patterns, susceptibility patterns, Gram positive, Gram negative, antibiotics resistance.

## Introduction

Microorganisms which include bacteria, fungi, viruses, protozoan and some metazoans are found in a wide of habitat. Although many microorganisms are beneficial and necessary for well of human being. So, microbial activities may cause undesirable cosequences such as food spoilage and disease, Prescott *et al.* (2008). Human and animals have abundant normal flora which is the mixture of microorganisms regularly found at any anatomical site that usually do not produce disease which can be resident. Various classes of organisms such as bacteria, viruses, fungi,and protozoa cause disease in man, Salle (2001). The importance of microorganism in disease was not immediately obvious to people and it took many years for scientist to establish the connection beetwen them and illnesses, Prescott *et al.*(2008).Under normal circumstances, microbes do not thrive, but the scratching of the surface of the skin provides oppourtunities for microbes to infect the tissue.For any aetiological agent to survive and spread, there must be an environmentally favoured place to reside, Murray (1990).Very few of the numerous microorganisms to which human are exposed to produce disease and the ability

 

to initiate disease is determined by an organism virulence and host factor, Murray (1990). The effect of infectious disease ranges from mild to severe or deadly depending on the physiology of the host and pathogen involved, Prescott *et al.*(2008). Investigations from the previous studies on microbiology on skin reveals *Staphylococcus epidermidis* as the dominant bacterial species found on hand. Other regular residents are *Staphylococcus hominis* and other coagulase-negative staphylococci, followed by coryneform bacteria such as propionibacteria, corynebacteria, dermabacteria, and micrococci, Hay (1993); Lee *et al*. (1994).

Furthermore, armpit is usually accompanied by body odour as a result of the normal functional of apocrine glands, one of the two type of glands that produce sweat which resulting to the body odour, Alkerman *et al.* (2005). Hence, the temporary resident skin flora often include nosocomial bacteria and fungi, Hay, (1993); Noble **(**1993). Fresh apocrine sweat is odourless but it develops it characteristics smell when sweat is broken by the hair and skin cell in the armpit and eaten by bacteria. It is actually the bacteria that make the smell by releasing a chemical called 3-methyl-2-hexenoid acid, Alkerman *et al.* (2005).

The microorganisms that are constantlypresent on body surfaces are commensal, these species are life-long members of the body's normal microbial community, but are not found everywhere.There are many areas of the human body that remain axenic, and in the absence of disease, are never colonized by microorganism. Resident microbiota typically colonize the surface of the skin, mucous membranes, digestive tract, upper respiratory system and distal portion of the urinogenital system; where they play major role in maintaining health and normal function, Tami and Port (2009).

The organisms are adapted to the non-invasive mode of life defined by the limitations of the environment. If forcefully removed from the restrictions of that environment and introduced into the bloodstream or tissue, these organisms may become pathogenic, Mandell *et al*. (2005).

Transient microflora may attempt to colonize thesome areas of the body as do resident microbiota, Tami and Port (2009). Transient microorganisms are also extremely important to understand. These include food borne micro organisms and even soil borne micro organisms that make their way into the human digestive tract and, depending upon the characteristics of the specific organism involved, either subtly or dramatically influence the overall health of the human system, Alkerman *et al.* (2005).

Infectious diseases, also known as communicable diseases, contagious diseases or transmissible diseases comprise clinically evident illness resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism, Abad *et al.* (1994). A common but most irritating body odour is armpit odour; it is so embarrassing; therefore hand hygiene cannot be overemphasized since it has been considered to be the most important tool in nosocomial infection control, Alkerman *et al.*, (2005); Nystrom (1994). Colonization of health care workers' hands with *Staphylococcus aureus* has been described to range between 10.5 and 78.3%. Up to 24,000,000 cells can be found per hand. The colonization rate with *Staphylococcus aureus* was higher among doctors (36%) than among nurses (18%), as was the bacterial density of *Staphylococcus aureus* on the hands (21 and 5%, respectively, with more than 1,000 CFU per hand). The carrier rate may be up to 28% if the health care worker contacts patients with an atopic dermatitis which is colonized by *Staphylococcus aureus,* Williams *et al.*(1999); Methicilin-resistant *Staphylococcus aureus* (MRSA) has been isolated from the hands of up to 16.9% of health care workers. Vancomycin resistance enterococcus (VRE) can be found on the hands of up to 41% of health care workers. However, every person has sweat gland which responsible for perspiration which eventually allow bacteria to thrive at the armpits, Alkerman *et al.* (2005).

 

Hand carriage of pathogens such as *Staphylococcus aureus*, methicilin resistance *Staphylococcus aureus* or *Staphylococcus epidermidis* has repeatedly been associated with different types of Nosocomial Infections (NI). Hence, boils (or furuncles) is a skin disease caused by inflammation of hair follicles, resulting in localized accumulation of pus and dead tissue, Alkerman *et al.* (2005). The analysis of outbreaks revealed that dermatitis on the hands of health care workers was a risk factor for colonization or for inadequate hand hygiene, resulting in various types of Nosocomial Infection. The hands and gloves of 44 health care workers were sampled after care of vancomycin resistance enterococcus positive patients. Gloves were vancomycin resistance enterococcus positive for 17 of 44 healthcare workers, and hands were positive for 5 of 44, even though they had worn gloves, Tenorio e*t al.* (2001). One health care worker was even vancomycin resistance enterococcus positive on the hands although the culture from the glove was negative; *Staphylococcus aureus* is the most common gram-positive bacterium causing Nis, Tenorio e*t al.* (2001). Its frequency among all pathogens in NIs varies between 11.1% and 17.2%, Wagner *et al.* (1997)*;* Voss e*t al*., (1994). Methicillin resistance *Staphylococcus aureus* (MRSA) is increasing worldwide, Wagner *et al.* (1997); Voss *et al.*, (1994); Sartor *et al*. (1995). *Staphylococcus aureus* can survive on hands for at least 150 minutes, whereas, about 90% of men and 70% of women have the bacteria that produces mulder odour in their armpit, Alkerman *et al.* (2005).

In addition, Staphylococcus is a genus of Gram-positive, non-motile, spherical bacteria occuring in grape-like clusters. They belong to family of Staphylococcaceae, and are facultatively anaerobic, Catalase-positive, Oxidase-negative, ferment glucose and have teichoic acid in their cell walls, Prescott *et al.* (2008).Two common species of Staphylococcus that live in association with human are *Staphylococcus epidermidis* which lives normally on the skin and mucous membranes and *Staphylococcus aureus*, which may occur normally at various locales, but in a particular on the nasal membranes, Kenneth (2008). Nevertheless, vancomycin resistance enterococcus survives on hands or gloves for up to 60 min. On inanimate surfaces, *Staphylococcus aureus* and methicillin resistance *Staphylococcus aureus* may survive for 7 months, with wild strains surviving longer than laboratory strains. Vancomycin resistance enterococcus may survive on surfaces for 4 months. The long survival on surfaces, together with the relatively short survival on hands, suggests that contaminated surfaces may well be the source of transient colonization despite negative hand cultures, Reboli *et al*. (1989).

*Staphylococcus aureus* is one of the major resistant pathogens; found on the mucous membranes and the human skin of around a third of the population, it is extremely adaptable to antibiotic pressure. Methicillin was then the antibiotic of choice, but has since been replaced by oxacillin due to significant kidney toxicity. CA-MRSA (Community-acquired MRSA) has now emerged as an epidemic that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most frequently identified antimicrobial drug-resistant pathogen in US hospitals. The epidemiology of infections caused by MRSA is rapidly changing. Outbreaks of community-associated (CA)-MRSA infections have been reported in correctional facilities, among athletic teams, among military recruits, in newborn nurseries, and among men who have sex with men, Bartzokas *et al*. (1983).

Meanwhile, with regards to the mechanism of Antibiotics Resistance Staphylococci, research by Zukaite *et al.* (2000); Alberts and Bruce (2008) reveals that hospital strains of *Staphylococcus aureus* are usually resistant to a variety of different antibiotics. So, a few strains are resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains are increasingly-reported. Methicillin resistance is widespread and most methicillin-resistant strains are also multiply resistant. Phagocytosis is the major mechanism for combating staphylococcal infection, Zukaite *et al.* (2000); Alberts and Bruce (2008). Hence, the treatment of hospital acquired infection is often caused by

 

antibiotic methicilin resistant strains (MRSA) and can only be treated with vancomycin or an alternative. However, many of the community acquired (CA) Staphylococcal infections are now methicillin resistant, (Chopra *et al*., 2005; Darren *et al*., 2006).

The control of Healthcare Associated Infections (HCAI) represents a major challenge to hospitals and healthcare providers. Transmission of pathogens on the hands of healthcare workers is the most common cause of cross infection, Damani (1997); hygienic hand wash should be performed in order to remove or destroy transient microorganisms and to substantially reduce resident micro-organisms during times when surgical procedures are performed and before all aseptic procedures on the ward. Hence, what should be used for performing a hygienic hand wash is an approved antiseptic detergent such as 4 % Chlorhexidine gluconate or 7.5% Povidone iodine) (Larson, 1995).

Armpits odour is caused by the bacteria which residence at the armpit the apocrine secretes viscous fluid which is kind of milky from the hair follicles while the eccrine gland secretes water fluid. The bacteria thrive more in the sweat under armpit leading to the odour. Other causes of the odour can be due to the digestive products which accumulated in the body, Alkerman *et al.* (2005)**.** If you sweat profusely try a solution of hydrogen peroxide ($H_2O$) 3% in concentration for washing underarms; use good deodorants and antiperspirant. Because, deodorant make the skin to be acidic and it disallow bacteria to thrive while anti-perspirant block the sweat gland to produce sweat skin floraare usually non-pathogenic are either commensal or mutualistic, Alkerman *et al.* (2005). Thus, the effect of extracellular products obtained from culture supernatant of *Staphylococcus aureus* strain products to a fresh medium stimulated growth already after 2 hours of incubation, with an approximately two-fold increase in cell density as compared to an unsupplemented medium, probably by promoting an initiation of growth accompanied by a reduction of the initial lag phase, Bukharin *et al.* (2000). Fibrinolysin is an enzyme derived from plasma of bovine origin or extracted from cultures of certain bacteria. It is used locally only and exclusively together with the enzyme desoxyribonuclease (extracted from bovine pancreas). Fibrinolysin and desoxyribonuclease both act as lytic enzymes. The combination is available as ointment containing 1 BU (Biological Unit) fibrinolysin and 666 BUs desoxyribonuclease per gram, Birk *et al.* (1983). More also, by catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier, hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability. Other studies suggest no contribution, or effects independent of enzyme activity, Csoka *et al.* (2001).Coagulase is an extracellular protein which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. Coagulase is a traditional marker for identifying *Staphylococcus aureus* in the clinical microbiology laboratory, Darren *et al.* (2006). With regards to staphylokinase;Many strains of Staphylococcus aureus express a plasminogen activator called staphylokinase. This factor lyses fibrin. The genetic determinant is associated with lysogenic bacteriophages. A complex formed between staphylokinase and plasminogen activates plasmin-like proteolytic activity which causes dissolution of fibrin clots, Chopra *et al*. (2005).

Furthermore, *Staphylococcus aureus* can express a toxin that specifically acts on polymorphonuclear leukocytes. Phagocytosis is an important defense against staphylococcal infection so leukocidin should be a virulence factor, Starr and Engleberg (2006). Exotoxins in *Staphylococcus aureus* can express several different types of protein toxins which are probably responsible for symptoms during infections. Those which damage the membranes of cells were discussed above under invasion or lyse erythrocytes, causing hemolysis, but it is unlikely that haemolysis is a relevant determinant of virulence *in vivo*. Leukocidin causes membrane damage to leukocytes, Alberts (2008).

 

Streptococcus is a genus of Gram-positive, nonmotile, spherical bacteria occuring in chains. Most species are saprophytes and some are pathogenic. Many pathogenic species are haemolytic.They are chemoheterotrophic, mesophilic, nonsporing, Gram-positive cocci, which are facultatively anaerobic and Catalase-negative, Prescott *et al.*(2008).Like Staphylococus, there are many species of Streptococcus found normally in the human body, many are part of the skin and upper respiratory tract of human, Elmer (2006). The family of Corynebacteriaceae, and the group contains aerobic and facultative anaerobes, catalase-positive, straight to slightly curved rods, often with tapperedvends, Prescott *et al.* (2008). A genus of Gram-positive, mostly aerobic, nonmotile, rod like bacteria that frequetly bear club-shaped swellings. The genus contains the species *Corynebacterium diphtheriae* and non-diphtherial corynebacterium, Coyle *et al.* (1990). Corynebacteria are non-sporulating, Pleomorphic bacillio. They are chemoorganotrophic and exhibit a fermentation metabolism under certain conditions; and are fastidious organism growing on enrichment medium, Kenneth (2008).

Hands with artificial fingernails harbour gram-negative bacteria more often than those without. Health care workers have three opportunities for the post contamination treatment of hands viz: the social hand wash, the hygienic and, the hygienic hand disinfection which normally consists of the application of an alcohol-based hand rub into dry hands without water, Hedderwick *et al*. (2000). About 5% of all patients admitted to hospitals develop a nosocomial infection. Contaminated hands of health care personnel are reported as a major route for the spread of nosocomial infections Aitken *et al*. (2001); Alfurayh *et al.* (2000). Notwithstanding, another bacteria is *Klebsiella species* which are gram negative rod shaped bacteria which exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate. Klebsiella was named after the German bacteriologist Edwin Klebs (1834–1913). Multiple-resistant *Klebsiella pneumoniae* have been killed in vivo via intra peritoneal, intravenous or intranasal administration of phages in laboratory tests. While this treatment has been available for some time, a greater danger of bacterial resistance exists to phages than to antibiotics, Cogen *et al*. (2008). Resistance to phage may cause a bloom in the number of the microbe in environment as well as among humans (if not obligate pathogenic). This is why phage therapy is only used in conjunction with antibiotics, to supplement their activity instead of replacing it altogether, Berrie (2007). Nowadays, many people may think bacteria are associated with unhealthy subjects only which is not at all. This research was carried out to examine bacteria associated with hand palms and armpits of healthy subjects, and to determine the in-vitro antibiotics sensitivity patterns of the bacteria encountered from the hand palms and armpits of the healthy subjects from Adekunle Ajasin university Akungba-Akoko, Ondo State, Nigeria.

## Materials and Methods

### Study Area

The study area comprises the town known as Akungba Akoko in Ondo State, Nigeria where a state government owned university is sited.

### Study Population

This study was executed among a few undergraduates from the *cital del* of higher learning in Akungba Akoko. So, the samples were collected from twelve healthy students of Adekunle Ajasin University Akungba-Akoko, Ondo State, Nigeria. Seven hand palm swabs and five armpit swabs at Akungba-Akoko. The individuals whose samples were collected ages range between 18 and 30 years old.

### Materials Used

The materials used in this study include petri-dishes, wire loops, sterile swab stick, bursen burner, autoclave, microscope, slides, oil immersion, McCartney bottle, test tubes, syringe and needle as well

 

as antibiotic sensitive disc which comprises augumentin (Aug), tetracycline (Trt), cloxacillin (Cxc), chloramphenicol (Chl), streptomycin (Str), cotrimoxazole (Cot), erythromycin (Ery) and gentamicin (Gen).

**Media and Reagents**
Nutrient agar, tryptone soya agar, eosin methylene, Mueller-Hinton agar, blood agar, mannitol salt agar, crystal violet, safranin , hydrogen peroxide, ethanol and saline water.

**Sterilization Techniques**
Glassware such as petri-dishes were washed with soap and rinsed thoroughly with clean water and were allowed to air-dry for few minutes. They were arranged in the canisters and put in hot air oven for two hours at $170^0$C. The media were sterilized using the autoclave at $121^O$C for 15 minutes.

**Collection of Samples**
The samples were collected using sterile swab sticks. Different swab sticks were used to gently rub the palms of the hands and armpits of the healthy subjects. The samples were collected within the shortest space of time and transported to the laboratory for further microbiological analysis.

**Media Preparation**
The media used for the isolation of organisms were nutrient agar, eosine methylene blue, mannitol salt agar, Mueller-Hinton agar, and all which were prepared following standard methods.

**Method of isolation**
With the aid of a sterile loop, a small or loopful portion of pure culture was taken from incubated tryptone soya agar medium and streaked again on the plates containing mannitol salt agar, eosin methylene blue. These plates of media were then incubated aerobically at 37°C for 24hours or more until growth was observed. After incubation, the cultural and morphological characteristic of distinct, well isolated colonies were studied. These included the shape, size, elevation, edges, opacity, surface and colour representative and well separated colonies obtained from sample were picked, heat fixed and gram stain. Stock cultures of pure isolates were labelled accordingly and stored.

**Methods of identification**
Preliminary characterization of bacteria isolates was based on gram stain, morphology and cultures. Furthermore, characterization was carried out with various biochemical tests. These tests included catalase, coagulase, indole production, citrate utilization, starch hydrolysis and growth characteristics on MSA and EMB agar, and blood agar haemolysis. All media were prepared according to the manufacturers' specification and sterilized unless where stated otherwise at 121°C for 15minutes.

**Gram Staining Technique**
A sterile wire loop was used to place a drop of sterile water on a grease-free slide. The loop was flamed and allow to cool, it was used to pick an inoculum from the plate and placed on the slide. The inoculum was gently emusified with the water forming smear. This was then heat fixed by passing it over the flame.The smears werethen flooded with crystal violet and left to stand for 60 seconds and were rinsed with water. Lugol's Iodine solution was poured on the slides and allowed to stay for another 60 seconds and later rinsed with water. The smears were then decoulorized with 95% ethanol which waswashed off immediately. The smears were counter stained with Safranin solution for 30 seconds. The slides finally rinsed with water and then allow ed to air dry. After air drying, the slides were then viewed under the micoscope using oil immersion (X100objective lens).Organisms that retain the purple colour of crystal violet are the Gram-positive organisms while those that retain red or

 

pick colour of ssfraning are Gram-negative organism. Other morphological characteristics were also examined and recorded as short or long rods, cocci in cluster or in chains.

**Catalase Test**
A sterile wire loop was used to transfer one drop of hydrogen peroxide on grease-free slide. A small portion of the inoculum of 24 hours old culture was picked and placed on the slide with hydrogen peroxide. The slide was observed for effervescence (i.e. production of bubbles). The result was recorded for each isolate as either Catalase-positive or Catalase-negative depending on the reaction shown. Positive result show the conversion of hydrogen peroxide to oxygen and water.

**Coagulase Test**
The test was performed with 24 hours old culture. A loopful of normal saline was placed on a clean slide and a small amount of the culture was emusified in the normal saline to get a homogenous suspension. A drop of human plasma was added and mixed for 5 seconds. A positive reaction was observed by the formation of easily visible white clumps; while organism without formation of white clump, is a negative for the reaction.

**Haemolysis Test**
All the isolates were streaked on the blood agar and they showed different growth characteristics on the agar. This test was done to show different kind of haemolysis which include alpha (α), beta (β) and gamma (γ) haemolysis. Some of the isolates tested lysed the red blood cells completely (β-haemolysis), some lysed it partially (α-haemolysis) other isolate did not lyse the red blood cell all (γ-haemolysis).

**Growth Characteristics on Mannitol Salt Agar**
Mannitol salt agar plates were streaked with the appropriate organism and inoculated at 37°C for 24 hours, after which the plates were examined for growthand fermentation of mannitol. Apparent growth of the organisms indicated salt tolerance. Fermentation of mannitol was indicated by a change in thecolour of the medium background of each colony from red to yellow.

**Carbohydrate test**
Phenol red (0.01g), sodium chloride (1.0g) and fermentable sugars (1.0g) were weighed into a conical flask containing 100ml of nutrient broth. The mixture was swirled to ensure that all the components in it dissolved. 5ml of the preparation was dispensed into test tubes containing inverted Durham tubes.
The tubes were covered with cotton wool and Aluminum foil and were sterilized using autoclave at temperature of 121$^{o}$C for 15 minutes. The sugars used include glucose, lactose and dextrose. In addition, all test tubes were inoculated with respective test organism aseptically and incubated at 37$^{o}$C for 3-5days depending on how fast the organisms can utilize the sugars. Changes in the colour of indicator (phenol red) from red to yellow indicate the utilization of the sugar (that is positive test), and if the gas is detected in the Durham tubes, it signifies the organism produces gas. Control test was done for each sugar by not inoculating any test organism in the sugar preparation.

**Antibiotic Sensitivity Test**
Sensitivity testing were carried out using comparative methods and based on Gram staining reaction. The bacterial isolates were subjected to an antimicrobial testing to determine their sensitivity pattern.

**Preparation of Inoculum**
Cultures of isolated organisms on nutrient agar slant were used to prepare the inoculum using a sterile wire loop. Approximately one isolated colony was transffered into 10ml of steriled distilled water

 

cointaining in the test tube and then mixed properly. The liquid now serve as source of inoculum containing 106CFU/ml of bacterial suspention.

**Inoculating of Plating Medium**
A sterile cotton woolo swab stick was dipped into the properly mixed inoculum and excess fluid was removed by rotating the swab against the inside wall of the surface of the prepared Mueller-Hinton Agar plates which was constituted according to manufacturers specification. The spread was done in four different planes by rotating the plane at 180°C each time. This was toensure that every part of the plate was inoculated.

**Placement of Disc**
A pair of forceps was flammed until red hot and cooled and it was then used to pick the antibiotics disc and it was as well used to place the discs on the inoculated plates. The discs were pressed down on the agar so as to be in complete contact with agar. The plates were incubated at 37°C for 18 hours.

**Results**
Identification of isolates was based on their cultural, morphological and characteristics. A total of sixteen isolates were cultured from all the eleven samples examined for the incidence of bacteria from hand palms and armpits of healthy individuals.

Out of the 16 isolates cultured 10(62.5%) were gram positive cocci while 4(25%) were gram positive rods and 2(12.5%) were gram negative rods. 4(40%) of the 10 gram positive cocci were smooth, convex, opaque, yellow with entire edges on tryptone soya agar while 1(10%) was opaque, circular, convex white porcelain with entire edges and 2(20%) were opaque, raised, smooth, white porcelain with entire edges whereas 3(30%) were opaque, raised, smooth, white porcelain with entire edges on tryptone soya agar.

Moreover, 2 (50%) of the four gram positive rods were opaque, raised, irregular with entire edges while 1(25%) was translucent, raised, creamy with lobate edge and 1(25%) was opaque, raised, irregular, curled edge and creamy. However, one (50%) of the two gram negative rods were was transparent, raised, irregular with entire edge while the other one (50%) was translucent, raised, irregular, creamy with curled edge.

In addition, the growth of the isolates encountered in this study was observed on both mannitol salt agar (MSA) and eosin methylene blue (EMB) agar. Hence, the gram stained reaction showed that 7(70%) of the 10 Gram-positive cocci appeared as cocci in clusters while the remaining 3(30%) appeared as cocci in short chain. Whereas, two (50%) of all the four Gram-positive rods appeared as long rods align along their axis in parallel in a manner somewhat similar to fence posts, one (25%) appeared as short rod and fat and one (25%) appeared as long chain of cell aligned along axis.

Furthermore, all the two gram negative rods in this study were gram stained as long rods. Also, the two isolates cultured showed large, mucoid, brownish growth on eosin methylene blue. The biochemical reactions of the bacterial isolates encountered in this study were also studied. Results of carbohydrate fermentation test (Table 1) show that out of the thirteen isolates tested, 10(76.92%) reduce glucose to acid without gas production and only 2(15.38%) isolates reduce glucose to acid with gas production, while 9(69.23%) and 2(12.23%) of the isolates tested from lactose and dextrose respectively.
However, only three (18.25%) of the 16 isolates were catalase negative while the rest thirteen (81.25%) isolates were catalase positive. 4(25%) were coagulase positive while the 12 (75%) isolates

 

were coagulase negative. Out of 13 isolates examined for haemolysis in blood agar, 4(30.77%) were alpha () haemolytic, 8(61.54%) were beta (β) haemolytic and 1(γ) was gamma haemolytic. Growth and fermentation of mannitol salt agar (MSA) in nine of the isolates were also studied. All the nine isolates tested showed tolerance by growing on the agar while 4(44.44%) of the isolates tested fermented mannitol in the agar.

Meanwhile, only two (12.5%) of all sixteen isolates were able to grow on eosin methylene blue (EMB) agar while the remaining 12(87.5%) isolates were not able to grow on the agar.

Based on these characteristics comparable to those enumerated in Bergey's manual (8th edition) and manual of Clinical Microbiology (5th edition), 7(43.75%) were identified as Staphylococci of which 4(57.14%) isolates were *Staphylococcus aureus* and 3(42.86%) were identified as *Staphylococcus species*. Other bacteria identified were 3(18.75%) *streptococcus species*, 2(12.5%) *Corynebacterium* species, 1(6.25%) *Clostridium* species, 1(6.25%) *Bacillus species*, 2(12.5%) and *Klebsiella species* (Table 1).

**Table 1: Profile of Bacterial isolates in relation to sources of Collection**

| Sources | Bacterial isolates | Number and prevalent rate (n= 16) |
|---|---|---|
| **Hand palm** | *Staphylococcus aureus* | 2 (12.5%) |
| **Armpit** | *Staphylococcus aureus* | 2 (12.5%) |
| **Armpit** | *Staphylococcus species* | 3 (18.75%) |
| **Armpit** | *Corynebacterium species* | 2 (12.5%) |
| **Hand palm** | *Bacillus specie* | 1 (6.25%) |
| **Hand palm** | *Clostridium specie* | 1 (6.25%) |
| **Hand palm** | *Streptococcus species* | 2 (12.5%) |
| **Armpit** | *Streptococcus species* | 1 (6.25%) |
| **Hand palm** | *Klebsiella species* | 2 (12.5%) |
| **Total** | | 16 (100) |

**Table 2: Incidences of Gram positive and Gram negative rods from Hand palms and Armpits**

| Bacterial isolates | Number and prevalent rate ( n=6) |
|---|---|
| *Corynebacterium species* | 2 (33.33%) |
| *Bacillus specie* | 1 (16.67%) |
| *Clostridium specie* | 1 (16.67%) |
| *Klebsiella species* | 2 (33.33%) |
| **Total** | **6 (100)** |

**Table 3: Incidences of bacteria from Hand palms and Armpits of Healthy Individuals In Relation to Age**

| Age (year) | Number of Individual | Frequency of bacteria |
|---|---|---|
| **18-21** | 2 (18.18%) | 4 (25%) |
| **22-25** | 6 (54.55%) | 7 (43.75%) |
| **26-30** | 2 (27.27%) | 5 (31.25%) |
| **Total** | **11 (100)** | **16 (100)** |

**Table 4: Frequency of bacteria cultured from Hand palms and Armpits in relation to Age and Sex**

| Age | Sex | | Frequency of bacteria |
|---|---|---|---|
| | Male | Female | |
| **18-21** | 1(14.29%) | 1 (25%) | 4 (25%) |
| **22-25** | 4(57.14%) | 2(50%) | 7 (43.75%) |
| **26-30** | 2(28.57%) | 1(25%) | 5 (31.25%) |
| **Total** | **7 (100)** | **4 (100)** | **16 (100)** |

**Table 5: Distribution of bacteria in relation to age, sex and sources of collection**

| Age | Sex | Sources of collection | Prevalent rate (%) |
|---|---|---|---|
| **18-21** | M | Hand palm | 2(12.5%) |
| **18-21** | F | Armpit | 1(6.25%) |
| **18-21** | M | Hand palm | 1(6.25%) |
| **22-25** | M | Hand palm | 4(25%) |
| **22-25** | M | Armpit | 1(6.25%) |
| **22-25** | F | Hand palm | 1(6.25%) |
| **22-25** | F | Armpit | 1(6.25%) |
| **26-30** | M | Hand palm | 2(12.5%) |
| **26-30** | F | Hand palm | 2(12.5%) |
| **26-30** | M | Armpit | 1(6.25%) |
| **Total** | | | **16 (100)** |

**M – Male F- Female**

**Table 6: Antibiotics resistance of the bacterial isolated from hand palms and armpits**

| Isolate code | Identification | Antibiotics to which isolates were resistance |
|---|---|---|
| **$HP_{1a}$** | *Streptococcus species* | Aug, Str, Tet, Cot, Chl, Cxc, Ery, Gen |
| **$HP_{1b}$** | *Klebsiella species* | Aug, |
| **$HP_2$** | *Klebsiella species* | Nt |
| **$HP_3$** | *Clostridium species* | Aug,Tet, Cxc |
| **$HP_{4a}$** | *Staphylococcus aureus* | Aug, Cxc, Cot |
| **$HP_{4b}$** | *Streptococcus species* | Aug, Cxc |
| **$HP_5$** | *Bacillus species* | Aug |
| **$HP_6$** | *Staphylococcus aureus* | Aug, Str, Tet, Cot, Chl, Cxc, Ery, Gen |
| **$AP_{1a}$** | *Staphylococcus species* | Aug, Cxc, Chl,Cot, Ery |

| | | |
|---|---|---|
| **$AP_{1b}$** | *Streptococcus species* | Aug, Cot, Ery |
| **$AP_2$** | *Corynebacterium species* | Aug, Cxc, Cot |
| **$AP_{3a}$** | *Corynebacterium species* | Aug, Cxc, Cot |
| **$AP_{3b}$** | *Staphylococcus species* | Nt |
| **$AP_{4a}$** | *Staphylococcus aureus* | Aug, Cxc, Cot, Ery |
| **$AP_{4b}$** | *Staphylococcus species* | Aug |
| **$AP_5$** | *Staphylococcus aureus* | Aug, Cxc, Cot, Ery |

**HP – Hand Palm** **AP - Armpit** augumentin (Aug), tetracycline (Trt), cloxacillin (Cxc), chloramphenicol (Chl), streptomycin (Str), cotrimoxazole (Cot), erythromycin (Ery), gentamicin (Gen) Nt – Not tested

**Table 7: pattern of resistance of bacterial isolates against antibiotics employed**

| **Bacterial isolates** | **Number of isolates tested** | **Aug** | **Gen** | **Cxc** | **Chl** | **Cot** | **Tet** | **Str** | **Ery** |
|---|---|---|---|---|---|---|---|---|---|
| ***Staphylococcus aureus*** | 4 | 4 (100) | 1 (25) | 4 (100) | 1(25) | 4(100) | 1(25) | 3(75) | Nt |
| ***Streptococcus species*** | 3 | 3 (100) | 1 (33.33) | 3 (100) | 1 (33.33) | 1 (33.33) | 1 (33.33) | 1 (33.33) | 2 (66.67) |
| ***Staphylococcus species*** | 2 | 2 (100) | 0(0) | 1 (50) | 1(50) | 1(50) | 0(0) | 0(0) | 1(50) |
| ***Corynebacterium species*** | 2 | 2 (100) | 0(0) | 2(100) | 0(0) | 2(100) | 0(0) | 0(0) | 0(0) |
| ***Clostridium species*** | 1 | 1 (100) | 0(0) | 1 (100) | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) |
| ***Bacillus species*** | 1 | 1 (100) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Klebsiella species*** | 1 | 1 (100) | 0(0) | Nt | Nt | 0(0) | 0(0) | Nt | Nt |
| **Total** | 14 | | | | | | | | |

Augumentin (Aug), tetracycline (Trt), cloxacillin (Cxc), chloramphenicol (Chl), streptomycin (Str), cotrimoxazole (Cot), erythromycin (Ery), gentamicin (Gen), Nt – Not tested

**Table 8: The profile of multiple antibiotics resistance pattern of isolates**

| **Bacterial isolates** | **Number of isolates tested** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| ***Staphylococcus aureus*** | 4 | - | - | 1 | 2 | - | - | - | 1 |
| ***Streptococcus species*** | 3 | - | 1 | 1 | - | - | - | - | 1 |
| ***Staphylococcus species*** | 2 | 1 | - | - | - | 1 | - | - | - |
| ***Corynebacterium species*** | 2 | - | - | 2 | - | - | - | - | - |
| ***Clostridium species*** | 1 | - | - | 1 | - | - | - | - | - |
| ***Bacillus species*** | 1 | 1 | - | - | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Klebsiella* species** | 1 | - | 1 | - | - | - | - | - | - |

**Information relating to the samples analysed**

A total of eleven individuals, consisting of 7(63.64%) males and 4(36.36%) females between age 18 and 30 years old were examined during this study (Table 3 and 4). Also, the distribution microbes associated with the sites under consideration was later on discovered to be either normal flora such as Staphylococci or contaminants *Klebsiella* species from the environment such as lower part of urinary tract. Despite the fact that Staphylococci are native or normal flora of the body they also serve as opportunistic pathogens. Hence, from this study, the incidence of *Staphylococcus aureus* 4(25%) were found to be the highest, followed by that of coagulase-negative *Staphylococcus* species *and Streptococcus* specieswhich were (18.75%) each as shown in table 1.

Moreover, there the incidence of *Corynebacterium* and *Klebsiella species* constituted 2(12.5%) each, while *Clostridium* speciesand *Bacillus* species are accounted 1(6.25%) each the lowest incident rate (Table 1). In addition, the prevalence of bacteria among individuals between age 22 and 25 years constituted the highest which was 43.75% while among individuals between ages 18 to 21 and 26 to 30 years constituted 25% and 31.25% respectively (Table 3). Therefore, among the samples analysed individuals between age 18-21, 4(25%) were cultured from a male (50%) and a female (50%). For age range 22-25, 7(43.75%) isolates were cultured from 6 individuals of which 4(66.67%) were males and 2(33.33%) were females. For age range 26-30 years, 5(31.25%) from 3 individuals, 2(66.67%) males and 1(33.33%) female (Table 3 and 4).

Nonetheless, incidence of bacteria is higher in males than females especially among individuals between age 22-25 years old (Table 4). In contrast, the incidence is lower among individuals between age18-21. Gram positive cocci were found be the most common organism recovered, then gram positive rods followed by gram negative rods as shown in table 3.

**Antibiotics resistance patterns of the bacterial isolates**

A total of fourteen isolates were tested with various antibiotics enumerated in the materials and methods. Out of these fourteen isolates two were resistance to one antibiotic, while five and two isolates were resistance to eight antibiotics respectively. Also another two were resistance to four antibiotics while only one isolate was resistance to five of the antibiotics employed (Table 7).

The patterns of resistance of each isolates against the antibiotics tested were evaluated and are shown in table 8. The incidence of *Staphylococcus aureus* to augmentin, cloxacillin and cotrimoxazole was highest involving all the four isolates tested. This was followed by resistance to erythromycin occurring in three of the four isolates. Only one *Staphylococcus aureus* was resistance to eight of the antibiotics which include augmentin, gentamicin, chloramphenicol, tetracycline, streptomycin, cotrimoxazole, erythromycin and cloxacillin.

Furthermore, all the three isolates of *Streptococcus* species tested were resistance to augmentin and cloxacillin while only one of the three isolates was resistance gentamicin, chloramphenicol, tetracycline, streptomycin and cotrimoxazole. Whereas only two of the three isolates were resistance to erythromycin. With regards to *corynebacterium* species, all the two isolates augmentin, cloxacillin and cotrimoxazole. In contrast, all the two isolates were sensitive to gentamicin, chloramphenicol, tetracycline, streptomycin and erythromycin as shown in table 6 and 7.

Meanwhile, the *Clostridium* specieencountered in this study was resistance to augmentin, cloxacillin and tetracycline while the *Bacillus* specie encountered show resistance to only augmentin and sensitive to gentamicin, chloramphenicol, tetracycline, streptomycin, cotrimoxazole, erythromycin and cloxacillin. With regards to *Klebsiella* species, all the isolates tested were resistance to augmentin and amoxicillin. Hence, the two isolates of *Klebsiella* species were sensitive to gentamicin, ofloxacin, nalidixic acid, cotrimoxazole, nitrofuratoin and tetracycline (Table 7 and 8).

**The profile of multiple antibiotics resistance patterns**

The profile of multiple antibiotics resistance of the isolates tested was studied. Out of the fourteen isolates evaluated two isolates were resistance to one antibiotic, one *Staphylococcus* species and one isolate of *Bacillus* specie. Also, two isolates were resistance to two antibiotics the isolates were *Streptococcus species* and *Klebsiella species.* In addition, one isolates of *Staphylococcus aureus* and *Streptococcus* species showed multi-resistance to eight of the antibiotics employed. Five isolates of which two were *Corynebacterium species* and one *Staphylococcus aureus*, *Streptococcus* speciesand *Clostridium* species showed multi-resistance to three of the antibiotics employed. However, the only isolates that showed multi-resistance to four of the antibiotics were two of the four *Staphylococcus aureus.* While of one isolates of the *Streptococcus* species showed multi-resistant to five of the antibiotics.

**Discussion**

The microbiology of hand palms and armpits is an important aspect of the area that need special attention in medical microbiology because of the function of hand palms and armpits in spreading of infectious agents and because of the odour produce under the arms as a result of the bacteria that the two sites of the body in under consideration. In human, bacteria are the most common and numerous component of the skin flora; even though, human skin is a remarkable organ, the largest but often taken for granted, Nesters *et al*. (2007). At hand palms and armpits, a total of 16 isolates were recovered from 11 individuals as shown in table 3. The results from this study showed that some diseases and pathogen is highly specific and may be due to any several causal agents at the site of the body. Therefore, disease does not necessary follow exposure to a given causal agents. In fact, factors including the degree of resistance of the host and virulence of the pathogen. This finding is in agreement with the report by Singleton Paul and Nesters and others (Singleton, 1997; Nesters *et al.*, 2007).

However, microorganisms which colonize a given habitat affect each other in various ways because they will have to compete with each other for nutrients, oxygen, and space. According to the report by Singleton, those that cannot survive will have to be eliminated from such habitat, as it can be seen from this study where most of the isolates 10 (62.5%) were gram positive cocci, Singleton, (1997).
Further investigation revealed that sweat from the armpit remains sterile when secreted but the activities of the native flora of the part of the body break the apocrine and thereby responsible for the unpleasant odour. As part of the bacteria encountered in this study, *Corynebacterium* species (12.5%) and other native flora such as *Staphylococcus aureus* (25%) that are consistently found on a specific area of the body such armpit have made site of the body sources of body odour as a result of the release of chemical called 3-methyl-2-hexenoid acid by the native microbes, Alkerman *et al*. (2005).
Moreover, according to findings in this study, not many are aware of the cause of armpit odour of which the main cause is the culprit bacteria which reside in the sweat on the skin. This finding is also in agreement with the report by Alkerman and others which revealed that some individual has odour all over their body, Alkerman *et al*. (2005). However, infection can be directed towards the Gram-positive cocci ten (62.5%) and most common two (12.5%) Gram-positive rods isolates encountered from hand palms and armpits respectively.

 

In addition, incidence of *Staphylococcus aureus, Streptococcus* speciesand *Staphylococcus* species were the predominant organisms isolated from the two sites of the body constituting (25%), (18.75%) and (18.75%) respectively (Table 1). This observation confirms the finding by Prescott *et al* (2008) that Staphylococci are pathogens that inhabit the membrane of skins and feeding on the sweat and dead skins. However, chance of infection by hand palms is high as a result of the gram positive rods isolates from the site of the body, since *Bacillus species* and *Clostridium* species constituting (6.25%) each. This must have being due to infection by air, water or soil contamination by the two isolates. Also, *Klebsiella* species(12.5%) isolated from hand palms must have as well be the result of contamination from the environment; because *Klebsiella* species is one of the native flora of the lower part of the intestinal tract.

The results of the age distribution of the individuals examined in this study shows that 54.55% of the 11 participants were around age 22-25 years with 7(43.75%) bacterial incidence from 4(66.67%) males and 2(33.33%) females (Table 5). This investigation reveals that both men and women have these bacteria on their armpit and produce mulder odour this is mostly common among young adults. The results obtained show the incidence of bacterial isolates to be higher in males (66.64%) than females (36.36%). The results is not apparently clear but could be associated with the nature of the sampling populated examined.

The antibiotic sensitivity testing on bacterial isolates cultured from the body sites viz: hand palms and armpits among the individuals between age eighteen and thirty years old was carried out to provide suggestions for practicing clinicians on the antibiotics to which these organism were sensitive. Although, resistance of infectious microorganisms of hand and armpits against antibiotics are regularly reported, it is difficult to compare results since variations in methodology may contribute to some extent to these differences. Therefore, it follows that the clinician who manages infections of the hand palms and armpits should be acutely aware of the changing antibiotic sensitivity patterns of the bacteria that cause such infections.

In addition, the in-vitro antibiotic sensitivity testing revealed that the *Staphylococcus aureus* strains had considerable resistance to many of the antibiotics employed. Resistance to augmentin (100%), cloxacillin (100%) and cotrimoxazole (100%) were particularly striking. With regards to *Klebsiella species* which happened to be the only Gram-negative bacteria from this study,all the two isolates encountered show resistance to augmentin (100%) as well as amoxicillin, hence this similar finding in which all *Klebsiella* species were resistant to amoxicillin has been reported by Monica (2004). With regards to *Bacillus* speciesonly one isolate cultured was resistance to only augmentin. In case of *Corynebacterium* species, the in-vitro antibiotics sensitivity testing showed that all the two isolates from this study were (100%) multi-resistance to augmentin, cloxacillin and cotrimoxazole.

Meanwhile, the results obtained show that only one the *Staphylococcus aureus* and *Streptococcus* species tested showed resistance to eight of the antibiotics employed. Whereas, *Clostridium* speciesfrom show 100% multi-resistance to augmentin and tetracycline. This findings is also in agreement with the report by Monica Chesbrough that misuse of drugs against infectious organisms can lead to multi-resistant of most the antibiotics employed against the infectious agents, Monica (2004).

In general, from this study, it is apparent that many of the organisms isolated were resistant to many of the antibiotics employed with a considerable number being resistant to as many as three, four and even five antibiotics. The high incidence of multiple antibiotics resistance in these organisms indicates an alarming case of multi-resistance and is of concern, because the information obtained

 

from the report by Monica that most of the antimicrobial resistance which is now making it difficult to treat some infectious agents is due to the extensive use and misuse of drugs. Therefore, the abuse of antibiotics could not be implicated as being responsible for the high incidence of antibiotics resistance encountered in this study.

**Conclusion**

This study shows that bacterial isolates particularly, gram positive cocci and gram positive rods were predominant on the skin. As a matter of fact, *Staphylococcus aureus* were predominant pathogens associated with hand palms and armpits part of the body while *Streptococcus* species and *Staphylococcus* species were found to be predominant at the same rate. This makes it easier for Staphylococcus to invade wounds sepsis whenever there is cut in the body because Staphylococcus is normal or native flora of the body but become pathogenic under certain condition as opportunistic infection such as in the case its infection on wound sepsis. Infection of hand palms and armpits is preventable by taken into consideration the genera such as Clostridium which may bring about infection from food as it can be seen in the case of *Clostridium perfrigens* or soil as in the *case Clostridium tetani*. Thus, the infection from these two genera is preventable because of the aetiologic factors such as poor hand and armpits hygiene which causes food infection and body odours respectively. Therefore, education and awareness of people on keeping proper hygienic condition through proper hand washing technique with soup or detergent and daily washing body with soup will therefore ameliorate bacterial infection by hand and body odours from the armpits.

Antibiotics susceptibility pattern of the bacterial isolates from the study indicates the presence of highly resistant species of bacteria which suggests the reliance on specific susceptibility patterns of bacteria for treatment. Some of the antibiotics such as gentamicin, chloramphenicol and tetracycline can still be prescribed with high success rates, but it is important that research in the field on antibiotics susceptibility should be continued because microorganisms may vary in different ecosystem.

**Contribution to knowledge**

This study reveals that bacteria were prevalent in the two sites of the body under consideration. The investigation also unveiled a high incidence of bacteria among people between the ages of twenty-two and twenty-five years, this could be due to the level of their hygiene. Besides, the results of the in-vitro susceptibility testing on commonly employed antibiotics such as gentamicin, streptomycin, tetracycline and erythromycin may not be effective for in treatment of hand and armpit infections. Therefore, information on resistance pattern of isolates encountered in the study to commonly available antibiotic employed will assist the clinicians in making improvement in management of hand and armpits hygiene.

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