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BACTERIOLOGICAL STUDY OF THE PALM AMONG MADONNA UNIVERSITY STUDENTS, ELELE CAMPUS

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ABSTRACT

Microorganism can be transferred to the human palms from contaminated surfaces. Contaminated hands can transmit disease to one self as well as to others. This study was done to isolate bacteria present in palms of students of Madonna University Elele Campus, Rivers State Nigeria. A total of 85 palms were swabbed, samples were taken from randomly selected students males (45%) and female (40%). Out of these 85 samples of palm swabs, 36 isolates were gotten. From the result, the most frequently isolated organism was *Staphylococcus aureus*20 (55.5%) and the least isolated organism was *Salmonella spp* 2 (5.5%), other organism isolated were *Klebsiellaspp*6(16.6%), *Escherichia coli* 8(22.2%). There was no significant difference $p>0.05$ among percentage occurrence among the organism isolated. Also this work shows that the highest number of isolates gotten from the males was 21(58.3%) and 15(41.6%) number of isolates from female students. The result show there was no significant $p>0.05$ difference among percentage occurrence of organism isolated when compared by gender between the types of organisms isolated and sex distribution of the organisms isolated. The subjects needed to be meticulously educated on need for proper hand hygiene and hand washing practice.

Keywords*: bacteriological study, palm, students*

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INTRODUCTION

Bacteria are types of biological cell. They constitute a large domain of prokaryotic microorganisms. Typically, a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste and the deep biosphere of the earth's crust (Woese and Fox, 2017).

Palm of the hand, the central region of the front of the hand and a subdivision of the cubit. The hand is the main body organ by which we work and maneuver through our everyday activities. Hands especially are the primary mode of transmission of many infectious diseases, particularly among those living and working in close proximity to one another such as class rooms, dormitories, hostels etc. The hand is the extremity of the superior 6 limb, hence, serves as a medium for the transfer of microorganisms from one location to the other and from one person to another. Close environments, doorknobs and other inanimate objects serving as resting vehicles of transmission all contribute to increased infection rates among these groups (Dodrill *et al.,* 2011).

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 (Hammond *et al.,* 2018). Human hands usually harbour microorganisms both as part of normal microflora and microorganisms contacted from the environment. These normal microflorae such as *Staphylococcus aureus* resident in the human skin can therefore be passed from one individual to another. 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 especially palm are of two types i.e. resident and transient (UNICEF, 2008). The resident types are the normal flora while the transient are contacted from the environment. Teaching appropriate hand hygienic practice has numerous benefits in various environments such as learning institution including high schools and college as well as promote general wellness (Curtis and Cairncross, 2013).

Appropriate hand hygiene practices can potentially result in minimal transmission of disease agents. Basic hand washing with soap and water is a brilliant way of reducing the risk and spread of infection through hand contact, but the problem of maintaining strict compliance to conventional hand washing practices is a major challenge to overcome, especially in the school premises. Hand hygiene practices are difficult to perform due to factors such as lack of sinks in most classroom environments. In these situations an alternative to the conventional hand hygiene practice of hand washing with soap and water is the use of a waterless alcohol gel hand sanitizer. Waterless hand sanitizers, such as alcohol gels, offer quick, easy and effective hand hygiene.

In classroom situations, students in residence hall may be less likely to regularly wash their hands due to the absence of sinks in their rooms and the inconvenience of walking to the washrooms to do so. Alcohol hand sanitizers have been shown to offer an effective alternative to conventional hand washing in high institutions (Aiello *et al.,* 2009).

Pathogens that may be present on the palm of hand as transient types includes *Salmonella* spp., *Shigella* spp., *Escherichia coli,* Since human hands usually habour 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 chance that other persons will acquire these organisms is dependent on how long the organism can survive in the environment (Nkang *et al.*, 2019).

Disinfection of surfaces is also necessary to prevent infections from transient microbes especially surfaces that the hand comes in contact with mostly and frequently. Studies have shown that although these surfaces cannot be totally free from microorganisms, they can be minimized (Kibret and Abera, 2012).

This study therefore seeks to know the types of microorganisms associated with contaminated hands of students of Madonna University Elele Rivers State, to create awareness on the possible consequences of the use of contaminated hands and to educate the students on the importance of sanitizing and proper hand washing.

This work aimed at isolating the bacteria present on the palm of Madonna University Student Elele Rivers State.

MATERIALS AND METHODS

Study Area

The area of studies is Madonna University Elele Campus.

STUDY POPULATION

85 palm swab was collected randomly from the palm of Male (45) and female (40)students of Madonna University Campus Elele Rivers State.

STERILIZATION OF MATERIALS

In order to avoid contamination, materials like spatula, conical flask and culture media were sterilized using autoclave. The hot air oven was used for sterilization of the measuring cylinder and drying plates. The weighing balance and working were sterilized by using methylated spirit to wipe them.

MEDIA USED

The media used in this study include MacConkey agar, mannitol salt agar, eosin methylene blue agar, salmonella shigella agar.

REAGENTS AND DYES USED

These include lugol's iodine, safranine, crystals violet, alcohol (70%) or acetone.

SAMPLE COLLECTION

A total of 85 palm swabs (samples) were collected from 85 persons using sterile swab stick dipped in peptone water. The swab stick was used to rub the palm for microbial analysis.

MICROBIAL ANALYSIS

Each specimen was cultured on different media for 24 hours at 37 degree celcius using the streak plate method.

IDENTIFICATION OF BACTERIAL ISOLATES

After incubation for 24hours at 37 degree celcius, colonies were collected and sub cultured by streaking on nutrient agar to obtain pure cultures. Representative's isolates from the pure cultures were further sub cultured into nutrient agar slant by streaking to preserve the isolates used as stock cultured for biochemical test. The isolates were identified based on their morphological characteristics, biochemical reactions and gram reaction.

Gram Staining

This technique as described by Cheesbrough (2006) is used differentiate gram positive organism from gram negative organism. Part of a colony of an organism was picked and smeared on a clean grease free dry slide containing drops of normal saline. The smear was air dried and heat fixed and flooded with crystal violet for 60seconds. It was washed with water and then covered with lugol's iodine solution for 60seconds and washed again, after this it was decolorized with 70% alcohol and wash off again and finally counter stained with safranine for 2 minutes and washed off with water. After this the film was blotted dry and immersion oil was added and viewed using 100x objective.

Result – Gram positive organism – blue-purple

Gram negative organism-reddish pink.

BIOCHEMICAL TEST

Catalase Test

This technique as described by Cheesbrough (2006) is used to differentiate those bacteria that produce catalase from those that do not. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. A loopful of normal saline was placed on a grease-free and dry glass slide and the test organism was added to it. Two drops of 3% hydrogen peroxide were added.

Oxidase Test

This test as described by Cheesbrough (2006) is used in the identification of organisms which produce the oxidase enzyme such as *Pseudomonas.* 10g/L solution of tetra methyl phenylene diaminedihydrochloride (oxidase reagent) was prepared and a piece of filter paper soaked with 2-3 drops of the reagent. A loopful of the test organism was smeared on the same point the filter paper was dropped. A positive result was indicated by the development of a deep purple color within 10 seconds. (This shows that the phenylene diamine has been oxidized).

Indole Test

This test as described by Cheesbrough (2006) was based on the ability of some organisms to breakdown the amino acid, tryptophan with the release of indole. Peptone water was prepared and dispensed in 3ml amounts into sterile test tubes. It was sterilized at 121°C for 15mins. The test organisms were inoculated and incubated for 48 hours at 37°C. 0.5ml of Kovac's reagent was added after the incubation period. Appearance of red ring color at the surface layer within l0min of gentle shaking is positive.

Coagulase test

This test as described by Cheesbrough (2006) is used to identify *Staphylococcus aureus*. A pathogenic *Staphylococcus aureus* has the power of clotting blood plasma due to the production of enzyme coagulase. Coagulase may be detected by the rapid slide test or tube methods.

Slide test

A colony of test organism was emulsified in one drop of normal saline on a clean glass slide in which a homogenous suspension was obtained. A loopful of human plasma was

added and mixed thoroughly. The reaction was obtained in which a positive result was detected by a clumping within 1-2 minutes while negative one shows no clumping.

Citrate Utilization Test

This test as described by Cheesbrough (2006) was based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen. Cimmon's Citrate agar was prepared according to manufacturer's specification. 5ml amounts were dispensed into bijou bottle and sterilized at 121°C for 15 minutes. The bijou bottles were kept in slanting position to obtain an agar slant onto which the test organisms were inoculated after gelling. It was incubated at 37°C for 48 hours. Development of a bright blue colour in the medium showed a positive result while no blue colour indicated a negative result.

Motility Test

This test as described by Cheesbrough (2006) is used to detect the true motility of an organism. The bacterial isolates were separately inoculated into a test tube containing 9ml of sterile nutrient broth. The tubes were incubated at 30°C for 6 hours after which 2 loopful of the young broth cultures were placed on clean grease-free slides. A cover slip was placed over the slides and viewed under x10 and x40 objective lens of the microscope to observe the motility of the organisms.

Urease Test

This test as described by Cheesbrough (2006) differentiates *proteus* species that produce Urease enzymes within 4-6hours inoculation. Urease is an enzyme that breaks carbon amino nitrogen bonds of amide to form carbon dioxide, ammonia and water. The test organism is inoculated into a test tube containing 3mls of sterile urea broth and phenol red as incubated for 24hours. A colour changes to pink indicates a positive result.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Sensitivity test was done on diagnostic sensitivity media Mueller Hinton agar using disk diffusion method. Antibiotic diffuse out of a disk placed on the surface of the agar. If bacteria are sensitive to the antibiotic, then a zone of growth inhibition forms around the

disk after incubation. The zone size depends on several factors and to methods is available to control this process, comparative disk testing (where both a test and control organism are tested on the same plate) and standardized disk testing. The antibiotics diffuse out of the disk into the agar, along a concentration gradient, as the plates are incubated (for 18-24h). If the bacterial stain is sensitive to the antibiotic, then a zone of inhibition (no growth) occurs around the disk. The control organism is of defined sensitivity to the antibiotics being tested, and this method allows a direct comparison of the diameter of the zones of inhibition between the test control organisms.

STATISTICAL ANALYSIS

Data obtained from the study was analyzed using the statistical package for social science (SPSS) version 20 for window 8.1. The results were expressed in frequencies of occurrence and percentage. Also, values were expressed as ± standard deviation. Anova was used to compare mean differences between the bacterial isolates.

RESULTS

A total of 85 palm swabs were collected from the palm of male (45) and female (40) students of Madonna University Elele Campus. The isolates identified were *Escherichia coli, Salmonella spp* and *Kiebsiella spp* for the gram negatives, while for the gram positive organism is *Staphylococcus aureus*.

Table 1 Shows the frequency of occurrences of the isolates from 85 samples from the results the most frequently isolated organism was *Staphylococcus aureus* and the least isolated was *Salmonella spp* others isolated was *Escherichia coli* and *Kiebsiella spp.*

Table 2 shows the frequency of occurrences of bacteria isolated from the palm of male and female students of Madonna University Elele Campus.

Table 3 shows the antibiotics susceptibility pattern of gram positive bacteria (*Staphylococcus aureus)* isolated from the palm of male and female students of Madonna University Elele Campus.

Table 4 shows the antibiotics susceptibility pattern of gram negative bacteria (*Escherichia coli, Salmonella spp, Kiebsiella spp*) isolated from the palm of male and female students of Madonna University Elele Campus.

TABLE 1: shows the percentage occurrence of bacteria isolated from the palm of male and female students of Madonna University Elele Campus

No of isolates	N (%)
Staphylococcus aureus	20 (55.5)
Klebsiella spp	6 (16.6)
Salmonella spp	2 (5.5)
Escherichia coli	8 (22.2)
TOTAL	36 (100)

P-value=>0.05, there was no significant difference among percentage occurrence among the organism isolated.

Table 2: Shows the sex distribution of bacteria isolated from the palm of male and female students of Madonna University Elele Campus

SEX	*Staphylococcus aureus* (%)	*Kiebsiella spp* (%)	*Salmonella spp* (%)	*Escherichia coli* (%)	Total (%)
Male Students	12 (60)	4 (66.6)	0 (0)	5(62.5)	21(58.3)
Female Students	8 (40)	2 (33.3)	2 (100)	3 (37.5)	15(41.6)
Total	20 (100)	6 (100)	2 (100)	8 (100)	36 (100)

P-value=>0.05, Also there was no significant difference among percentage occurrence of organism isolated when compared by gender.

Table 3: Antibiotics susceptibility pattern of gram positives bacteria (*Staphylococcus aureus)* isolated from the palm of male and female students of Madonna University Elele Campus

	Staphylococcus aureus **n=20**	
Antibiotics	**S (%)**	**R (%)**
Rocephin	12(60)	8(40)
Ciprofloxacin	11(55)	9(45)
Streptomycin	11(55)	9(45)
Septrin	10(50)	10(50)
Erythromycin	10(50)	10(50)
Peflacin	11(55)	9(45)
Gentamycin	10(50)	10(50)
Ampiclox	12(60)	8(40)
Zinacef	10(50)	10(50)
Amoxacillin	12(60)	8(40)

KEY: n= no of organisms, S= Sensitive, R= Resistant

Table 4: Antibiotic Susceptibility Test for Gram-negative organisms

Antibiotics	*E coli* n=8 S(%) R(%)	*Salmonella spp* n=2 S(%)R(%)	*Klebsiella spp n=6* S(%)R(%)
Septrin	4(50) 4(50)	0(0)2(100)	2(33.3)4(66.6)
Chloramphenicol	6(75)2(25)	0(0)2(100)	3(50) 3(50)
Ciprofloxacin	2(25)6(75)	2(100) 0(0)	4(66.6)2(33.3)
Amoxacillin	4(50)4(50)	0(0)2(100)	4(66.6)2(33.3)
Augumentin	6(75)2(25)	1(50)1(50)	2(33.3)4(66.6)
Gentamycin	2(25)6(75)	1(50)1(50)	2(33.3)4(66.6)
Peflacin	6(75)2(25)	2(100)0(0)	4(66.6)2(33.3)
Tarivid	2(25)6(75)	1(50)1(50)	3(50) 3(50)
Streptomycin	2(25)6(75)	1(50)1(50)	2(33.3)4(66.6)

KEY: n = no of organisms, S= Sensitive, R= Resistant

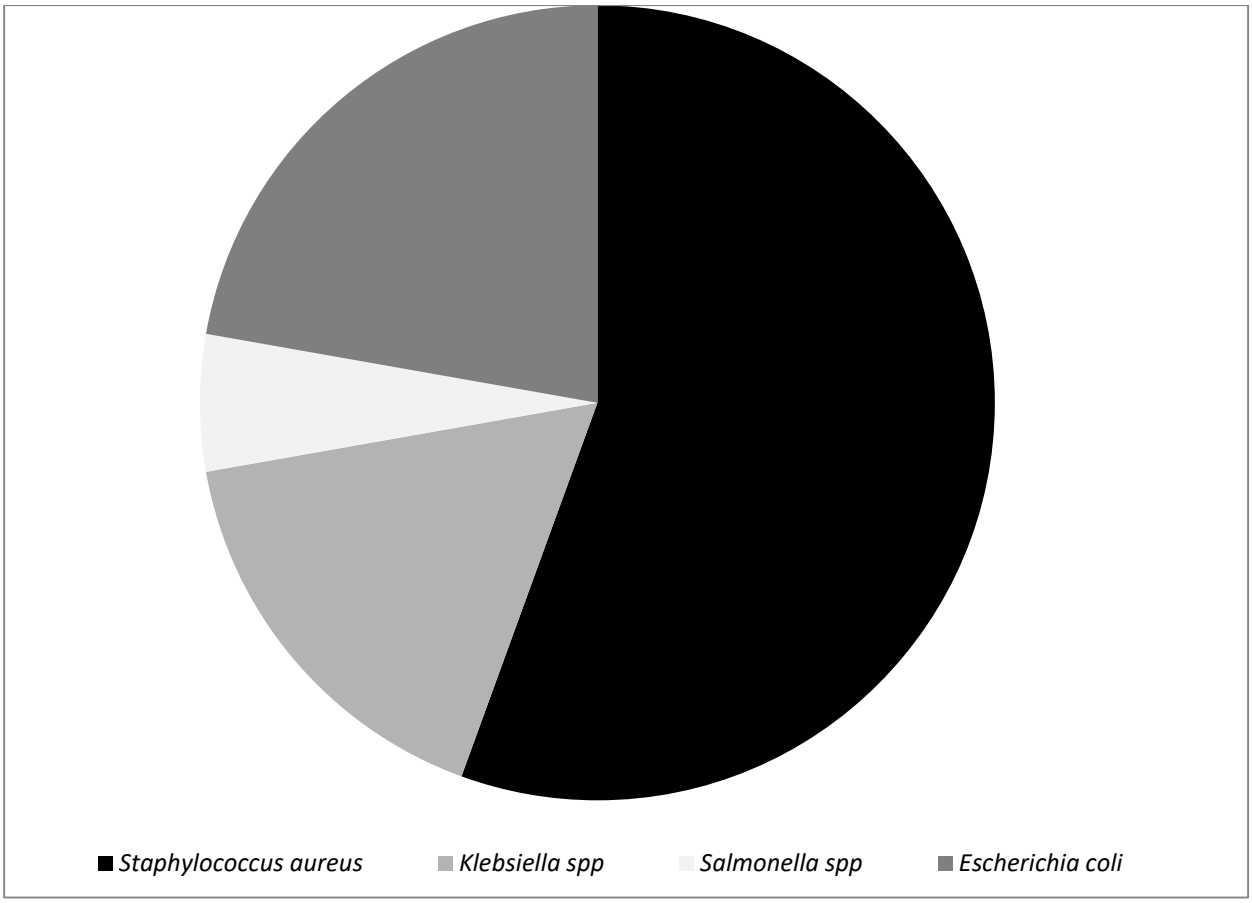

Figure 1: Pie chart showing occurrence of bacteria isolated from the palm of male and female students of Madonna University Elele Campus

DISCUSSION

The human hands harbor microorganisms both as part of a person's normal microbial flora as well as transient microbes acquired from the environment. Some pathogenic organisms are spread by contaminated hands. Hygiene has a measurable impact on reducing the burden of infections in the developing world. In this research, the practice of hand washing either with water or with soap and water is very low as compared to what is obtainable in studies from other countries. The result of the study shows that most of the students hands was contaminated with one bacterial pathogen or the other, and this is an indication of poor personal hygiene particularly hand hygiene. This has been attributed to lack of appropriate hand washing facilities or poor location of these facilities (Mathieu *et al.,* 2013).

The result obtained in this study shows that the palm of students of Madonna University Elele Campus contains both the normal flora and transient flora of the hands. The presence of *Staphylococcus aureus, Kiebsiella spp, Salmonella spp, Escherichia coli*. The presence of these bacteria indicates possibility of poor hand hygiene by the students involved.

In this study, a total of 85 samples were collected from palms of students, Out of these 85 samples of palm swabs 36 isolates were gotten. From the results the most frequently isolated organism was *Staphylococcus aureus*20 (55.5%) and the least isolated organism was *Salmonella spp*2 (5.5%), other organism isolated were *Klebsiellaspp*6 (16.6%), *Escherichia coli* 8 (22.2%). There was no significant difference $p>0.05$ among percentage occurrence among the organism isolated.

Also this work shows that the highest number of isolates gotten from the males was 21(58.3%) and 15(41.6%) number of isolates from female students. This has similarities with the work done by (Wututantrige *et al.,* 2012) who had a total of (36.5%) for male students and (30.15%) for female students. The result show there no significant $p>0.05$ difference among percentage occurrence of organism isolated when compared by gender between the types of organisms isolated and sex distribution of the organisms isolated. Rocephin (60%), Ampiclox (60%) and Amoxacillin (60%) were highly susceptible against

gram positive bacteria and rather resistant to septrin (50%), Zinnacef (50%), Gentamycin (50%), Zinacef (50%) and Erythromycin (50%). This was also in line with the work of (Reyner *et al.*, 2005). Also from my work the gram negative bacteria was more susceptible to pefloxacin (75%) and Chloramphenicol (75%). The bacteria isolated could be as a result of negligence of proper hand hygiene as ignorance cannot be a factor as it concerns students.

CONCLUSION

According to the research study human palms have been discovered to be the abode of different types of microorganisms. The palm of most students was seen to harbour more *Staphylococcus aureus* which was capable of producing enterotoxins which may lead to food poisoning and provoke vomiting. It is therefore not safe to be ignorant to hand hygiene benefits.

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