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Microbial Assessment of Female Covered and Open Shoes in University Of Agriculture, Makurdi, Benue State, Nigeria

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Abstract: **Microbial assessments of female open and covered shoes in University of Agriculture Makurdi were investigated to ascertain microbial loads. A total of thirty (30) open and close shoes sample were swabbed and cultured on blood agar and nutrient agar for both bacteria and fungi. Three bacteria were identified namely Stapylococcus aureus, Streptococci spp, Baccilus spp and Psuedomonas spp, four fungi were also identified namely Aspergillus spp, Penicillium spp, Mucor spp and Yeast cell. Staphylococcus aureus tend to dominate both in open and covered shoes among bacteria with frequency of 28 and percentage of 49.1 and the least among the bacteria was Streptococci spp with frequency of 7 and percentage of 12.2 on this research study, bacteria is more dominant in open shoes than close shoes while on fungi Aspergillus spp appears more frequently with occurrence of 15 and percentage of 57.6 and Penicillium spp appears as the least among fungi with frequency of 2 and percentage of 7.6, Mucor spp occurred with frequency of 6 and percentage of 23.0 while Yeast cell occurred with frequency of 3 and percentage of 11.5. Fungi tend to be more dominant in covered shoes than open shoes. Based on the findings of this study, shoes especially covered shoes should be air dried and disinfectant wipes can also be used to avoid infections from these organisms.**

Keywords: **Microbial, Female, Open shoes, Covered shoes.**

1. INTRODUCTION

A shoe is a protective covering for the foot, with a bottom part composed of thick leather or plastic sole and often a thicker heel or synthetic materials.(Sally, 1993). A shoe is an item of footwear intended to protect and comfort the human foot while doing or carrying various activities in his or her daily life. Feet may be compromised by bacterial and fungal infections, chronic disease, obesity, immune suppression, vascular disease, and uncomfortable or tight shoes (Akiba et al., 2006). Tight shoes can injure the feet and make them prone to contamination and infections. Unventilated shoes are prone to bacterial and fungal proliferation. Sweat is a nutrient for bacteria, and bacterial metabolism gives feet, socks, and shoes a strong odor (Akiba et al., 2006). Bacterial and fungal infections proliferations are influenced by microclimate, temperature, humidity, life- style and individual predisposition. Feet have a rich bacterial flora, most of which is not normally pathogenic if the feet are in good health (Stewart, 2015). Lifestyle factors can expose the feet to higher risks of contamination by certain bacteria. For example, going barefoot exposes feet to contamination by Escherichia coli and other potential pathogens. Patients with circulatory problems and certain chronic conditions are susceptible to infection by Staphylococcus aureus, Staphylococcus epidermidis, and the coliform group of bacteria especially Enterococcus spp (Akiba et al., 2006). Shoes are also used as an item of decoration. The designer shoes varies enormously through time and from culture to culture, with appearance originally been tied to function. Additionally, fashion has often dictated many designed elements, whether shoes has a very high heels or flat ones (Akiba et al., 2006). Contemporary, shoes vary widely in style, complexity and lust. Basic shoes and sandals may consist of only a thin soul and strap. High fashion shoes may

be made of very expensive materials in complex construction and sale for thousands of Naira or Dollars in pair. Other shoes are for very specific purpose such as boots designed specifically for mountaineering or skipping (Tom, 2002). Traditionally, shoes have been made from leather, wood or canvas, but are increasingly made from rubber, plastic and other petrochemical derived material (Tom, 2002).

In early civilization, however, sandals were said to be the most common footwear in most part of the world only a few had shoes in Mesopotamia (c.1600-1200BC). A type of soft shoes was worn by the mountain people who lived on the boarder of Iran. The soft shoes were made up of wraparound leather similar to moccasin. As late as 1850, most shoes were made on absolute straight lasts, therefore no difference between right and left shoes (Cameron, 1999).

Microorganisms such as bacteria tend to live more on shoes than in other places. As we walk, we constantly pick new debris that feeds the growth of more bacteria. (Weber, 2008). A research carried out by (Gerba, 2008). An environmental microbiologist at the University of Arizona says he doesn't put on his shoes up on his desk anymore after completing a study dealing with the accumulation and elimination of Escherchia coli and other nasty fecal – based bacteria on shoes (Pohla, 2008). Beside Escherchia coli which is known to cause intestinal and urinary tract infections, the sole of shoes picked up Klebsiella pneumonia bacteria, a source of wound and blood stream infection as well as pneumonia, and Serratia a rare cause of infections in the respiratory tract and wounds (Pohla, 2008). Back here in Nigeria, Nigerian cobblers are making exquisite piece that are competing favorably with footwear manufacturing in any part of the world especially female open and covered shoes and also highly meeting up in terms of quality, design and patronage (Chuks, 2003). Nigerian shoe makers are manufacturing exquisite pieces that are equal or sometimes even higher quality with shoes manufacturer in any part of the world (Chucks, 2003).

Shoes are major protector of the foot and microorganisms such as bacteria tend to live more on shoes than in other places. As we walk, we constantly pick new debris that feeds the growth of more bacteria. And such microorganisms are Escherchia coli, Klebsiella pneumonia, Serratia and these microorganisms can also cause harm to our health and environment. The shoes are now becoming a bacteria cafeteria because they survive longer than they would on a desk top. And most bacteria love growing in a moist, warm environment they like to live in sauna (heat environment) and that is basically what most shoes have becomes (Gerba, 2008). Shoes are vulnerable to be contaminated by micro -organisms when worn and a more appropriate circumstance is provided by physical contact as well as sweat dipping (Mayan et al., 1999). Hence various microorganisms which grow and breed in micro-environment of inner shoes have no barrier to location or ethnicity. The role of these microbes has been clearly recognized by Chris (2010) in the paper "Identification of fungi from children's shoes" as undesired and produces odor, leading to stinking foot, mosses foot and a series of other diseases directly affecting physical and mental health ultimately.

It is important to determine the kind of microbes on foot wears, verify bacteria levels on footwear and the effectiveness on open and closed female shoes in reducing those levels inside and outside the shoe surface. (Chris, 2010). Some of the bacteria found on the shoes are known to cause intestinal and urinary tract infections, meningitis and diarrheal disease; a common source for wound and bloodstream infections as well as pneumonia and Serratia a rare cause of infections in the respiratory tract and wounds (Chris, 2010).

Study has shown that there is limited research about foot wears we put on in our daily life. And it's highly alarming that it has been professionally neglected. So a proper research is to be carried out to determine the microbial contamination of females open and covered shoes in the University of Agriculture Makurdi, Benue State.

Aim of the Study:

To determine the microbial contamination of female open and covered shoes in the University of Agriculture Makurdi.

2. MATERIALS AND METHODS

Study Area:

This study was conducted and samples were collected from female students in the University of Agriculture Makurdi Benue State. Makurdi town is the capital of Benue State of Nigeria**,** situated in central Nigeria along the Benue River, with a population of about 292,664 people. The distance from Makurdi to the major city of Lagos is about 585km.

Sample Collection:

Thirty (32) samples of swab stick, each were used among students in university of Agriculture Makurdi Benue state. The swab sticks were wet with normal saline and each was used to swab the shoes of female student (Both open and close

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shoes). They were labeled with unique identification number. The swab were transported to the advanced micro biology laboratory were the swab were cultured all on Sabaroud Dextrose Agar (SDA), Cled, and nutrient Agar (NA) respectively. The plate were incubated at 27^{o}C for 24 hours and observed for morphological and biochemical characteristics. The Sabaroud Dextrose Agar (SDA) was incubated 25^{o}C for 3-5 days (i.e 3days for yeast and five days for dermatophy).

Materials Used:

The materials used for this project work comprises of the following; sterile disposable swab sticks, petri dishes, universal bottles, beakers, conical flasks, measuring cylinders, glass slides, wire loop, Bunsen burner and microscope. Others are autoclave, weighing balance, hydrogen peroxide, blood plasma, disinfectant, Gram staining reagents, Nutrient agar, Sabatroured Dextrose Agar, masking tapes, hand gloves, nose masks.

Preparation of Media:

Nutrient agar, CLED and Sabouraud agar were prepared according to manufacturer's instructions.

Gram Staining:

Isolated colonies on prepared nutrient agar slant were gram stained as described by a manual of laboratory exercise for introductory and general microbiology 2004.

Then Coagulase test, catalase test, motility test and oxidase test were done..

Hydrogen Sulphide Production:

Few colonies each suspected organism were transferred TSA and incubated at 37^{o}C for 24hours. It was then observed for presence of blacking hydrogen sulphide and gas production. TSA was observed for maltose, sucrose lactose fermentation. Shigella shows red slope, yellow both positive and negative production. E. coli shows yellow book and yellow. No hydrogen sulphide but gas production the evidence of gas production shows crack.

Fungal Identification:

The growths on the Sabouraud dextrose agar plates were observed morphologically for color, size of growth, pigment production. Lacto phenol cotton blue test was carried out for identification of molds.

Lacto phenol Cotton blue Test:

A drop of methanol was placed on a clean slide and a portion of fungi growth was cut and was tested in the methanol. A drop of lacto phenol cotton blue was added, a cover slip was then placed on it gently. It was observed under microscope with ×40 objective. The picture seen was compared with an identification chart (Cheesbrough, 2000).

Data Analysis:

Data was analyzed using descriptive statistics (SPSS version 20)

3. RESULTS

A total of thirty (32) open and covered shoes samples were swabbed and cultured on blood agar and nutrient agar from the Federal University of Agriculture in Makurdi metropolis in Benue State. The cultured plates were further analysed by observing the morphological characteristics, Gram staining, percentage of bacteria and fungi, average colony count and other biochemical tests and the following results were obtained as shown in the Tables 1, 2, 3,4,5 and 6.

Table 1 shows the morphological characteristics of the isolated bacteria and also the colony characteristics of each microorganisms namely; Staphylococcus aureus with shiny yellowish, & spherical, Streptococcus spp with cream gold, shiny, smooth, spherical, Bacillus spp with Creamy with rough edge and Psuedomonas spp with bluish rod and green like in colour.

TABLE 1: Morphological Characteristics of Bateria Isolated

S/NO	**Colony Characteristics**	**Probable Organism**
1	Shiny Yellowish, & spherical	Staphylococcus aureus
2	Creamy with rough edge.	Bacillus spp
3	Cream gold, shiny, smooth, spherical.	Streptococcus spp
4	Bluish rod and green like.	Psuedomonas spp

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The morphological characteristics of fungi with their colony characteristics and their microscopic appearance The colony characteristics of Aspergillus spp was black in colour, large mass of colony, Penicillium spp had cotton, fuzzy, green, dark in colour, Mucor sp had cotton, fuzzy, green, dark in colour, and Yeast cell has moist, shiny whitish colonies (Table 2).

TABLE 2: Morphological Characteristics of Fungi Isolated

S/NO	**Colony Characteristics**	**Microscopic Appearance**	**Probable Organism**
1	Black in colour, large mass of colony	Conidiosporesaeptate with hyphae	Aspergillus spp
2	Cotton, fuzzy, green, dark in colour	Green spores with whitish hyphae	Penicillium spp
3	Cotton thick , creamy colour, black at maturity	Non-septate hyphae	Mucor sp
5	Moist, shiny whitish colony	Ova , spherical shape	Yeast cell

Table 3 shows the biochemical characteristics of Gram positive and Gram negative microorganisms isolated from female open and covered shoes and also the various tests which were carried out such as catalase test, coagulase test, oxidase test and motality test. While the suspected organisms are Streptococcus spp, Staphylococcus. aureus, Bacillus spp and Pseudomonas spp.

TABLE 3: Biochemical Characteristics of Gram Positive and Negetive Organisms Isolated From Female Open and Covered Shoes

S/no	**Shape**	**Gram reaction**	**Catalase test**	**Coagulase Test**	**Oxidase test**		**Motality Test**	**Suspected organism**
1	Coci	+	-	+	-	-		Streptococcus spp
2	Coci	+	+	+	-	-		S. aureus
3	Coci, Rod	+	+	+	-	+		Bacillus sp
4	Rod	-	-	-	+	+		Pseudomonas spp

The frequencies and percentages of bacteria isolated from female open and covered shoes from Table 4: shows that Staphylococcus aureus occurred more frequently and equal on both open and covered shoes followed by Bacillus spp which occurred more on covered shoes than open shoes and Pseudomonas spp appeared more on open shoes than close shoes while Streptococcus spp occurred least among all.

TABLE.4: The Frequencies of Isolated Bacteria from Female Open and Covered Shoes

S/NO	**Organisms**	**Open shoes**	**Covered shoes**	**Total frequency**	**Percentage**
1	Staphylococcus aureus	14	14	28	49.1
2	Bacillus species	3	10	13	22.8
3	Streptococci spp	5	2	7	12.2
4	Pseudomonas spp	8	1	9	15.7
	Total	**30**	**27**	**57**	**100.0**

Table 5 shows the frequencies and percentages of isolated fungi from female open and covered shoes whereby Aspergillus spp had the highest number of occurrence with the frequency of 15 and percentage of 57.6% followed by Mucor spp with the frequency of 6 and percentage of 24%. The least is Penicillium spp with frequency of 2 and percentage of 7.6%, Mucor spp appeared as the second highest with frequency of 6 and percentage of 23% and Yeast cell with frequency of 3 and percentage of 11.5%. Aspergillus spp occurred more on covered shoes than open shoes, Mucor spp appeared more on covered shoes than open shoes while Yeast cell is only dominant in covered shoes and none occurred in open shoes. The occurrence of Penicillium spp is equal on both shoes.

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TABLE 5: The Frequencies of Isolated Fungi from Female Open and Covered Shoes.

S/NO	Organisms	Open shoes	Covered shoes	Total frequency	Percentage
1	Aspergillus spp	6	9	15	57.6
2	Penicillium spp	1	1	2	7.6
3	Mucor spp	2	4	6	23.0
4	Yeast cell	0	3	3	11.5
	Total	**9**	**17**	**26**	**100.0**

Table 6 explains the average numbers of colonies of isolated bacteria and their colony forming unit per mile and the organisms are Staphylococcus aureus with colonies of 66 and Bacillus species with 12 colonies, Streptococci species with colonies of 8 and Pseudomonas species with colonies of 6.

Table 6: Average Numbers of Colonies of Isolated Bacteria with their Percentage

Organisms	Colonies	Percentage
Staphylococcus aureus	66	71.7
Bacillus species	12	13.0
Streptococci species	8	8.6
Pseudomonas species	6	6.5
Total	**92**	**100**

4. DISCUSSION

This study demonstrates that shoes are frequently colonized with bacteria such Staphylococcus aureus, Streptococci spp, Bacillus spp and Pseudomonas spp and fungi such as Aspergillus spp, Penicillium spp, Mucor spp and Yeast cell which are of great risk to human health. With the percentage occurrence of each bacterium, Staphylococcus aureus (49.1%) has the highest frequency followed by Bacillus spp (22.8%), Psuedomonas spp (15.7%) and the lowest is Streptococci spp (12.2%). With the percentage occurrence of each fungi, Aspergillus spp frequency (57.6%) has the highest followed by Mucor spp (23.0%), Yeast cell (11.5%) and the lowest is Penicillium spp (7.6%). Staphylococci are widespread in the environment and can be cultured from shoes and cloths and virtually all environmental surfaces (Bakker, 2013).

Staphylococcus aureus accounted for the highest prevalence rate among bacteria isolated for both open and covered shoes because it dominates our entire environment. In the recent study by Agbulu et al., (2015) on isolation and characterization of microorganism associated with second hand female undergarment and children wear sold in makurdi metropolis,the only bacteria isolated from samples was Bacillus spp. According to the studies, the survival of microbes on formites is influenced by intrinsic and extrinsic factors which might be a reason for survival of Bacillus spp. But in this very study, Staphylococcus aureus, Bacillus spp, Streptococci spp and Psuedomonas spp were all present in the samples. Staphylococcus aureus is a common bacterium that lives on the human body, in most situations, Staphylococcus aureus is harmless but it may cause death in some cases.

Aspergillus spp accounted for the highest prevalence rate among fungi isolated for both open and covered shoes because there are frequently found in air and soil. According to Summerbell et al., (1992) Aspergillus spp is capable of causing infections in human and other animals in our surrounding because it is generally believe that the amount of airborne spore of Aspergillus spp in indoor air is higher than outdoor air at any given time so when shoes are keep in door for over a long period of time there are meant to be affected with Aspergillus spp.

This research is agreed with the study by potera (2001) according to his research, cooten fabrics spread more of Aspergillus spp better than other fabrics and most shoes are been produced with cotton fabric especially here in Nigeria. Aspergillus spp is a common mould that causes the infection aspergillosis. Most people breath in Aspergillus spore every day without getting sick but it does affect people with wreaked immune system or lungs disease that have high risk of developing problems due to Aspergillus.

Mucor spp is the second with the highest frequency and percentage (23.0%). Mucor spp is a microbial genus of approximately 6 species of mould commonly found in soil which tend to live in our shoes as we step out every day. Most species of Mucor are unable to infect humans and endothermic animals due to their inability to grow in warm environment close to 37^0C (Summerbell et al., 1992).

Yeast cell is the third on the list with percentage (11.5%), it grows typically in moist environments and most times our shoes are not cross ventilated so it gives room for Yeast cell to grow faster (Bakker, 2013).

Penicillium spp was the fungi with the least percentage (7.6%) as carried out in this research. They are also commonly found in soils, decaying vegetables, air and are common contaminants on various substances and they tend to also live on shoes.

5. CONCLUSION

This study has shown that shoes harbour microorganisms such as bacteria and fungi. There were strains of fungi and bacteria isolated from ladies shoes and the bacteria are Streptococcus aureaus, Streptococci spp, Bacillus spp and Psuedomonas spp while the fungi are Aspergillus spp, Penicillium spp, Mucor spp and Yeast cell and the organisms are highly pathogenic the entire environment.

The following is therefore recommended:

1. Shoes should be wiped with disinfectant before and after use.
2. Do not wear shoes with wet feet because water stuck between toes is a common culprit.
3. Give your shoe a break for at least 24 hours after wearing them just to air out some festering bacteria.
4. Do not wear shoes into your homes, leave them by the door side or carry them with hands to your closet then wash your hand with disinfectant.
5. Shoes should be approach with caution since the study has demonstrated that shoes have high bacteria and fungi load.

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