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Microbial assessment of the armpits of some selected university students in Lagos, Nigeria

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ABSTRACT

A study of the carriage of microorganisms in armpits and prevailing factors was carried out on 80 students of the University of Lagos. The armpits were swabbed and the microbiological analyses were carried out on the swab samples. The organisms isolated include *Staphylococcus epidermidis* (35%), *Staphylococcus aureus* (3%), *Staphylococcus cohnii* (3%), *Staphylococus haemolyticus* (15%), *Staphylococcus hominis* (25%), *Micrococcus luteus* (9%), *Staphylococcus capitis* (6%), *Staphylococcus saprophyticus* (3%) and *Candida tropicalis* (1%). Questionnaires on gender and health related factors were administered to the subjects. Most students regardless of sex, used toilet soap (62.5%), had their bath twice daily (60%), used sponge for body scrubbing (87.5%) and shaved regularly (78.75%) but these did not have any significant influence on the carriage of microorganisms (P = 0.05). More female participants used deodorants, than the males. The bacterial and fungal counts in the armpits of females were lower than the counts from male armpits, which means that the use of deodorant reduced the carriage of microorganisms. From the antibiotic sensitivity tests carried out on *S. aureus*, the highest sensitivity was recorded for Ofloxacin while the least was for Cotrimoxazole. However the bacterium was resistant to most antibiotics tested. The DNA profile of *S. aureus* showed that none of the strains had a plasmid thereby suggesting that the antibiotic resistance genes in these strains could be chromosomally-encoded.

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Keywords: Microorganisms, armpit, university students, Lagos, Nigeria.

INTRODUCTION

Large numbers of microorganisms live on and in the various components of the normal skin. Depending on the body location and the amount of skin moisture, the number of skin bacteria may range from only about 1000 organisms per square centimeter on the back to more than 10 million in the groin and armpit, where moisture is more plentiful. The number of microorganisms increases after a hot shower because of increased flow of secretion from the skin glands where many reside (Nester et al., 2004). In humans, the formation of body odors is mainly caused by skin glands excretions and bacterial activity (Lundstrom and Olsson, 2010). The axilla, a skin region around the armpit usually differs from other regions of the body with respect to the presence, identity and number of sweat glands. The axillary region is of particular

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interest, as it contains dense aggregations of eccrine, apocrine, and sebaceous glands that nurture diverse communities of microbiota thought to play an important role in generating individual odour (Taylor et al., 2003).

Between the different types of skin glands, the human body odor is primarily the result of the apocrine sweat glands, which secrete the majority of chemical compounds needed for the skin flora to metabolize it into odorant substances (Lundstrom and Olsson, 2010). This happens mostly in the axillary (armpit) region, although the gland can also be found in the areola, anogenital region, and around the navel (Turkington and Dover, 2007). In humans the armpit regions seem more important than the genital region for body odor which may be related to human bipedalism. The genital and armpit regions also contain springy hairs which help diffuse body odors (Claus, 2007). Body odor is influenced by the actions of the skin flora, including members of *Corynebacterium*, which manufacture enzymes called lipases that break down the lipids in sweat to create smaller molecules like butyric acid. These smaller molecules smell, and give body odor its characteristic aroma (Buckman, 2003). Propionic acid (propanoic acid) is present in many sweat samples. Propionibacteria in adolescent and adult sebaceous glands can turn its amino acids into propionic acid. Isovaleric acid (3-methyl butanoic acid) is the other source of body odor as a result of actions of the bacteria *Staphylococcus epidermidis* (Ara et al., 2006). A wide variety of deodorant and antiperspirant products are sold for the purpose of mitigating this odour. Factors such as food, drink, and diseases can affect body odor (Claus, 2007).

The flourishing of body flora in a given area depends upon the physiological factors of temperature, moisture, and the presence of certain nutrients and inhibitory substances. Microbes of the normal resident flora are harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. On mucous membranes and skin, the resident flora may prevent colonization by pathogens and possible disease through "bacterial interference" (Brooks et al., 2004). These organisms are adapted to the non invasive mode of life defined by the limitations of the environment. However, resident microbes may produce disease if introduced into foreign locations in large numbers and if predisposing factors are present (Brooks et al., 2004). They can cause skin diseases and enter the blood system creating life threatening diseases particularly in immunosuppressed people (Cogen et al., 2008).

Axillary infections include abscess (bacteria commonly causing abscesses are *Staphylococcus aureus* and *Streptococcus)* (Bolognia et al., 2008), boils (furuncles) usually caused by *Staphylococcus aureus*, *Acanthosis nigricans*, allergic contact dermatitis, erythrasma (caused by an infection by the bacterium C*orynebacterium minutissimum*, hidradenitis suppurativa, intertrigo (intertrigo may be complicated by superficial skin infection with yeast or bacteria, irritant contact dermatitis, psoriasis, melanoma, Tinea infections and a skin tag (acrochordon) (Freedberg, 2003).

This study was undertaken to: (i) Determine the populations and distribution of microorganisms in the armpits of some students of the University of Lagos, (ii) determine the role of gender and health related characteristics such as the use of soap, sponges, deodorant, and frequency of bathing and shaving of armpit hairs on the carriage of microorganisms and (iii) determine the resistance pattern of *Staphylococcus aureus* isolate to antibiotics.

MATERIALS AND METHODS

Collection of samples

Armpit swabs of eighty students from University of Lagos, between the age group of 18 – 25 years were collected before and after bath. Sterile swab sticks moistened with 1.0 ml of sterile normal saline were rubbed

vigorously with rotation over the armpits (approximately 2 cm^2) of participants.

Questionnaires on health related characteristics of the participants were completed by the researcher before sample collection. Information was obtained on gender, use of soap, sponge, deodorant and frequency of bathing and shaving of armpit hairs.

Isolation and identification of microorganisms

Swabs were inoculated immediately after collection onto appropriate agar plates, which include mannitol salt agar, blood agar, nutrient agar, and Sabouraud dextrose agar. All inoculations were done in duplicates and incubated for 24 h at 37 °C for bacteria and 72-96 h at 25-27 °C for fungi. Typical colonies observed after incubation were picked aseptically with inoculating loop and purified by sub-culturing. Bacterial isolates were identified on the basis of colonial morphology, and biochemical tests such as catalase, coagulase, and oxidase tests, DNase activity, and sugar fermentation tests. Fungi were identified microscopically.

Antibiotic susceptibility tests

Antibiotic susceptibility test for the isolated strains of *Staphylococcus aureus* was done using the disc diffusion technique. The most common pathogen causing skin infection is *Staphylococcus aureus* and the incidence of multiplication of resistance strains of *Staphylococcus aureus* has been increasing (Nishima et al., 1995). For this reason and most especially because of their known multiple resistance to antibiotics, we tested antibiotics only on *Staphylococcus aureus* strains in this study. The antibiotic impregnated disc (multi disc) was placed on sensitivity test agar plate previously inoculated with the test organism and incubated at 37 °C for 24 hours. Zones of inhibition were measured to the nearest millimeter (mm). The antibiotics tested include amoxicillin (25 µg), cotrimoxazole (25 µg), gentamycin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), ofloxacin (30 µg), augmentin (30 µg) and tetracycline (30 µg).

Plasmid DNA extraction

The miniprep method was used. This is a combination and modification of the methods described by Maniatis et al. (1982), Lech and Brent (1987) and Kraft et al. (1988). From overnight broth culture 1.5 ml was spinned for 1.0 min in a micro-centrifuge to pellet cells. The supernatant was decanted leaving 50-100 µl together with cell pellet and vortexed at high speed to re-suspend cells completely. This was followed by the addition of 300 µl of tris EDTA sodium hydroxide sodium dodecyl sulphate (TENS) and mixed by inverting the tube 3-5 times until the mixture became sticky. To this was added 150 µl of 3.0 M sodium acetate (pH 5.2) and again vortexed to mix completely. It was spinned for 5.0 min in a micro-centrifuge to pellet cell debris and chromosomal DNA. The supernatant was transferred into a fresh tube, mixed well with 900 µl of ice cold absolute ethanol. It was spinned for 10.0 min to pellet plasmid DNA (white pellet). Pellets were re-suspended in 20-40 µl of tris acetate electrophoresis (TAE) buffer or distilled water for further use.

Agarose gel electrophoresis of plasmid DNA

Agarose powder was mixed with electrophoresis buffer to the desired concentration, and then heated in a microwave oven until completely melted. Ethidium bromide was added to the gel (final concentration 0.5 µg/ml) to facilitate visualization of DNA after electrophoresis. After cooling the solution to about 60 °C, it was poured into a casting tray containing a sample comb and allowed to solidify at room temperature. After the gel had solidified, the comb was removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, was inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer were then pipetted into the sample wells, the lid and power leads were placed on the apparatus, and current was applied. DNA migrated towards the positive electrode, which was coloured red. When

adequate migration had occurred, DNA fragments were visualized by staining with ethidium bromide. This fluorescent dye intercalates between bases of DNA and RNA. It was incorporated into the gel so that staining occurred during electrophoresis, but the gel can also be stained after electrophoresis by soaking in a dilute solution of ethidium bromide. To visualize DNA or RNA, the gel was placed on an ultraviolet transilluminator and the photograph was taken using a photo documentation system. All molecular weights were calculated using an online molecular weight marker at insilico.ehu.es (Kraft et al., 1988).

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Data analysis

Data generated in the course of this study were analyzed using SPSS for windows, 15.0 version. The student's t-test statistical tool was used to test for significant differences between the variables with regard to the hypotheses tested. The P-value was used as decision rule for accepting or rejecting the null hypothesis. The level of significance for the test was 0.05.

RESULTS

There were no significant differences in most of the variables examined between male and female student subjects including soap usage (P = 0.342), bathing habit (P = 0.273), shaving pattern (P = 0.226) and sponge usage (P = 0.105). However, there was significant difference in the use of deodorant (P = 0.001) among males and females, with more females using it. Majority of the participants used toilet soaps (62.50%), had bath twice daily (60.0%), used sponge (87.50%), shaved regularly (78.75%), while 47.50% used deodorant (Table 1). The bacterial and fungal counts of male armpits were higher than for the female armpits. There was no significant difference in bacterial counts before and after bath for both male and female participants (Table 2).

The use of deodorants affected bacterial counts. Most of the participants did not use deodorants and bacterial count was significantly high among them. The other variables such as soap usage, sponge usage, bathing habit and shaving frequency had no significant effect on bacterial counts (Table 3). From Table 4, the occurrence of *Staphylococcus epidermidis* in the samples was the highest (35%) while that of *Candida tropicalis* was the lowest (1%) compared to other microbial isolates. *S. aureus* was resistant to most of the antibiotics tested (Table 5). The DNA profile of *S. aureus* (Figure 1) showed that none of the strains examined possessed a plasmid.

Table 1: Percentage distribution of characteristic habits of student subjects by gender.

Health related characteristics	Male n=40 (%)	Female n=40 (%)	Total N=80 (%)	P value
Soaps usage:				
Medicated soap	12 (30.00)	18 (45.00)	30 (37.50)	0.342
Toilet soap	28 (70.00)	22 (55.00)	50 (62.50)	
Deodorant usage:				
Yes	8 (20.00)	30 (75.00)	38 (47.50)	0.001
No	32 (80.00)	10 (25.00)	42 (52.50)	
Bathing habit:				
Once daily	18 (45.00)	14 (35.00)	32 (40.00)	0.273
Twice daily	22 (55.00)	26 (65.00)	48 (60.00)	
Sponge usage:				
Yes	32 (80.00)	38 (95.00)	70 (87.50)	0.105
No	8 (20.00)	2(5.00)	10 (12.50)	
Shaving pattern:				
Once a week	2 (5.00)	10 (25.00)	12 (15.00)	0.226
Once a month	23 (57.50)	28 (70.00)	51 (63.75)	
Once in 2 months	15 (37.50)	2 (5.00)	17 (21.25)	

n= number of student subjects

Table 2: Microbial counts of armpits of student subjects.

	Male armpit		Female armpit	
	Before bath	**After bath**	**Before bath**	**After bath**
Bacteria				
Mean bacterial count (cfu/cm^2)	2.74×10^2	2.70×10^2	2.44×10^2	2.36×10^2
S.D	32.034	31.067	30.893	30.534
Fungi				
Mean fungal count (cfu/cm^2)	4.40×10^1	4.10×10^1	3.90×10^1	3.80×10^1
S.D	11.547	10.747	8.231	8.230

S.D = Standard deviation

Table 3: Effects of variables on the carriage of microorganisms.

	Mean bacterial count (cfu/cm^2)	**Mean bacterial count (cfu/cm^2)**
Variables	Male	Female
Soap usage		
Medicated soap	2.67×10^2	2.42×10^2
Toilet soap	2.79×10^2	2.64×10^2
Deodorant usage		
Yes	2.33×10^2	2.30×10^2
No	2.92×10^2	2.59×10^2
Bathing habit		
Once daily	2.78×10^2	2.49×10^2
Twice daily	2.72×10^2	2.44×10^2
Sponge usage		
Yes	2.72×10^2	2.45×10^2
No	2.81×10^2	2.59×10^2
Shaving pattern		
Once a week	2.54×10^2	2.39×10^2
Once in two weeks	2.61×10^2	2.46×10^2
Once in three months	2.97×10^2	2.86×10^2

Table 4: Species diversity of microorganisms in the armpits of student subjects.

Organisms isolated	**Occurrence of microbial isolates (%)**
Staphylococcus epidermidis	35
Staphylococcus hominis	25
Staphylococcus haemolyticus	15
Micrococcus luteus	9
Staphylococcus capitis	6
Staphylococcus cohnii	3
Staphylococcus saprophyticus	3
Staphylococcus aureus	3
Candida tropicalis	1

Table 5: Antibiotic resistance patterns and zones of inhibition of strains of *Staphylococcus aureus.*

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Antimicrobials	Disk potency	Sensitivity of control strains (mm)	Zones of inhibition (mm)								
			S1	S2	S3	S4	S5	S6	S7	S8	S9
Amoxicillin	25 µg	16-22	12	12	8	12	8	10	5	12	10
Cotrimoxazole	25 µg	24-32	8	10	5	5	8	5	8	5	8
Erythromycin	15 µg	22-25	10	12	10	10	15	12	15	10	15
Gentamycin	10 µg	19-26	18	15	18	15	10	10	12	8	12
Tetracycline	30 µg	24-30	10	8	5	5	8	12	10	10	8
Chloramphenicol	30 µg	22-28	10	10	12	15	15	10	12	15	18
Ofloxacin	30 µg	21-27	25	22	18	8	15	12	20	21	10
Augmentin	30 µg	19-25	15	8	12	10	12	8	10	10	8

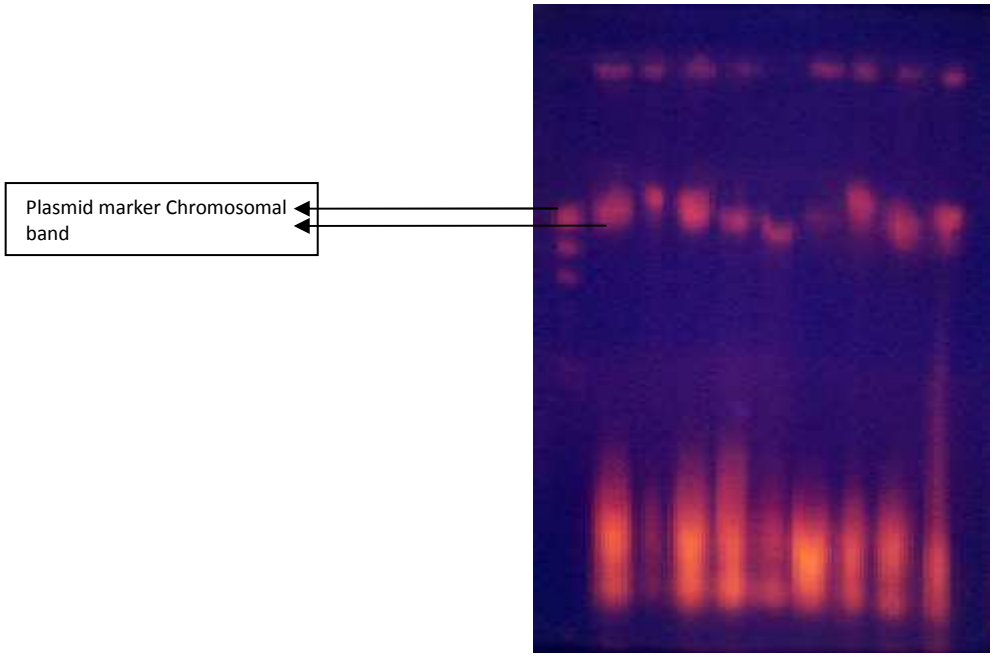

Figure 1: Agarose gel electrophoresis profiles of DNA extracts of *Staphylococcus aureus* strains (S1-S9) from the armpits of student subjects.

DISCUSSION

The bacterial/fungal population in the armpits of males was higher than in females. However, there was no significant difference in bacterial/fungal population before and after bath and in the use of soaps, sponges, frequency of bathing or shaving for both male and female participants. Neither profuse sweating nor washing and bathing can eliminate or significantly modify the normal resident microorganisms. The number of superficial microorganisms may be diminished by vigorous daily scrubbing with soap containing hexachlorophene or other disinfectants, but the flora is rapidly replenished from sebaceous and sweat glands even when contact with other skin and areas or with the environment is completely excluded (Brooks et al., 2004).

From this study we observed that both males and females that used deodorants had lower bacterial counts in their armpits than

those that didn't use deodorant. Deodorants are known to work by suppressing the growth of microorganisms and hence armpit odors. Amongst the microbial isolates from the armpit, *Staphylococcus epidermidis* occurred most frequently (35.0%) while *Candida tropicalis* was the least occurring organism (1.0%). *S. epidermidis* although a normal flora of the skin, skin glands, anterior nares, and mucous membranes of humans and animals, is an opportunistic pathogen for humans that can cause urinary tract infections, wound infections, endocarditis, and septicemia. Due to contamination, it is probably the most common species found in laboratory tests (Queck and Otto, 2008). *S. epidermidis* is also a major concern for people with catheters or other surgical implants because it is known to cause biofilms that grow on these devices (Salyers and Whitt, 2000). *Candida tropicalis* is also a pathogen of concern.

Staphylococcus aureus recorded 3% frequency of occurrence in the armpits of the subjects. It is a known commensal on human skin, but it is an opportunistic pathogen causing a wide range of infections among which are furuncles (boils), carbuncles, impetigo, epidermal necrosis, osteomyelitis, staphylococcal food poisoning and toxic shock syndrome. Following the disc diffusion assay, the highest sensitivity was recorded for ofloxacin in this study, while the least was for co-trimoxazole. *S. aureus* showed resistance to most of the antibiotics tested for. The diameter of inhibition zone obtained, ranged from 5 to 25 mm. Drug resistance may be natural or acquired characteristics of a microorganism. This may result from impaired cell wall or cell envelope penetration, enzymatic inactivation, altered binding sites or active extrusion from cell as a result of efflux mechanisms (Finch, 2004).

Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme which breaks down the β-lactam ring of the penicillin molecule. Penicillinase-resistant penicillins such as methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase. Methicillin resistance requires the presence of the chromosomally localized *mecA* gene (Kernodle, 2000), which codes for an altered penicillin-binding protein (PBP) that has a lower affinity for binding β-lactams (penicillins, cephalosporins and carbapenems). The plasmid profile of *S. aureus* isolates in this study showed that none of the strains had a plasmid and this may suggest that the resistance genes could be chromosomally encoded.

Conclusion

In our communities where teenagers hawk various food items, the role of carriers of enterotoxin producing strains of *S. aureus* in food poisoning should be of concern. The public should be made aware of the importance of personal hygiene. We found that more female participants used deodorants, than the males, and the bacterial and fungal counts in the armpits of females were lower than the counts from male armpits, which mean that the use of deodorant reduced the carriage of microorganisms. The number of superficial microorganisms may be diminished by vigorous daily scrubbing with sponge and soap but, the flora is rapidly replenished from sebaceous and sweats glands. Washing our skin to prevent the transmission of organisms is important. However, the total elimination of organisms on our skin surfaces can at times be harmful. The bacteria that normally dwell on our skin surfaces help us by preventing potential disease-causing organisms from dwelling on our skin.

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