Research Paper

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Effect of Deodorant on Microflora of The Armpit of Female Students of A University in Nigeria

Science

KEYWORDS : Deodorant, microflora, armpit, susceptibility.

*** ISITUA Chinwe Christy**	Department of Microbiology, College of Sciences, Afe Babalola University, Nigeria * Corresponding author
AZEGBEOBOR Margaretmary Ideselu	Department of Microbiology, Faculty of Life Sciences, University of Benin, Nigeria
UMOH Elizabeth Edem	Department of Microbiology, College of Sciences, Afe Babalola University, Nigeria

ABSTRACT

Majority of deodorants incorporate an antimicrobial agent as active ingredients to inhibit the growth of microbial populations responsible for sweat degradation and malodor generation. Research about deodorants are becoming more popular but very little is known about these products; and a multiple of reports and possible harmful effects of their use are circulating in the media. In a microbiological perspective, our goal was to evaluate the effects of deodorant on the microflora of the armpit. The study lasted for one month and was divided into 2 phases: Phase I consist of two weeks without deodorant usage, while phase II consist of two weeks of deodorant usage. The brand of deodorant used was 'Bouquet Deodorant'. Axillae swabs were collected from 20 consented female students of the University under study. Samples were cultured and incubated at 37oC both aerobically and anaerobically for 24 – 48H on chocolate, blood and MacConkey agars. Antibiotic susceptibility test was done on nutrient agar using disc diffusion method of Bauer and Kirby. The isolates identified consist of Staphylococcus albus which was predominant and accounted for 52.4% and 50.0% of the bacterial isolates for the first and second weeks respectively. Other bacteria isolated include Staphylococcus aureus (42.9% and 46.1%) and Klebsiella spp (4.7% and 3.9%) respectively for the successive two weeks. All the isolates showed some degree of susceptibility to the antibiotics used. There was a sustained reduction of the Gram positive cocci and a general elimination of the Gram negative bacteria during two weeks usage of deodorant.

INTRODUCTION

The skin is the primary external coating of the human body. In adults, skin occupies approximately 2.4 square yards and because it is exposed to the environment, the skin is inhabited by a number of bacteria. Over much of the body there are hundreds of bacteria per square inch of skin. In moisture laden regions such as the armpit, groin, and in between the toes, bacteria can number up to one hundred per square inch (Ibe and Wariso, 2005). Most human skin is populated with Staphylococcus epidermidis, propionibacterium acnes and other microorganisms. These bacteria are normal microflora and are beneficial to skin. They help maintain the low pH of skin which inhibits the growth of more harmful bacteria. They also consume the limited amounts of nutrients available on skin, making it hard for other bacteria to establish themselves (Barrett-Hill, 2004).

There are four main groups of bacteria that predominate almost everywhere on the skin: Diphtheroids (e.g. corynebacteria like Corynebacterium diphtheria; Propionibacterium acnes was once classified as a Corynebacterium is considered part of this group), micrococci (which include the staphylococci such as Staphylococcus epidermidis), streptococci (either alpha (α) or gamma (γ) hemolytic), and the enterococci. Besides bacteria the skin also is the home to yeast (like Candida) and fungi (Barrett-Hill, 2004). The populations of microbes vary over the body's skin due to differences in pH, oxygen, water, and secretions. Certain groups, such as the diphtheroids, are found mainly in the groin and armpits. The densities of microbes vary considerably. The armpit is home to about 500,000 bacteria per square inch; the forearm - about 12,000 bacteria per square inch (Taylor et al., 2003).

Beyond its protective function for the human body, the skin serves as a colonization site for commensal, symbiotic pathogenic microorganisms with cellular densities in the range of 106 per cm3 (Eugenie et al., 2010). The surface of the skin itself comprises several distinct environments. Areas such as the axillae (armpit), the perineum (groin) and the toe webs provide typically moisture regions for bacterial growth. These "tropical forest" environments often harbour the largest diversity amongst the skin flora. Typical organisms include Staphylococcus aureus, Corynebacterium and some Gram-negative bacteria. The bulk of the human skin surface, however, is much drier and is predominantly inhabited by Staphylococcus epidermidis and Propionibacterium (Barrett-Hill, 2004).

Body odours originate from many sources. Axillary odor is the most characteristic and stigmatized of these odours. Breakthroughs in controlling axillary malodour came in the latter part of the 1900s when scientists explained the structure and function of human sweat glands and the role of bacterial microflora on the skin. Characterization of underarm secretions and skin bacteria led to various odour strategies, such as reducing perspiration by blocking sweat glands and reducing microorganisms on the body surface (Ibe and Wariso, 2005).

In the course of the earliest studies of human body odour, the odoriferous steroids 5α- androst-16-en-3-one and 5α-androst-16-en-3α -ol have been detected in human axillae secretions (Natch et al., 2004). Another source of axillary malodor are volatile thiols, which are released from precursors by the action of C-S-lyases. Even though these odorous compounds are present only in trace amounts, they play an important role in malodour formation due to their low perception thresh holds (Eugenie et al., 2010).

Axillary malodour is formed upon the biotransformation of human secretion by the skin microbiota. In the process, initially odourless substrates are metabolized by commensal microbes' resident on the axillary skin. Culture dependent investigations of the composition of the axillary microbiota revealed the presence of four bacteria phyla Staphylococcus, Micrococcus, Corynebacterium and Propionibacterium

and yeasts of the genus Malessezia (Leyden et al., 1981). Generally, the armpit microflora is dominated by either Staphylococcal or aerobic Corynebacterium species, the latter being associated with axillary malodour (Taylor et al., 2003).

The offensive smell of malodorous skin has become a major concern for many individuals. Although a variety of materials have been suggested to reduce perception of body and underarm malodour. Over the last century, a whole range of varying deodorants has been developed (Andreas et al., 1990). The majority of deodorants (aerosols, pumps, sticks, roll-ons, creams, soaps) incorporate an antimicrobial agent as active ingredient. Such compounds are included to inhibit the growth of the microbial populations responsible for sweat degradation and malodour generation (Ashley, 1987).

Deodorants are substances applied to the body to reduce axillary odour by exerting an anti-bacterial action on the organisms which decompose apocrine axillary secretions while antiperspirants both reduce axillary odour and the amount of perspiration released to keep the armpit dry and comfortable (Philip and Jack, 2000). It is unquestionable that deodorants have helped many people to mask body odour since their advent in the 19th century. Masking of body odour is a natural part of the human species, common even among the ancient Greeks, and probably something unchangeable. Deodorants were regarded as "cheap miracle products" that helped mankind to get rid of body odour (Arthiha, 2004). Since then the use of deodorants has flourished rapidly, with the increasing emphasis on personal hygiene among people.

Consequently, all over the world, studies are performed to analyze and get a deeper understanding of the commonly consumed deodorants. Researches about deodorants are becoming more popular because of the simple reason that more and more people are using them regularly. However, still many people know very little about these products and a multiple of reports and possible harmful effects of the use of deodorants are circulating in the media. The ideas are divided among scientists, but they all agree about one point, in the long run consumption of deodorants may and probably not have harmful effects on the human body. This study was therefore carried out with the view to determine the various types of bacteria present in swab samples of the axillae and the population and antibiogram of the isolates identified.

MATERIALS AND METHOD

This study was conducted at the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. Benin City is a cosmopolitan City and capital of Edo State.

Sterile swab sticks was used to collect samples from twenty volunteer female students in the University of Benin. A total of four hundred (400) samples were collected with twenty (20) samples a day for five (5) days in a week. The period of collection of samples lasted for four (4) weeks, this four (4) weeks period was divided into two phases: Phase one lasted for two (2) weeks and involved collection of samples from volunteers who have stayed away from using any form of deodorant on their axilla (armpit), while Phase two (2) lasted for two weeks and involved collection of samples from the same volunteers who were now allowed to use deodorant. All volunteers used the same kind of deodorant with the brand name Bouquet deodorant. The composition of this deodorant was: Water, Tiethylcitrate, Cyclomethicone, Ceteareth-12, Fragrance, Glycerin, Isopropyl Palmitate, Xanthan Gum, Aluminium Chlorohydrate, Triclosan, Trisodium Citrate, Methylchloroisothiazolinone, Methylisothiazolinone, and Sodium Hydroxide. Samples were collected from volunteers as from 2pm. This is the time period for maximum perspiration activity. Collection of samples spanned a period of one month. The first two weeks, volunteers did not use deodorants and the next two weeks volunteers used same type of deodorant. Swab sticks were used to touch the axillae and thereafter stored for onward microbiological analysis at the laboratory.

The microbiological samples obtained from the armpit were plated on chocolate agar (Oxoid No Cm 271), blood agar (Oxoid No Cm 271) and Mc Conkey agar (Oxoid No 7), while nutrient agar was used for susceptibility testing (Cheesbrough, 2006). The agar were prepared according to the manufacturer's instruction and were incubated aerobically and anaerobically as the case may be. Samples inoculated on chocolate agar required 5 – 10 % carbon dioxide to create partial anaerobic conditions for microbial growth; and in this study the candle jar method at 37oC was used to generate carbon dioxide (Cheesbrough, 2006).

The various agar plates were streaked aseptically with sterile wire loop and well-spaced out to form discrete colonies. The inoculated plates were incubated at 370C. The plates which required anaerobic incubation were put in anaerobic jar filled with hydrogen gas, while others were incubated aerobically. Grease free slides were smeared with each specimen for Grams staining. The blood agar plate was incubated both aerobically and anaerobically. Plates were examined after 24 hours, and those which did not have growth were reincubated for another 24 hours. All the isolates were identified using colonial morphology and biochemical reactions (catalase, coagulase tube and slide tests as well as indole, citrate and urease tests) according to the methods of Barrow and Feltham (1999). Susceptibility test was done by the disc diffusion method of Bauer et. al. (1996). With a sterile forceps, commercially prepared antibiotic discs were placed at least 25mm apart on nutrient agar plates for all isolates. The different antimicrobial agents used were: Imipenem, Gentamycin, Cefuroxime, Ofloxacin, Erythromycin, Amoxicillin clavulanate, Ciprofloxacin and Cloxacilin. Plates were incubated at 37oC for 24 hours, after which the zones of inhibition in each case were measured and compared to determine sensitive and non-sensitive organisms. "R" represents resistance, while "S" represents sensitivity to the antibiotic. Results were presented descriptively.

RESULTS

Of the two hundred (200) swab samples examined during the phase one period, one hundred and ninety-one (191) were positive for microorganisms and nine (9) negative; while one hundred and seventy-eight (178) were positive and twenty-two (22) negative for microorganisms during the phase two period of the study.

Three hundred and sixty-nine (369) isolates belonging to three different species were recovered from the 400 axillae swabs cultured before and during deodorant application. All the bacterial isolates were facultative anaerobes in nature with Gram positive cocci being the most prevalent isolates accounting for 95.7% of total isolates while Gram negative rods were recovered in 4.3% of total isolates. Staphylococcus albus was the predominant organism recovered accounting for 52.4% and 50.0% of total isolates for phase one and phase two periods of study respectively. Other bacteria recovered from the axillae region after cul-

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turing were Staphylococcus aureus (42.9% and 46.1) and Klebsiella sp. (4.7% and 3.9%) respectively for both phases (Figure 1 and 2).

The effects of daily deodorant application on total axillae bacterial count is shown in table 1 with a marked reduction in the total number of isolates (from 9.60 x 102/cm2 skin to 2.16 x 102/cm2 skin) during daily application from day 1 to day 10 of the phase two period; there was an average value of 9.69 x 102/cm2 skin and 4.73 x 102/cm2 skin of the total number of isolates before and after deodorant application respectively. Also, the effect of daily deodorant application on individual bacterial species is shown in Figure 3.

The sensitivity of the isolates to selected antibiotics are shown in table 2; all the isolates showed a high degree of susceptibility to imipenem, gentamycin and ofloxacin, Klebsiella sp. was most susceptible to imipenem. Staphylococcus aureus and Klebsiella sp. showed some degree of susceptibility to cefuroxime, while Staphylococcus albus was resistant. Klebsiella sp. and Staphylococcus albus showed some degree of susceptibility to ciprofloxacin, while Staphylococcus aureus was resistant.

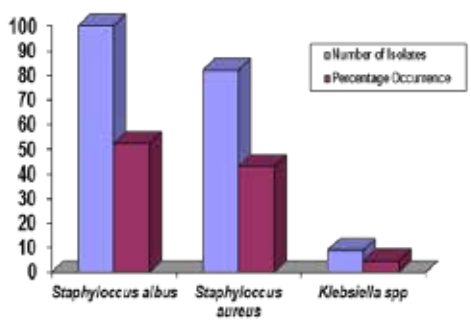

Figure 1: Graphical representation of the percentage of occurrence of bacterial isolates for the phase one period.

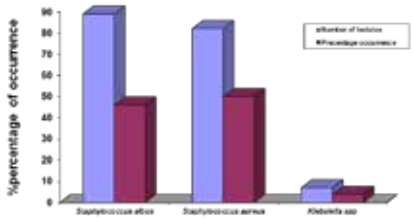

Figure 2: Graphical representation of the percentage of occurrence of bacterial isolates for the phase two period

Table 1: Effect of daily deodorant application on total axillae bacterial count

Application period	Bacterial isolates/cm^2 skin	
	Before application (10^2)	During application (10^2)
Day 1	9.11	9.60
Day 2	9.78	7.81
Day 3	8.79	4.85
Day 4	7.05	5.12
Day 5	9.01	3.93
Day 6	12.12	3.95
Day 7	10.48	3.56
Day 8	10.82	3.64
Day 9	9.95	2.68
Day 10	9.76	2.16

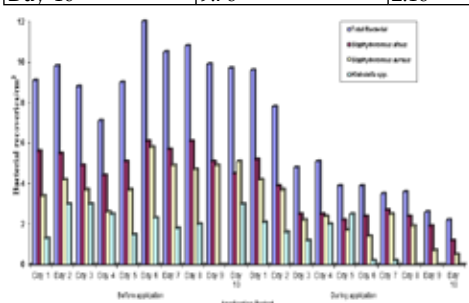

Figure 3: Graphical Representation of individual bacterial species recovered for both phase 1 and 2 period

Table 4: Antibiogram of bacterial isolates from axillae region

Isolates	No.	IPM	CN	CXM	Antibiotics OFX	E	AUG	CIP	OB
No. of *S. aureus* No. of *S. aureus* susceptible % Susceptibility % Resistance	164	153 93.3% 6.7%	132 80.5% 19.5%	155 94.5% 5.5%	148 90.2% 9.8%	147 89.6% 10.4%	105 64% 36%	0 0% 100%	39 23.8% 76.2%
No. of *S. albus* No. of *S. albus* susceptible % Susceptibility % Resistance	189	172 91% 9%	117 61.9% 38.1%	0 0% 100%	147 77.8% 22.2%	175 92.6% 7.4%	104 55% 45%	61 32.3% 67.7%	179 94.7% 5.3%
No. of *Klebsiella* sp. No. of *Klebsiella* sp. susceptible % Susceptibility % Resistance Total isolates	16 369	16 100% 0%	14 87.5% 12.5%	12 75% 25%	8 50% 50%	1 6.3% 93.7%	4 25% 75%	2 12.5% 87.5%	1 6.3% 93.7

Key:
IPM: Imipenem
CN: Gentamycin
CXM: Cefuroxime
OFX: Ofloxacin

E: Erythromycin
AUG: Amoxicillin clavulanate
CIP: Ciprofloxacin
OB: Cloxacillin

DISCUSSION

Previous studies have demonstrated that the unwashed axillae maintain a fairly constant microbial population over three days, varying form 4.9 x 104 to 5.9x105 bacteria/cm2 skin. When the axillae are washed with soap, the microbial count is initially reduced, but the intermediate counts each day show a buildup, with the axillary population increasing up to the unwashed level by the end of the day (Fearnley and Cox, 1983). The same tendency is evident following three days application of an ethanol-based aerosol spray, although the initial reduction in microbial count is much greater, presumably due to the intrinsic antibacterial action of the alcohol itself (Ashley, 1987).

In the study of two weeks duration (phase 1 period) and prior to use of deodorant, the total aerobic bacteria count was found to be on average 9.69 x 102/cm2 skin (Table 1). Of the twenty test subjects, 8.6% carried gram-negative bacteria in the axillae, in particular Klebsiella sp. The gram-negative bacteria were eliminated following deodorant application (Figure 3), suggesting that in this case gram-negative carriage was only of a transient nature. With nineteen of the twenty subjects, a simplification of the gram-positive microflora was evident with deodorant usage, Staphylococcus sp. predominating. From 369 primary isolates characterized during the course of the study, 353 could be identified into the predominant genera, Staphylococcus. Antibacterial efficacy of the deodorant was believed to be significantly enhanced prior to the active ingredients such as triclosan which slow bacterial growth, aluminum chlorohydrate that promote underarm dryness, etc. particularly following repeated application.

This study clearly showed that S. albus is the predominant organism in swab specimens analyzed within the survey period. This situation may not be unconnected with the fact that S. albus is the dominant species that lives mostly on the skin (Gill et al., 2005). Due to contamination, it is probably the most common species found in laboratory tests. Although S. albus is not usually pathogenic, patients with compromised immune systems are often at risk for developing an infection. These infections can both be nosocomial or community acquired, but they pose a greater threat to hospital patients. S. albus 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 (Hedin, 1993). The ability to form biofilms on plastic devices is a major virulence factor for S. albus which is caused by surface proteins that bind blood and extracellular matrix proteins. This occurs mostly on intravenous catheters and on medical prostheses (Otto, 2009).

S. aureus was next numbering 164(86.11%). It is a member of the family Staphylococcus like Staphylococcus albus. This situation may be unconnected with the ubiquity of Staphylococcus aureus as it colonises body surfaces. Although some strains of Staphylococcus are usually harmless, injury or break in the skin enables the organism to invade the body and overcome the body's natural defenses. The consequences can range from minor lesions to deep seated infections (Maree et al., 2007). S. aureus is a hardy bacterium as it was shown in a study where it survived for three months on a piece of polyester, a material, being the main material used in hospital privacy curtains (Mordi et al., 2012). The other organism isolated was Klebsiella. Its clinical relevance lies in the fact that it is a gram-negative organism which has the capability of producing extended spectrum betalactamases (ESBALS) (DeChamps et al., 1999).

All the isolates showed some degree of susceptibility to impenem, gentamycin, ofloxacin, erythromycin, cloxacillin, and amoxilillin clavulanate. S. aureus and Klebsiella showed some degree of susceptibility to cefuroxime while Staphylococcus albus was resistant. All the isolates showed some degree of susceptibility to ciprofloxicin while S. aureus was resistant.

Humans sweat as a means of thermoregulation to prevent body temperature elevation when heat is generated by strenuous activity, sweat when left to sit can produce a strong odour contributing heavily to the scent known as body odour (Sadahiko et al., 2010). Hence, a wash in the morning cannot efficiently prevent body odour all day which is caused by bacterial growth that use the secretion from apocrine, eccrine, and sebaceous glands as the source of nutrient. The produced malodour is characteristic for each person depending on many factors such as intrinsic skin structures, food eaten, and types of bacteria. In order to prevent this body odour and to significantly reduce the growth by microorganisms on the armpit which is the major site of malodour in human, cosmetic products called antiperspirants and deodorants were introduced.

CONCLUSION

This study therefore has provided evidence to show the efficacy of the use of deodorant on helping to prevent body odour and significantly reducing microbial population of the axillae. Hence it is suggested that attention be paid to personal hygiene by bathing frequently and paying special attention to underarms using underarm deodorants.

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