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THE EFFECTS OF SOME MEDICATED SOAPS ON SOME NORMAL MICRO-FLORA OF THE HUMAN SKIN

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ABSTRACT
This project investigates the effects of selected medicated soap (*Delta, Tetmosol* and *Tura*) commonly used at the study area on some normal micro flora (*Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes*) of the human skin. A total of seventy two samples were collected from three body regions (armpit, cubital and finger web) and subjected to both microbial and biochemical tests. Out of the total samples, Thirty six samples each were collected from Bingham University, Karu and Auta-Balefi communities also in Karu. All the samples were cultured in standard Blood and MacConkey agar at 37^0 C for 24 hours. The result showed that there were differences in the prevalence of both *Staphylococcus aureus* and *Staphylococcus epidermidis* at the two locations. Null hypothesis was therefore rejected following t-test (*t-cal= 0.75, df=1, 0.05* and *t-tab=12.7*). *Staphylocccus pyogenes* was isolated from only one sample after treatment. The result also indicated that application of treatment reduced the prevalence of the skin micro flora. On the basis of treatment, the Null hypothesis was also rejected again, for this sample batch (*t-cal= 1.0, df=2, 0.05* and *t-tab=4.03*). These results seem to suggest that, the use of these medicated soaps should be in moderate levels because, over-use may reduce the resident micro flora thereby giving way to transient micro flora, which may grow opportunistically above the normal threshold level, creating a disease situation especially in immune-compromised individuals.

KEY WORDS: Medicated, Soap, Normal, Skin, Flora, Microorganisms and Anti-bacterial.

INTRODUCTION

Normal micro flora (microorganisms) are found on the surface of all human skin (Prescott *et al* ., 2008) . The skin is an important organ of the body that serves for protection against infections by germs and shields delicate underlying tissues against injury (Speers and Dawson, 1965). If an individual loses this protection either by injury or by surgical operations, the person is much more susceptible to infections on the skin. Most of micro flora found on the human skin is harmless and some are beneficial. It is important to note that every organism is a potential pathogen because; even the most innocuous microbe may cause infection particularly if the skin is broken or infected. Some of the normal micro flora of the skin that are of bacterial origin include, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus aureus, Candida albicans, Mallasseiza furfur and Mycobacterium spp.* This study is limited to some normal skin flora of bacterial origin. Bacteria are ubiquitous in nature, and live in the bodies of plants and animals (Fredrickson *et al*., 2004). There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body, with a larger number of the skin flora. The normal skin micro flora are non – pathogenic, but could either be commensals (not harmful to their host) or mutualistic (beneficial). However, resident microbes can cause skin diseases and enter the blood system creating life-threatening diseases particularly in immunosuppressed people. (Alam *et al.,* 1990). Suppression of the normal flora by use of antibiotics or other antimicrobial agents indicates that the normal flora may serve as defense against colonization by potential pathogens. For instance, treatment of the skin of humans with antibacterial agents such as hexachlorophene results in suppression of the normal Gram positive flora and promotes colonization and clinical infection by Gram negative bacilli and other organisms that cannot normally establish themselves on the skin. Common hygiene practice such as use of soap can change the prevalence of a particular skin flora and probably replace them with other microflora. Soap is a cleaning or emulsifying agent made by reacting animal or vegetable fats or oils with potassium or sodium hydroxide. Soap often contains coloring matter and acts by emulsifying grease and lowering the surface tension of water so that it readily penetrates to remove dirt. Medicated soap contains additional ingredients, usually for the treatment of skin disorders. Soap cleanses because molecules of fat are attracted to the fatty part of the anions of soap in water solution and are pulled off by the dirty surface into water (Eckburg *et al.,* 2005).

MATERIALS AND METHODS

Sample area and size

A total of 72 samples (24 from each sampling site) were collected from armpits, cubital and finger webs of 12 volunteers from Bingham University and Auta-Balefi community, Karu, Nasarawa, Nigeria. All the samples were labeled appropriately. These individuals were selected because, they volunteered to be sampled and also accepted to apply the treatments (use of the soap for bathing for two weeks). The two communities were selected because the volunteers were readily available and could be contacted easily without an appointment.

Materials

In this investigation, materials used include incubator set at 37^0C, autoclave, reflected and transmitted light microscope, Bunsen burner, wire loop, swab stick, Petri dish, latex hand gloves, slides, cotton wool, weighing balance and foil paper were used. The media were MacConkey and blood agar. The reagents included crystal violet Reagent No. 28, Lugols iodine Reagent No. 53, Acetone – alcohol decolorized Reagent No. 1, Safranin, Hydrogen peroxide, Plasma, Distilled water and 70% Ethanol.

Sampling and the treatment of samples

Three types of soaps were administered as treatment. These include *Tura, Tetmosol* and *Delta.* Each volunteer was given two tablets of soap to bathe with three times a day for two weeks depending on the treatment. Swab technique was used to collect samples because it is non-destructive, reproducible and economical (Scholefield *et al.*, 1981). Sterile global swab sticks were moistened in sterile distilled water and eight strokes were made on each of the sites (armpit, cubital and finger webs) before and after two weeks of treatment. Swab sticks (samples) were kept in a covered rubber container and transported to the Bingham University Microbiology Laboratory within 30 minutes for analysis. The samples were then subjected to microbial and biochemical tests.

Microbiological analysis

The samples were then cultured on Blood and MacConkey agar (Fluka chemie GmbH CH-947 Buchs), using the streak plate method (Cheesbrough, 2005). The inoculated plates were then incubated at 37^oC for 24 hours, (Raygada and Levine, 2009). Distinct colonies were isolated and sub-cultured into appropriate agar media and kept at 4^oC before the plates were read for identification purposes.

Macroscopic and microscopic analysis of isolates

The cultured plates were observed macroscopically for physical appearance of the colonies. These physical appearances include colour, size, shape, transparency, consistency and nature of the surface (Raygada and Levine, 2009). The isolates were also observed microscopically after Gram staining as described by Cheesbrough, 2005. Using the ×100 oil immersion objective of the light microscope, isolates were identified and assigned to their genera as per the procedure outlined by (Raygada and Levine, 2009).

BIOCHEMICAL ANALYSIS OF THE ISOLATES

Coagulase test

In this study, the slide method test was used. A drop of saline on two separate spots was placed on a grease-free slide. Then, a speck of growth of the test organism was picked and emulsified in both spots, to one spot a drop of plasma was added and to the other a drop of saline was added. Both treatments mixtures were mixed thoroughly by rocking. Coagulation was an indication of positive test to which plasma was added. The presence of clotting indicates positive test for *Staphylococcus aureus* (Cheesbrough, 2005). This test was based on our understanding that the microorganism has the capability to produce Coagulase enzyme which causes the coagulation of human blood plasma.

Catalase test

This was carried out to determine the ability of the microorganisms to produce Catalase enzyme and degrade hydrogen peroxide (H_2O_2). A drop of 3% Hydrogen peroxide was placed on a clean glass slide. A speck of growth of each isolate was collected from the medium using a wire loop and the growth was emulsified in the drop. A positive test was indicated by bubbling and frothing (Cheesbrough, 2005). The principle behind the Catalase test is to differentiate between pathogenic and non-pathogenic Staphylococcus.

RESULTS AND DISCUSSION

Normal Microflora Isolated from skin of Volunteers sampled in Bingham University and Auta-Balefi communities before the Application of the treatment.

As shown in Table 1, results of samples collected from Bingham University indicated that *Staphylococcus aureus* was isolated from twelve (12) samples collected from the armpit regions and none was isolated from the cubital and finger web regions. *Staphylococcus epidermidis* was isolated from twelve (12) samples collected from each of the cubital and finger web regions. None was isolated from samples collected from the armpit.

In Auta-Balefi, result shows that *Staphylococcus aureus* was isolated from the twelve samples collected from the armpit region, while *Staphylococcus epidermidis* was isolated from the 12 samples taken from cubital and finger web regions.

TABLE 1: Microflora isolated from skins of volunteers sampled in Bingham University and Auta-Balefi communities before the application of *Delta, Tetmosol* and *Tura Soaps.*

	Microbial Test			Biochemical tests						
	Gram Staining			Catalase			Coagulase			
Isolate No	Ap	Cu	FW	Ap	Cu	FW	Ap	Cu	FW	Isolates
BU1-BU12	12	0	0	12	0	0	12	0	0	*S. aureus*
BU1-BU12	0	0	0	0	0	0	0	0	0	*S. epidermidis*
AB1-AB12	12	0	0	12	0	0	12	0	0	*S. aureus*
AB1-AB12	0	12	12	0	12	12	0	0	0	*S. epidermidis*

Key: Ap = Armpit, **Cu**=Cubital, **FW**=Finger Web
BU1-BU12 = Number of individuals sampled in Bingham University Community
AB1-AB12 = Number of individuals sampled in Auta-Balefi Community

Normal Microflora isolated from the samples after the Application of the treatment.

As shown in Table 2 below, results indicated the prevalence of the three microflora after treatment for two weeks in Bingham University and Auta-Balefi communities. Samples labeled **BD1- BD4, BT1-BT4** and **Bt1-Bt4**, and collected from three sites (**Ap**, **Cu** and **FW**) were subjected to three tests (Gram staining, catalase and coagulase). In Bingham University, out of 36 samples, *Staph aureus* was isolated from twelve samples collected from the armpit. The breakdown of the result based on the treatment showed that, the highest prevalence of *Staph aureus* was observed on samples treated with *Tetmosol* i.e. 6 samples, however, *Delta* and *Tura soap* have three samples each from which *Staph aureus* was isolated from. The prevalence of *Staph epidermidis* was observed on a total of four samples (1 sample from armpit and three samples from cubital). The breakdown of the result based on treatment showed that samples treated with *Delta* and *Tetmosol soap* were observed to have equal prevalence i.e two samples each. *Strep pyogenes* was not isolated from any of the samples after the treatment in the Bingham University community and no microflora was isolated on the finger web region. In Auta-Balefi community, results in table 2 showed that out of the 36 samples labeled (**AD1-AD4, AT1-AT4** and **At1-At4**) and collected from three sites (**Ap**, **Cu** and **FW**), 24 samples collected from the armpit region tested positive for presence of *Staph aureus*. The breakdown of the result showed that highest prevalence (nine samples each) was observed on samples treated with *Delta* and *Tura*. *Staph aureus* was isolated from only six samples treated with *Tetmosol*. *Staph epidermidis* was isolated from a total of eleven samples (six from the armpit and five from the cubital regions). The breakdown of the result according to treatments showed that the highest prevalence was observed on samples treated with *Tetmosol soap*. *Tura* and *Delta soap* have the prevalence on two and three samples respectively. *Strep pyogenes* was isolated from two samples (one from cubital and one from the armpit) treated with *Tura soap* in Auta-Balefi and no micro flora was isolated from the finger web region.

TABLE 2: Microflora isolated from the samples after the Application of the Treatment in Bingham University and Auta-Balefi communities

	Microbial test			Biochemical tests						
	Gram Staining			Catalase			Coagulase			
Isolate No	Ap	Cu	FW	Ap	Cu	FW	Ap	Cu	FW	Isolates
BD1- BD4	1	0	0	1	0	0	1	0	0	*S. aureus*
BD1-BD4	0	1	0	0	1	0	0	0	0	*S. epidermidis*
BT1-BT4	2	0	0	2	0	0	2	0	0	*S. aureus*
BT1-BT4	0	0	0	0	1	0	0	0	0	*S. epidermidis*
Bt1-Bt4	0	0	0	1	0	0	1	0	0	*S. aureus*
AD1-AD4	3	0	0	3	0	0	3	0	0	*S. aureus*
AD1-AD4	0	3	0	0	0	0	0	0	0	*S. epidermidis*
AT1-AT4	2	0	0	2	0	0	2	0	0	*S. aureus*
AT1-AT4	0	3	0	3	0	0	0	0	0	*S. epidermidis*
At1-At4	3	0	0	3	0	0	3	0	0	*S. aureus*
At1-At4	0	1	0	0	1	0	0	0	0	*S. epidermidis*
At1-At4	0	1	0	0	1	0	0	0	0	*S. pyogenes*

Key: Ap = Armpit, **Cu** = Cubital, Fw = Finger Web,
BD1-BD4 = Samples treated with *Delta soap* in Bingham University community
BT1-BT4 = Samples treated with *Tetmosol soap* in Bingham University community
Bt1-Bt4 = Samples treated with *Tura soap* in Bingham University community
AD1-AD4 = Samples treated with *Delta soap* in Auta-Balefi Community
AT1- AT4 = Samples treated with *Tetmosol soap* in Auta-Balefi Community
At1-At4 = Samples treated with Tura *soap* in Auta-Balefi Community

DISCUSSION

Normal microflora isolated from samples before and after the treatment with Medicated Soaps.

Bactericidal effect of three medicated soaps (*Delta, Tetmosol* and *Tura*) was investigated on three micro-flora of the skin namely, *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes*. Before the treatment, the skin flora distribution was observed as follows, **Ap: 12**, **Cu: 6** and **FW: 6**, however after the treatment, the new skin flora distribution was **Ap: 6**, **Cu: 1** and **FW: 0**. This is an indication that the treatment had reduced the normal flora see Fig 1 below. As shown in the results (Tables 1 and 2), *S. aureus* was isolated from the 12 samples collected from the armpit before the application of the treatment in the Bingham University Community. However, after the treatment, *S. aureus* was isolated from 4 samples only (fig 1). The breakdown of the result according to the treatment showed that higher prevalence of *S. aureus* was observed on samples treated with *Tetmosol i.e.* 2 samples. *Delta* and *Tura* have equal value of three, (fig 2). This result is an indication that medicated soaps have the potential to reduce the occurrence of micro-flora on human skin. This implies that normal skin flora can be replaced with transient flora.

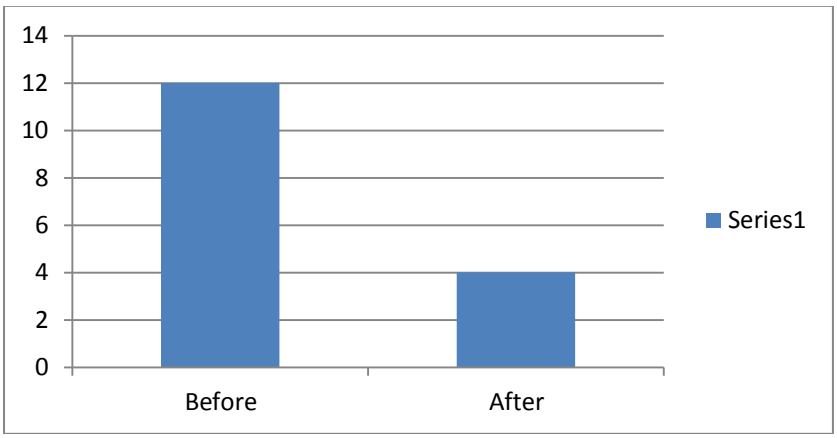

FIGURE 1: Occurrence of *S. aureus* in Bingham University Community before and after the application of treatment.

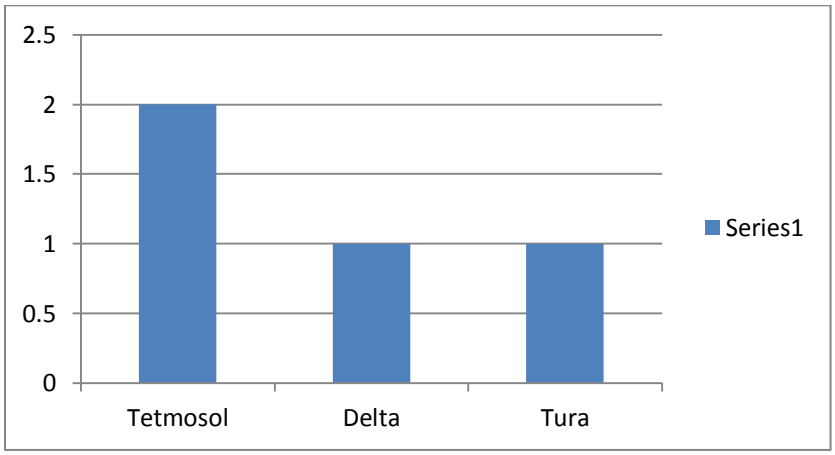

FIGURE 2: Occurrence of *S. aureus* based on treatment in Bingham University community

Similarly, as observed in (fig 3) below, before the application of the treatment, *S. epidermidis* was isolated from 24 samples (**Cu: 12, FW: 12),** however, after the application of treatment (*Delta*, *Tura* and *Tetmosol*), it was observed that only two samples collected from the cubital had the prevalence of *S. epidermidis* (fig 4). No micro-organism was isolated from *Tura.* This is also an indication that medicated soap can reduce normal skin flora, especially *Tura*.

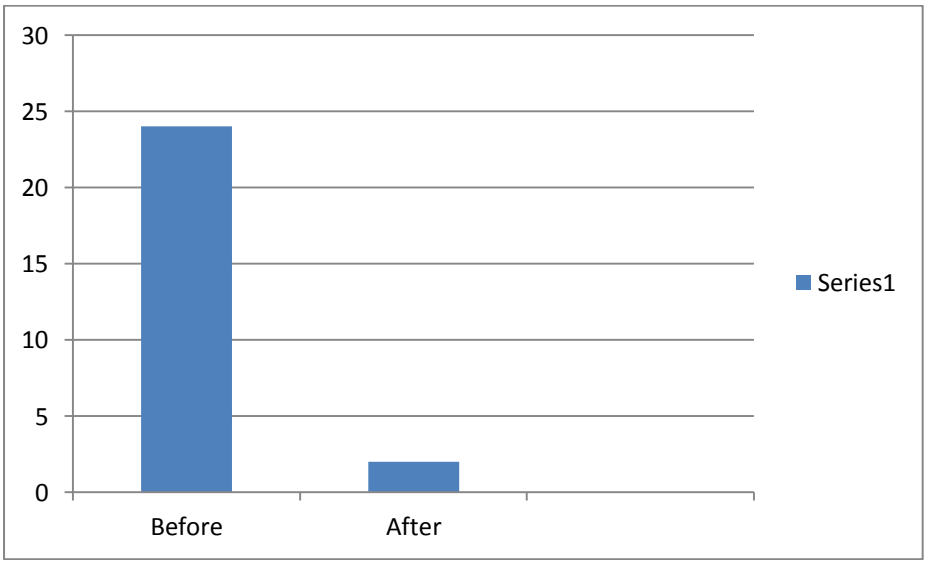

FIGURE 3: Occurrence of *S. epidermidis* in Bingham University Community before and after the application of treatment.

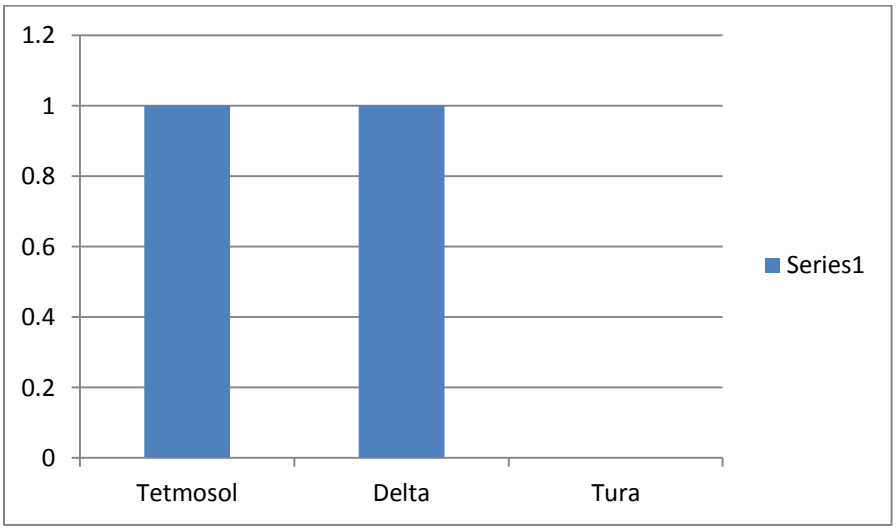

FIGURE 4: Isolate of *S. epidermidis* following treatment in Bingham University community

At Auta-Balefi, before the application of the treatment, *S. aureus* was isolated from 12 samples collected from the armpit region (fig 5). As indicated in fig 6 below, after the application of treatment it was observed that *S. aureus* was isolated from 8 samples collected from the armpit region. This result indicated more than 20% reduction in the inhabitant skin flora. Null hypothesis was therefore rejected after t-test (*t-cal= 0.75, df=1, 0.05* and *t-tab=12.7*). On the bases of treatment, Null hypothesis was also rejected (*t-cal= 1.0, df=2, 0.05* and *t-tab=4.03*)

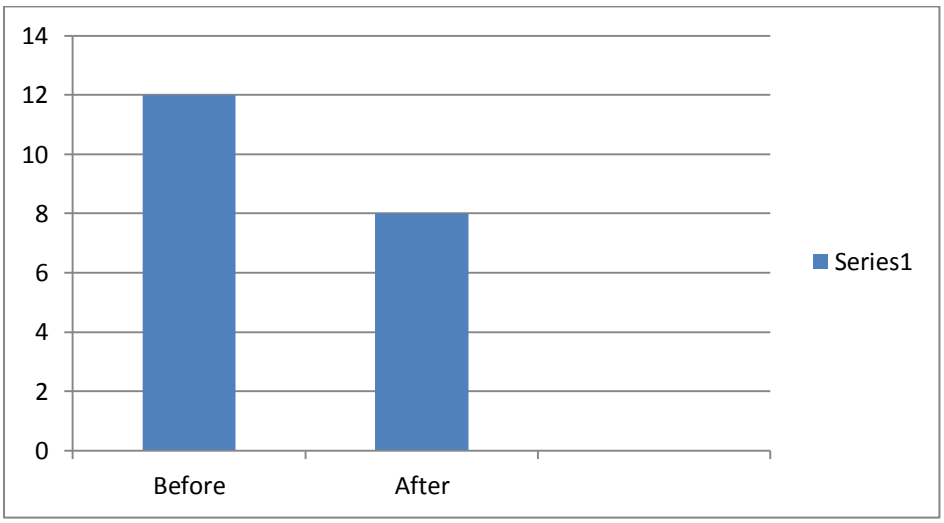

FIGURE 5: Occurrence of *S. aureus* in Auta-Balefi Community before and after the application of treatment.

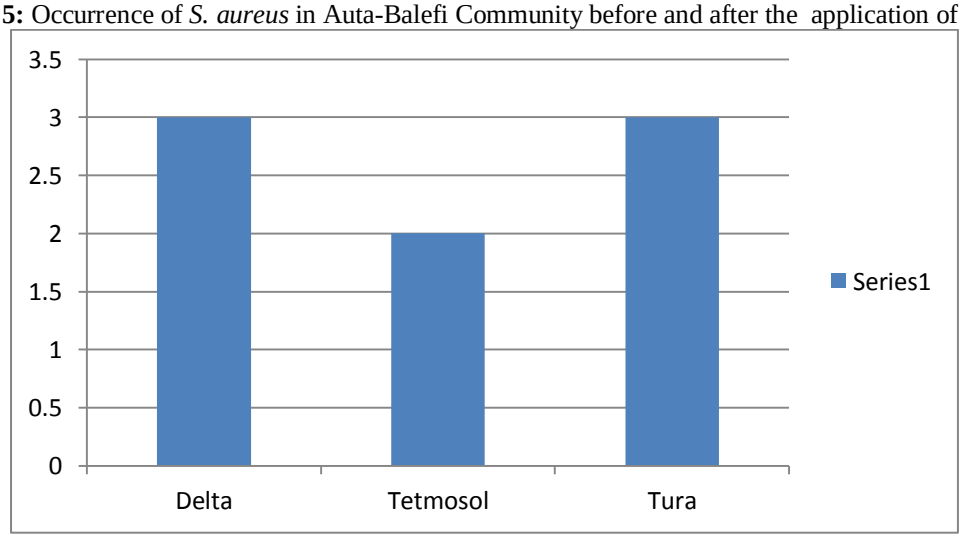

FIGURE 6: Isolates of *S. aureus* following treatment in Auta-Balefi community

As indicated in fig 7 below, before treatment was applied, *S. epidermidis* was isolated from 24 samples (**Cu:12, FW:12**), however, upon application of treatment, *S. epidermidis* was isolated from 7 samples collected from the cubital region. The breakdown of the result shows 3 out of the samples were treated with *Delta soap* while *Tetmosol* and *Tura* were 3 and 1 samples respectively (see fig 8 below). *S. pyogenes* appeared in only 1 sample treated with Tura soap in Auta-Balefi community. It was not observed before the treatment. Null hypothesis was therefore rejected after t-test (t-cal=2.5, df=1, 0.05 and t-tab=12.7). On the bases of treatment, Null hypothesis was also rejected (t-cal= 0.71, df=2, 0.05 and t-tab=4.303).

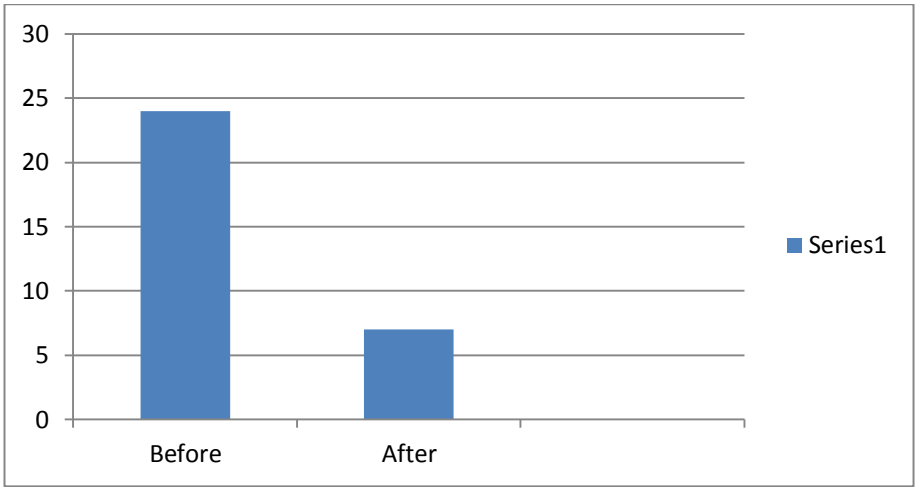

FIGURE 7: Occurrence of *S. epidermidis* in Auta-Balefi Community before and after treatment.

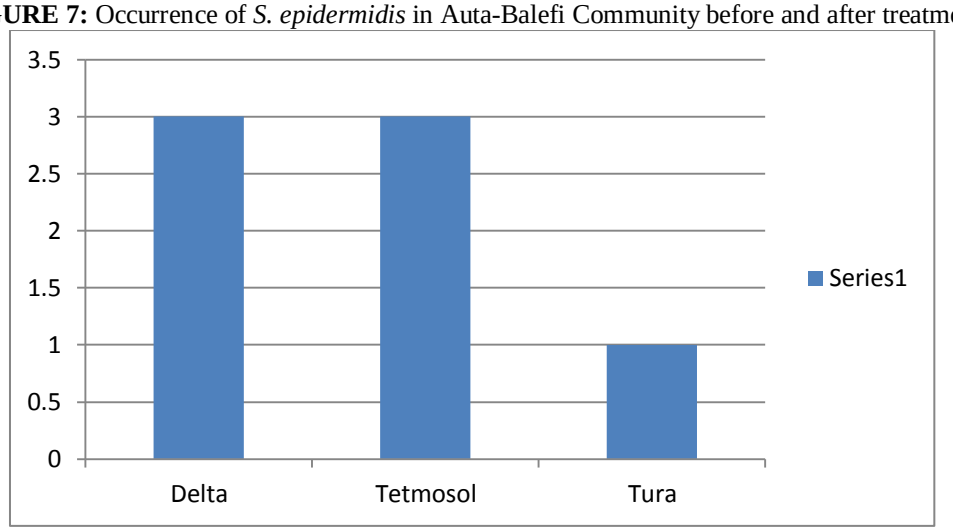

FIGURE 8: Occurrence of *S. epidermidis* following the treatment of sampling subjects in Bingham University community

It is worthy of note that, comparing the above result with that of Evans 1975, where it was shown that the use of medicated soap reduced the normal flora of the body compared to the use of mild toilet soap. *Tura* contains sodium *sulfite* which causes skin irritation with prolonged exposure, a good reason why it reduces the normal flora on the skin than *Delta* and *Tetmosol soap*s. Few literatures showed that the use of medicated soaps reduces the number of normal flora of the skin which may subsequently result in exposure to infection of the skin, because the normal flora serves as a defense against invading organisms (Evans, 1975). From the above result, it indicates that, the 3 medicated soaps used in this research reduce the number of the normal flora of the skin compared with the control.

CONCLUSION

The above findings indicated that among the 3 soaps used in the study, *Delta* soap retained more normal flora than *Tetmosol* or *Tura*, even though it is one of the only species. Other than using this findings and that of (Hickman *et al.*, 2001), the use of mild toilet soap will be preferable because of high retention of normal flora.

RECOMMENDATION

Future investigation is likely to improve understanding of the interaction between skin physiology, microbiology and ecology and the role of the skin in transmission of infectious disease. Frequent bathing has aesthetic and stress relieving benefits but serves little microbiological purpose. Mild non- antimicrobial soaps should suffice for routine bathing.It is therefore recommended that people should not consistently use the same kind of soap for a long time, so that the skin environment can change from time to time.People should also be careful to take care of their armpits so that the growth in armpit cannot exceed the threshold level that could create a disease situation.

Personal hygiene is encouraged. More work should be done on other kinds of soaps and other locations.

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