# ANTIBACTERIAL ACTIVITIES OF DIFFERENT ANTISEPTIC SOAPS SOLD IN ABA ON *STAPHYLOCOCCUS AUREUS* FROM CLINICAL SAMPLES.

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## ABSTRACT

The antibacterial activity of different types of antiseptic soaps on *Staphylococcus aureus* isolated from wound infections and eczematous lesions was evaluated. The samples were collected from fifty individuals with wound and eczema infection within the age range of 9-73 years. Swabs were collected and streaked on appropriate agar and incubation temperature. Out of the fifty (50) individuals sampled, 25 persons were mostly infected with *Staphylococcus aureus* (50% ±1.05) followed by *Pseudomonas aeruginosa* (22% ±0.91), *Staphylococcus epidermis* (10% ±0.33) and the least was *Escherichia coli* (18% ±0.72). This study revealed that *Staphylococcus aureus* isolated and subcultured were sensitive to the three different antiseptic soaps. The zones of inhibition were highest in sample A with 25.0mm ±0.45 followed by sample B with 20.0mm ±0.98 and least in sample C with 10.0mm ±0.65. The results showed that antiseptic soaps were effective against *Staphylococcus aureus* involved in wound and eczema infection in humans. Therefore, antiseptic soaps could be used in cleaning the skin during time of hurt or cut, and should be used moderately by consumers to avoid irritation and resistance to pathogens.

**Key words:** Wound infections, Eczema infections, Zone of inhibition, *Staphylococcus aureus.*

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## 1.0 Introduction

Antibacterial activity is the ability to either destroy or inhibit the growth of microorganisms. This can be referred to as either cidal or static effects respectively. This is significant with respects to the human body in preventing sepsis and skin infections (Higaki *et. al.,* 2000). Soap cleanses because molecules are attracted to the fatty part of the anions of the soap solution and are pulled off by dirty surface into water. Antiseptic soaps contain additional ingredients, usually for the treatment of skin disorders (Eckburg *et. al.,* 2005). Antiseptic soaps have germicidal substances like, irgasan, trichlorocarbanlide, (TCC) etc, incorporated into them to enhance their antibacterial activity (Friedman and Wolf, 1996). These germicidal substances are added in a specific amount and their percentages are always stated on the soap case or leaflet which contains the information on how to use the soap for various purposes.

Normal microflora is found on the surface of all human skin (Prescott *et. al.,* 2008). The normal skin bacterial flora in human is composed of three major groups of gram-positive bacteria: the *coliform bacteria*, the *micrococci* and the *staphylococci* with only a minor component of gram-negative bacilli (Nobel, 1998). This is because the skin is a comparatively dry habitat, with available water as the major factor controlling growth. Occlusion of the skin is a potent way to increase the number of bacteria on the skin (Breuer *et al.,* 2002).

*Staphylococcus* species, though a common cause of human infections are found as non-pathogenic microorganisms in human samples. *Staphylococcus aureus* is the most important member of this group (Diekema *et. al.,* 2001) and has been associated with different clinical conditions and syndromes (Javid *et. al.,* 2006). In Ikegbunam *et al*., (2013), *Staphylococcus aureus* is an opportunistic pathogen affecting both immunocompetent and immunocompromised individuals frequently resulting in high morbidity and complications which constitutes problems to health. It is a gram-positive, non-spore forming cocci bacterium that is a member of the firmicutes, which are found as normal human microbiota of the skin and nasal cavity. It is the most frequently encountered bacterial species in hospitals (Emmerson, 2004). The major reservoirs of *Staphylococcus aureus* in hospitals are colonized in infected patients and hospitals workers (Javid *et al*., 2006). Carriers of *Staphylococcus aureus* are at risk of developing endogenous infections or transmitting infections to health care workers and patients. Its disease manifestation ranges from minor skin infections to life threatening diseases such as folliculitis, furuncle (boil), dermitis (eczema) carbuncle, ulcers, pneumonia sepsis and wound infections. *Staphylococcus aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome (TSS) through production of toxins.

Wound is defined as break in integrity of the skin or discontinuity of the skin as a result of breakage. Dermatitis or Eczema syndrome is an inflammation of the skin characterized by itching and scalded-skin and an infection caused by *Staphylococcus aureus.* Wound healing or restoration of skin continuity, a biological process can be accomplished by regeneration, cell proliferation and collage production which can be encouraged by washing the wound surface and other infected skin lesions like atopic dermatitis especially with antiseptic soap which due to its content of phenolic compounds help in keeping off organisms like *Staphylococcus aureus*, *Escherichia coli* and *pseudomonas aeruginosa* away from the sites (Al-saimary *et al.*, 2013).

Baker *et al.* 2004, described antisepsis as the most convenient way of preventing infection usually by inhibiting the growth of bacteria. Sterilization is described as the complete destruction of all living matters. This description is often restricted to destruction pathogenic organisms only. Therefore, the aim of this work is to determine the antibacterial activities of different antiseptic soaps on *Staphylococcus aureus* from clinical samples. In achieving this, the effectiveness of the different antiseptic soaps on the clinically isolated *Staphylococcus aureus* was determined through measurements of zone of inhibitions.

## 2.0 Materials and Methods.

### 2.1 Study area.

The study area is Aba Metropolis, Abia State, in the South-East Geopolitical zone of Nigeria. The Aba town which has been known for ages as a major commercial center in the Eastern Nigeria is of the Igbo tribe and inhabited by Ngwa people. They are predominantly traders in the popular Ariria market. They have rich cultural history. Aba town is about 49km away from its state capital city, Umuahia and about 52km away from Port Harcourt city, the capital of Rivers State. The geographical coordinates are $5.1167^0$N, and $7.3667^0$E. The area is of tropical climatic conditions with rain forest features. The soil type is silt-clay and the weather is typical of rain forest, with an average annual temperature ranging between 25 - 35°C as lowest and highest values, respectively.

### 2.2 Sources of sample.

The samples for the study were randomly purchased from drug stores and pharmaceutical shops within the Aba city.

### 2.3 Sample collection and preparation.

A total of 12 samples of four (4) different brands of commonly used antiseptic soaps designated A, B, C and normal beauty soap sample tagged D as control were randomly purchased from drug

stores and pharmaceutical shops within the Aba City. The soaps were designated A, B, C and D as control respectively. The soaps were reconstituted with a sterile distilled water to make a stock solution prior to the microbiological assay. The test organisms used in the study are those obtained from wound infections and eczematous lesions from System Medical Laboratories, Aba, in Aba-North Local Government of Abia State, Nigeria. The test organisms were further identified and the biochemical and morphological characteristics were confirmed (Cheesbrough, 2005). The isolates were subcultured into Mannitol salt agar slant and were maintained at $4^0$C on their respective slants.

**2.4 Sterilization of materials**

The glass wares used were washed in soapy water, rinsed, dried and then sterilized by dry-heat method in hot air oven at 160ºC for 1½ hours. Wire loops and needles were sterilized by heating to red hot in open gas flame. Cup borers were dipped into 70% ethanol before flaming to burn off the alcohol and then cooled beside the flame before use.

**2.5 Media and reagent preparation**

The media used were obtained in the commercially prepared powdered (dehydrated) form and were prepared according to the manufacturer's instructions. Specified quantity of each powdered media were reconstituted in specified volume of distilled water in a conical flask and mixed properly by shaking. The flask was then stoppered and sterilized by autoclaving at 121ºC and 15psi for 15 minutes. The autoclaved media were allowed to cool to 45-50ºC in a water bath before dispensing into pre-sterilized petri dishes and allowed to solidify.

**2.6 Biochemical analysis of the test organism**

**2.6.1 Coagulase test**

The slide method of Cheesbrough, 2005 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 with sterile wire loop and emulsified to form a smear. To one spot, a drop of plasma was added, while to the other a drop of saline was added. The treated mixtures were mixed thoroughly by rocking. Coagulation was an indication of positive test to slide. The presence of clotting indicates positive test for *Staphylococcus aureus*. This test was based on the capability of test organism to produce coagulase enzyme which causes the coagulation of human plasma.

**2.6.2 Catalase test**

The slide method test of Cheesbrough, 2005 was adopted. This was carried out to determine the ability of the test organism to produce catalase enzyme and degrade hydrogen peroxide ($H_2O_2$). A drop of 3% hydrogen peroxide was place on a clean glass slide. A speck of growth of each isolate was collected from the medium using wire loop and was emulsified in the drop. A positive test was indicated by the appearance of bubbles of gas (oxygen) indicating free oxygen liberation and is used to differentiate between pathogenic and non-pathogenic *Staphylococcus.*

**2.7 Microbiological analysis of samples.**

The method of disc agar-diffusion as described by Cheesbrough, 2005 was adopted using a sterile Mannitol salt agar and pour plate method. One (1) ml of the standardized suspension of the test organism was put into the empty sterile petri dish that had been sterilized. After autoclaving, the sterile medium was allowed to cool at 45ºC, and 19ml of sterile molten agar was dispensed from the autoclavable bottles into each of the plates containing the suspension of the isolate. These were mixed thoroughly and were allowed to solidify for 20 minutes. 6mm cup borer was used to bore three (3) holes at definite intervals in the plates, and various concentrations of soap samples were introduced into the bored holes and were labeled. The plates were allowed to stand for at least 30 minutes at room temperature for proper diffusion, after which they were incubated in duplicates for each of the antiseptic soaps at 37ºC for 24 hours. After incubation, the resultant diameter of zone of inhibition were measured and recorded.

**2.8 Data analysis**

Data obtained from this research work were analysed using ANOVA. Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 16.

**3.0 Results**

The biochemical analysis and colony description for the identification of *Staphylococcus aureus* was shown in Table 1. Catalase and coagulase tests were positive for the test organism.

Table 2 showed the zone of inhibition of Staphylococcus aureus using different concentrations of sample A Results showed that the zone of inhibition is in direct relationship with soap concentrations. The higher the soap concentration, the higher the zone of inhibition, meaning the two parameters are directly proportional. The highest zone of inhibition (25.0±1.41mm) was recorded in the soap concentration of 500mg/ml.

Table 3 and 4 showed the zone of inhibition of Staphylococcus aureus using different concentrations of sample B and sample C respectively, Results showed that the zone of inhibition and soap concentrations are directly proportional. The highest zones of inhibition (20.0±0.98mm/10.0±0.65mm) were recorded with concentration of 500mg/ml. Table 5 showed summary of zones of inhibition of Staphylococcus aureus using different concentrations of soaps.

Table 6 showed the percentage occurrence of different clinical isolates. Out of the fifty (50) individuals sampled, *Staphylococcus aureus* had the highest percentage occurrence (50% ±1.05) followed by *Pseudomonas aeruginosa* (22% ±0.91), *Staphylococcus epidermis* (10% ±0.33) and the least was *Escherichia coli* (18% ±0.72). The values obtained between the various soap samples, when compared were statistically significant ($p<0.05$).

**4.0 Discussion**

Soap is a water soluble compound made by a reaction (called saponification) between caustic soda (sodium hydroxide) or caustic potash (potassium hydroxide) with animal and/or vegetable fats (oils). Soap has surface active properties to wet a greasy (oily) soiled surface and suspend the oil and dirt in the water for rinsing off. Soap as a cleansing agent can be manufactured in bars, granules, or tablets as it is the case with most antiseptic soaps. Antiseptic soaps have germicidal substances like, irgasan, trichlorocarbanlide, (TCC) etc, incorporated into them to enhance their antibacterial activity (Friedman and Wolf, 1996).

The best in antibacterial activity of all the soaps used is sample A exhibiting maximum zone of inhibition for the test isolates. This could be attributed to its unique formulation of duo combination of irgasan and potassium mercuric iodide. Sample B is primarily used for its scabicide effect; however, it exhibited moderate antibacterial activity which is attributed to monosulfiram within its formulation.

Generally, antiseptic soap could be any cleaning soaps to which AAIs have been added. These chemicals kill bacteria and other microorganisms, though they are not effective at deactivating viruses just like any other kind of soaps. Soaps are intended for reduction of the inoculum sizes of both pathogenic and non-pathogenic microorganisms; the latter include the normal flora. Of these, two types are well known viz. resident flora that are the normal flora of the skin and other human body parts, and transient flora that are usually picked up from objects or other human being. Thus, it is routine practice to wash hands prior to eating, after examining a patient and before surgery, in order to

remove some potentially harmful transient flora as well as reduce a number of resident floras, which might cause opportunistic infections (Nobel,1998).

Results revealed that three (3) different soap samples assayed, displayed varying degrees of inhibition on the test organisms they contain different concentrations of antimicrobial active ingredients (AAIs). The antimicrobial active ingredients (AAIs) included irgasan, trichlorocarbanlide (TCC), mercuric iodide, monosulfiram, and trichloroxylenol which are considered manufacturer dependent.

When the efficacy of the antiseptic soap were assayed using the disc agar diffusion method, sample A were found to be most effective against the test organism having the highest zone of inhibition (25mm±0.45), followed by sample B (20.0mm±0.98) and C (10mm±0.65) respectively. The result of minimum inhibitory concentration (MIC) showed that sample A had better MIC of 62.5 mg/ml and 125 mg/ml respectively on *Staphylococcus aureus*. Analysis of variance for the means of antibacterial activities among the soaps revealed positive correlations ($P<0.05$). It was observed that significant differences exist among the different concentrations of soap samples with samples A having higher zones of inhibition when compared with others. The results obtained in this study are in agreement with the work of Obi, 2014 on antibacterial activities of some medicated soaps on selected human pathogens.

The assayed antiseptic soaps have demonstrated satisfactory antimicrobial effect, particularly in the antibacterial activity. Presumably, the observed variability in antimicrobial activity is due to difference of AAI contents, and type of formulations. Other studies have found that soaps containing AAIs to remove more bacteria than simply washing with beauty soap and water (Obi, 2014). Results from the control in this study has proved the use of beauty soap in cleaning wounds and other skin infections as inappropriate as susceptibility tests reveals.

The control for the experiment which is the beauty soap sample showed no observable inhibition against the test organism. This justifies why people do not use the control soap as medication in control of pathogens via bacteristatic or bactericidal activities even though it possess saponin effects for which reason they are used a regular soap to primarily wash out dirt from body surfaces and leave perfume on the skin. There is statistical significance among different values obtained in the results ($p<0.05$).

### 4.1 Conclusion

Antiseptic soaps based on the findings of this work could be used during medication in cleaning the skin during time of hurt, cut or eczema lesions and other bacterial infection sites, as control for pathogens through bacteristatic or bactericidal activities.

### 5.0 References

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**Table 1: Biochemical analysis of *Staphylococcus aureus***

| Isolate | Colony description | Catalase | Coagulase | Possible isolate |
|---|---|---|---|---|
| X | small smooth yellow colonies with glittering surface | + | + | *Staphylococcus aureus* |

**Table 2: Zones of inhibition of *Staphylococcus aureus* using different concentrations of A**

| Soap concentrations (mg/ml) | Zones of inhibition (mm) |
|---|---|
| 500 | 25.0 ± 0.45 |
| 250 | 21.0 ± 0.12 |
| 125 | 18.0 ± 0.48 |
| 62.5 | 14.0 ± 0.83 |

Legend: A- Antiseptic soap 1,

**Table 3: Zones of inhibition of *Staphylococcus aureus* using different concentrations of B**

| Soap concentrations (mg/ml) | Zones of inhibitions (mm) |
|---|---|
| 500 | 20.0 ± 0.98 |
| 250 | 17.0 ± 0.83 |
| 125 | 14.0 ± 1.24 |
| 62.5 | 9.0 ± 0.09 |

Legend: B- Antiseptic soap 2,

**Table 4: Zones of inhibition of *Staphylococcus aureus* using different concentrations of C**

| Soap concentrations (mg/ml) | Zones of Inhibition (mm) |
|---|---|
| 500 | 10.0 ± 0.65 |
| 250 | 8.0 ± 0.44 |
| 125 | 8.0 ± 0.24 |
| 62.5 | 7.0 ± 0.78 |

Legend: C- Antiseptic soap 3,

**Table 5: Diameter of zones of inhibition (mm) of *Staphylococcus aureus* by different soap samples at different concentrations**

| Soap concentration | A | B | C | D |
|---|---|---|---|---|
| (mg/ml) | **Zones of Inhibition (mm)** | | | |
| 500 | $25.0 \pm 0.45^{a}$ | $20.0 \pm 0.98^{b}$ | $10.0 \pm 0.65^{c}$ | $1.0 \pm 0.0^{d}$ |
| 250 | $21.0 \pm 0.12^{a}$ | $17.0 \pm 0.83^{b}$ | $8.0 \pm 0.44^{c}$ | $0.3 \pm 0.0^{d}$ |
| 125 | $18.0 \pm 0.48^{a}$ | $14.0 \pm 1.24^{b}$ | $8.0 \pm 0.24^{c}$ | $0.0 \pm 0.0^{d}$ |
| 62.5 | $14.0 \pm 0.83^{a}$ | $9.0 \pm 0.09^{b}$ | $7.0 \pm 0.78^{c}$ | $0.0 \pm 0.0^{d}$ |

Legend: A- Antiseptic soap 1, B- Antiseptic soap 2, C- Antiseptic soap 3, D- Beauty soap

**Table 6: Percentage occurrence on clinical isolates.**

| **Organisms** | **No of patients infected** | **% Occurrence** |
|---|---|---|
| Staphylococcus aureus | 25 | 50.0±1.05 |
| Pseudomonas aeruginosa | 11 | 22.0±0.91 |
| Staphylococcus epidermis | 05 | 10.0±0.33 |
| Escherichia coli | 09 | 18.0±0.72 |
| Total | 50 | 100 |