



Effect of Antiseptic and Herbal Soaps on Daily Encountered Human Skin Flora

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ABSTRACT

The present study was carried out to isolate the organisms present in the skin of female students at the age group of 18-23 from STET Women's College, Sundarakkottai, Mannargudi, Thiruvarur (Dt), Tamilnadu. India. The samples were streak plated onto different agar medium. The colonies thus formed on the plates were identified using Motility, Gram's staining and Biochemical tests and confirmed as *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis* and *M.luteus* by Bergey's Manual of Determinative Bacteriology. Totally 46 isolates of bacteria were obtained from 30 skin swab samples. The isolated bacterial pathogens were maintained as pure culture for further studies. Antiseptic and Herbal soaps such as Dettol, Lifebuoy, Margo, and Chandrika were collected from the Cholan super market, Mannargudi, Thiruvarur (Dt), Tamilnadu, India. Isolated organisms were subjected to sensitivity test using 4 different soaps viz Dettol, Lifebuoy, Margo and Chandrika. The antiseptic soap, Dettol showed better response against the bacterial species when compared to others. Dettol was followed by Margo, Chandrika, and Lifebuoy in controlling all the organisms. Totally 46 isolates of bacteria were obtained from 30 skin swab samples.

Keywords: skin swab, Dettol, Lifebuoy, Margo, Chandrika.

INTRODUCTION

Every person has a different complement of friendly bacteria on their skin surface and there can be as many as 180 different species growing there. These include the friendly species of *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Micrococcus luteus*, *Arcanobacterium haemolyticum* and

Propionbacterium acnes. Other commensally is part of the *Corynebacterium* group, the *Brevibacterium* species and the *Dermabacter* group (Lambers *et al.*, 2009)

Transient bacteria are deposited on the skin surface from environmental sources and cause skin infections. Examples of such bacteria are *Pseudomonas aeruginosa* (Fluit *et al.*, 2001) and *Staphylococcus aureus* (Higaki *et al.*, 2000).

Scrubbing body or hands, particularly with soaps is the first of defense against bacteria and other pathogens that can cause colds, the flu, skin infection and even deadly communicable diseases (Kimmel, 1996).

Using of medicated soap impacts the good bacterial species since medicated soap kills both the beneficial and harmful bacteria species. By killing the beneficial ones that keeps the bad bacterial species in check, these soaps ultimately leaves the skin more vulnerable (Grice *et al.*, 2009).

Soap plays an important role in removing and killing bacteria. Although fats and oils are general ingredients for soap making, but detergents are added to enhance the antibacterial activities of soaps. According to Roth and James (1988) medicated soaps can remove 65 to 89% bacteria from human skin (Nester *et al.*, 2004).

In our today society, it is discovered that the use of medicated soap for bathing is widely accepted by individuals of all ages because it is understood as

protecting the skin from skin infection and as being helpful in the fight against germs (Bibel, 2003).

Soaps and other cleansing agents have been around for quite a long time. For generations, hand washing with soap and water has been considered a measure of personal hygiene. Bacteria are very diverse and present in soil, water, sewage and on human body and are of great importance with reference to health (Johnson *et al.*, 2002).

MATERIALS AND METHODS

Skin swab specimens were collected from the female students of age group 18-23 from STET Women's College, Sundarakkottai, Mannargudi, Thiruvarur (Dt), Tamilnadu. India. One millilitre of normal saline was dispensed into various bijou bottles (the small bottle shaped vials typically used in laboratories). The specimen was collected by wetting the sterile swab stick with the normal saline and pressed against the bijou bottle to reduce the excess fluid before swabbing the surface of the patient's skin and the swab stick placed back into its jacket. Isolation of bacteria (Ronald Atlas, 1998), Identification of bacteria (Hans Christian Gram, 1884) Gram staining and Biochemical test, Motility test (Bailey and Scott, 1966).

COLLECTION OF SOAPS

The antiseptic and herbal soaps such as Dettol, Lifebuoy and Margo, Chandrika were collected from the cholan super market, Mannargudi, Thiruvarur (Dt), Tamilnadu. India.

ANTIBACTERIAL SUSCEPTIBILITY TEST

PREPARATION OF ANTIBACTERIAL DISC (Talaro and Talaro, 2002)

Disks of 6mm in diameter were punched off from Whatman's no 1 filter paper. All disks were collected into a Petri dish and sterilized in hot air oven at 160⁰ C for 1 hour. A sterile blade was used to scrap 1g each of the soaps and was dissolved in 9ml of sterile distilled water to give a stock solution of 10⁻¹. These stock solution were then stored in a refrigerator in a sealed sterile container prior to use. The discs were then picked and impregnated into the different prepared medicated soap solutions and were carefully removed with forceps afterwards, dried at 28⁰C and stored in an air-tight container.

SUSCEPTIBILITY TEST (Prescott *et al.*, 2008)

The antibacterial susceptibility was tested using modified disc diffusion technique; 1% dilute H₂SO₄ and 1% Barium chloride were mixed to give a standard turbidity of about 10⁶ cells. Isolated bacteria were sub cultured in normal saline for 3 hours interval to obtain a solution with turbidity equal to 0.5 MacFarland standards. Sterile swab stick were used to evenly spread the organism across the Muller Hinton agar plate and allowed to dry. The impregnated disks was carefully picked with sterile forceps and carefully placed on the inoculated Muller Hinton agar plate. The plates were then incubated at 37⁰C for 24 hours.

The diameters of the zones of inhibition were measured and recorded for all the medicated soaps. The zones of inhibition were measured using a meter rule. All measurements were recorded in millimetres.

MINIMAL INHIBITORY CONCENTRATION AND MINIMAL BACTERICIDAL CONCENTRATION TEST

The dilution susceptibility test was used to determine the MIC values.

A series of Mueller-Hinton broth tubes containing varying concentrations of the various soap samples in the range of 500mg/ml to 62.5mg/ml was prepared and incubated with a standard density of the test organisms. The lowest concentration of the soap sample resulting in no growth after 18-24 hours of incubation is the MIC.

STATISTICAL ANALYSIS

All the experiment were repeated as triplicates. The results obtained in the present study were subjected to statistical analysis like mean \bar{X} and standard deviation (SD)

$$\bar{X} = \frac{(\sum \bar{X})}{N}$$

Mean (\bar{X}) = Sum of all values of the variable
(N) = Number of observation

Where, add together all values of variable X were added together and was divided the total by the number of observation (N). The standard deviation (SD) was calculated by the

$$\text{formula (SD) } \sigma = \sqrt{\frac{\sum (X - \bar{X})^2}{N}}$$

X- Number of value

Table: 1 Morphological and Biochemical Characteristics of Isolated Bacteria

S. No	Characteristics	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>M. luteus</i>
Morphological characteristics						
1.	Gram staining	-	+	+	+	-
2.	Motility	+	+	-	-	+
3.	Shape	Rod	Rod	Cocci	Cocci	Cocci
Biochemical Characteristics						
4.	Indole	-	-	-	-	-
5.	MR	+	+	+	-	-
6.	VP	-	-	-	+	-
7.	Citrate	-	+	-	-	-
8.	Catalase	+	+	+	+	+
9.	TSI	A/A	A/A	A/A	A/A	A/A
10.	Urease	-	-	-	+	+

(+) Positive, (-) Negative, A/A – Acidic slant and acidic butt

Table: 2 Effective of Antiseptic and Herbal Soaps on Bacteria

S. No	Organisms	Antiseptic soap		Herbal soap	
		Dettol	Lifebuoy	Margo	Chandrika
1.	<i>E.coli</i>	31.0±1.00	15.0±1.5	19.05±1.74	17.00±1.29
2.	<i>B.subtilis</i>	05.00±0.75	06.03±0.84	08.03±0.96	11.01±1.10
3.	<i>S.aureus</i>	22.9±1.00	09.0±1.0	14.04±1.26	17.00±1.29
4.	<i>S.epidermidis</i>	18.0±1.00	08.4±1.2	09.01±1.0	15.01±1.33
5.	<i>M.luteus</i>	06.05±0.85	08.4±1.95	07.00±0.88	09.01±1.0

Values are triplicates, represented as Mean ± Standard Deviation

Table: 3 Growth Of Bacterial Species In Various Concentrations Of Dettol

Concentration of soaps µg/ml Dettol	Observation of growth on plates				
	<i>E.coli</i>	<i>B.subtilis</i>	<i>S.aureus</i>	<i>S.epidermidis</i>	<i>M.luteus</i>
25.00	-	-	-	-	-
12.50	+	-	-	-	-
06.25	+	+	+	-	+
03.38	+	+	+	+	+
0.169	+	+	+	+	+

(+) Presence of Growth of bacterial species, (-) Absence of Growth of bacterial species

Table: 4 Growth Of Bacterial Species In Various Concentrations Of Margo

Concentration of soaps ($\mu\text{g/ml}$) Margo	Observation of growth on plates				
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>M. luteus</i>
25.00	-	-	-	-	-
12.50	+	-	+	-	-
06.25	+	+	+	+	+
03.38	+	+	+	+	+
0.169	+	+	+	+	+

(+) Presence of Growth of bacterial species, (-) Absence of Growth of bacterial species

Table-5 Growth of Bacterial Species in Various Concentrations of Chandrika

Concentration of soaps ($\mu\text{g/ml}$) Chandrika	Observation of growth on plates				
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>M. luteus</i>
25.00	-	-	-	-	-
12.50	-	-	-	-	-
06.25	+	-	-	+	-
03.38	+	-	+	+	+
0.169	+	+	+	+	+

(+) Presence of Growth of bacterial species, (-) Absence of Growth of bacterial species

Table: 6 Growth Of Bacterial Species In Various Concentration Of Lifebuoy

Tube No.	Volume of water	Dilution	Intermediate Concentration of antibiotics($\mu\text{l/ml}$)	Final observation of antibiotic medium ($\mu\text{l/ml}$)
T1	2ml	1:01	0500.00	25.00
T2	2ml	1:02	0.250.00	12.50
T3	2ml	1:03	0.125.00	06.25
T4	2ml	1:04	0062.50	03.38
T5	2ml	1:05	0031.25	0.169

(+) Presence of Growth of bacterial species, (-) Absence of Growth of bacterial species

Table: 7 Number And Percentage Of Bacterial Isolates

S. No	Organisms	Age group			Total No of isolates	Percentage
		18-19	20-21	22-23		
		No of isolates				(%)
1	<i>E. coli</i>	5	6	5	16	34.78
2	<i>B. subtilis</i>	2	2	1	5	10.87
3	<i>S. aureus</i>	4	5	5	14	30.43
4	<i>S. epidermidis</i>	3	2	2	7	15.22
5	<i>M. luteus</i>	1	2	1	4	08.70
Total					46	100%

Table:8 Minimum Inhibitory Concentration

Tube No.	Volume of water	Dilution	Intermediate Concentration of antibiotics($\mu\text{l/ml}$)	Final observation of antibiotic medium ($\mu\text{l/ml}$)
T1	2ml	1:01	0500.00	25.00
T2	ml	1:02	0.250.00	12.50
T3	2ml	1:03	0.125.00	06.25
T4	2ml	1:04	0062.50	03.38
T5	2ml	1:05	0031.25	0.169

DISCUSSION

The present study was carried out to isolate the organisms present in the skin of female students from STET Women's College, Sundarakkottai, Mannargudi, Thiruvavur (Dt), Tamilnadu. India. The organisms thus isolated were tested against the antiseptic and herbal soaps to test the efficiency of the soaps to remove or kill the bacterial species.

The normal human skin harbours microorganisms that can be grouped into transient and resident flora. Microorganisms differ in their nutritional requirements and level of susceptibility to antimicrobial agents. The effect of soaps on the skin micro flora has not been widely studied. There is no soap that contains the required ingredient that suits all individual skin. Here skin is the main site of exposure to soap come into focus.

The human skin is the main site of exposure to soaps, therefore reactions exhibited by individual skin differs from soap to soap. The different concentration tested on the different organisms isolated from human skin shows that the soaps contained antimicrobial activities which inhibited the growth of the organisms, *Corynebacterium spp*, *E. coli*, *Bacillus spp*, *Staphylococcus aureus* and *Staphylococcus epidermidis* to different degrees 9.05, 8.87, 9.83, 9.9 and 10.2 respectively (Tachibana 1976). Likewise, in our study, different soaps showed different degrees of inhibition zone on the isolated bacterial species.

Soaps are generally used for the removal of germs and for cleaning purposes. Antibacterial soaps have long been favored by consumers as the result of marketing. The study showed medicated soaps were effective against all the Gram positive bacteria identified; Coagulase negative *staphylococcus species*, *Staphylococcus aureus*, *Streptococcus species* and *Bacillus species* (Grice *et al.*, 2009). This research work was also in line with the above said findings.

The inhibition of the growth pattern of the different isolates indicates the varying abilities of the organism to resist the antimicrobial effect of the soaps; these could be due to differences in the nature and structures of the bacterial cell wall which is the main target of any antimicrobial agent. This study revealed Coagulase negative *staphylococcus species* which may be other species of *Staphylococcus* and *Staphylococcus aureus* to be the highest occurring bacteria and shown to be more sensitive to TCP as seen in a study work done by Obi, 2014; who reported that *Staphylococcus aureus* was more sensitive to antibacterial soap. The cell wall nature of the Gram negative and Positive bacteria differs, So that the cleansing activity of the soaps also differs according to the nature of the organisms.

Therefore, with the use of antibacterial soap can function as removal as well as killing of bacteria indicating they have bacteriostatic activity and can inhibit the growth of bacteria. The antibacterial soap (TCP) was the most effective to the isolates, but this does not mean other antibacterial soaps are not effective. This may be due to differences in their active antibacterial components and type of formulations used (Nwambete and Lyombe, 2011). The antiseptic soaps used in this study was very effective when compared to herbal soaps, because antiseptic soaps have many chemicals in their formulations which may be effective in killing or removing the microbes on the skin.

It could also be a result of different species of microorganisms being harboured on the skin of individuals as microorganisms differ in their nutritional requirements and level of susceptibility to antimicrobial agents; and no soap contains the required ingredient that suits all individual skin (Ikpo *et al.*, 2012).

Most of the medicated soaps used, have shown satisfactory effect and also their antibacterial activity.

However, this reveals that manufacturers of these medicated soaps do actually incorporate those active ingredients that possess antibacterial activity as seen in the labels.

The inhibition of the growth pattern of the isolates indicates the varying abilities of the organism to resist the antimicrobial effect of the soaps. However these variations could be due to the differences in the nature and structures of the bacterial cell wall since it is the ultimate target of any antimicrobial agent or disinfectant. The result shows that the black soaps exhibited high levels of antimicrobial activity which is the ability of the soaps to inhibit the growth or destroy the normal micro biota. The active ingredient in the soap is what distinguishes one type of the soap from another. The medicated soaps were found to contain triclocarban and triclosan as the active antimicrobial agents. These chemical compounds function by denaturing all disrupting cell activity and interfering with microbial metabolism. These depend on a number of factors such as the inherent properties of the organisms, contact time, the composition of the soaps (e.g. triclosan), concentration of individual formulation and skin sensitivity (Ikpo, 2012).

Traditional black soap lacks a key ingredient used in killing microorganisms such as triclosan, instead when the soap is scrubbed into the skin; it helps release oils on the surface of the skin that can kill bacteria and rinsing microorganisms away on the skin and preventing the emergence of mutating bacteria. It was also observed that black soap has the largest antimicrobial action against the isolates with zones of inhibition (10.65). However, the other soap samples possess very efficient antimicrobial agent but to a lesser degree. For the other medicated soaps used effectiveness in inhibition of growth of the different organisms decreased in the order Zee, Dudu osun, Tura and Dettol with zones of inhibition 10.57, 9.78, 9.27 and 7.58 respectively (Ikpo, 2012).

In general, soaps are used for cleaning purposes and in order to remove dust and microbes present on the surface of skin. The choice of soap varies from person to person but it should not affect the sensitive skin and it should be effective against disease causing microbes present on skin. The antimicrobial efficacy of antiseptic soaps like Dettol, Savlon, Lifebuoy Plus and herbal soaps like Haldi Chandan, Aloe Vera and Neem against skin micro flora isolates *Staphylococcus*

aureus, *Bacillus subtilis*, *E.coli* and *Pseudomonas aeruginosa*. (Varsha (Chaudhari, 2016).

Results obtained from the experimental data revealed that most of the studied antiseptic soaps have antimicrobial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the isolates. Varied levels of effectiveness by soaps were observed against the isolated skin flora pathogens. When the efficacy of the antibacterial soaps were compared using the disc agar diffusion method, analysis of variance for the Means of antimicrobial activities among the different soaps revealed the positive correlations ($P < 0.05$). The results of the zone of inhibitions using the organisms showed that there were significant differences ($P < 0.05$) on the various microorganisms used for the study. *Staphylococcus aureus* have more zone of inhibition (42mm) while *Bacillus* have zone of inhibition (30mm). Significant differences were observed in the zone of inhibition in all types of antiseptic and herbal soaps used for the study. (Varsha Chaudhari, 2016).

Dettol was found to be most effective against all the pathogenic strains tested having the highest zone of inhibition (42mm) against *Staphylococcus aureus* and 30 mm against *Bacillus subtilis* at the highest concentration 500 mg/ml when used. Followed by Dettol, Savlon also inhibits the growth of *Staphylococcus* but least zone was appeared for *Bacillus* species. Among the antiseptic soaps Lifebuoy Plus showed least zone of inhibition against *Staphylococcus* and *Bacillus* but it inhibits the growth of *E. coli* and *Pseudomonas* species. Among the different herbal soaps studied Neem showed highest antimicrobial activity against all pathogen studied as compared to Haldi Chandan soaps. Haldi Chandan exhibited the least antibacterial activity with zone of inhibitions of 10.2 mm for *S. aureus*, 11.4 mm for *Bacillus* and 11.8 for *E. coli* species. In herbal soaps Aloe Vera was found to effective against *E. coli* but *Pseudomonas* was not found to be inhibited by Haldi Chandan and Aloe Vera soaps also. *Pseudomonas* exhibited higher resistance to soaps, no zone of inhibitions were recorded for this isolate. Savlon was found to be as efficient as Dettol in inhibiting the growth of pathogens. (Varsha Chaudhari 2016).

Most of the assayed medicated soaps have antibacterial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the isolates. When the efficacy of the antibacterial soap

were compared using the disc agar diffusion method, Crusader was found to be most effective against all the bacteria strains tested having the highest zone of inhibition (25 mm) against *Staphylococcus aureus* and 20 mm against *Escherichia coli* at the highest dilution used. Antigal exhibited the least antibacterial activity with zone of inhibitions of 9 mm 5 mm respectively against *S. aureus* and *E. coli* respectively.

The control soap samples (Key and Truck) had no observable inhibition against the test pathogens. This justifies why people do not use the control soaps as medication in control of pathogens via bacterostatic or bactericidal activities even though they possess saponin effects for which reason they have been employed as trusted washing soaps for decades now

It was clearly seen from this study that Gram positive bacterium (*S. aureus*) was killed at low concentrations of soaps than Gram negative bacterium (*E. coli*). This observation according to Rama Bhat *et al.*, (2011) may be explained by the fact that triclosan exhibits particular activity against gram positive bacteria (Bhargava and Leonard, 1996) due to differences in the cell wall composition.

In a similar work, Nwambete and Lyombe (2001) reported that Dettol, Lifebuoy and Tetmosol had inhibitory activities against *E. coli* and *S. aureus* at lower concentrations than that tested in this work. Lifebuoy and Dettol were also reported to have inhibitory effects against *E. coli* and *S. aureus* and also against *Pseudomonas aeruginosa* (Feroze *et al.*, 2014)

Majority of the assayed medicated soaps have demonstrated satisfactory effect, particularly the antibacterial activity, hence buttressing the information written on the soap labels that they possess antibacterial activity. This is due to differences in the active antibacterial ingredients and type of formulations used (Nwambete and Lyombe, 2011). However, repeated use of the agents might have caused some resistance.

The best in antibacterial activity of all the soaps used is Crusader exhibiting maximum zone of inhibition for the test isolates. This could be attributed to its unique formulation using potassium mercuric iodide. Tetmosol is primarily used for its scabicide effect; however, it exhibited moderate antibacterial activity which is attributed to monosulfiram within its formulation (White house Station, 2008).

Soaps are intended for reduction of the inoculums sizes of pathogenic and non-pathogenic microorganisms, the later include the normal flora. Of these, two types are well known viz resident 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 beings (White house station, 2008).

Thus, it is routine practices 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 flora, which might cause opportunistic infections (Saba Riaz *et al.*, 2009).

The inhibition of the growth pattern of the isolates indicates the varying abilities of the organism to resist the antimicrobial effect of the soaps. However, these variations could be due to differences in the nature and structures of the bacterial cell wall since it is ultimate target of any antimicrobial agent or disinfectant. The active ingredient in the soap is what distinguishes in the antimicrobial agents. The indicated soaps in this study were found to contain trichlocarban and triclosan as active antimicrobial agents. These chemical compounds function by denaturing cell activity and interfering with microbial metabolism. These depend on a number of factors such as the inherent properties of the organisms, contact time, the composition of the soaps concentration or individual formulation and skin sensitivity. (Ikoph, 2012).

CONCLUSION

The present study was carried out to isolate the organisms present in the skin of female students at the age group of 18-23 from STET Women's College, Sundarakkottai, Mannargudi, Thiruvarur (Dt), Tamilnadu, India. The samples were streak plated onto different agar medium. The colonies thus formed on the plates were identified using Motility, Gram's staining and Biochemical tests and confirmed as *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis* and *M. luteus* by Bergey's Manual Of Determinative Bacteriology. Totally 46 isolates of bacteria were obtained from 30 skin swab samples. The isolated bacterial pathogens were maintained as pure culture for further studies. Antiseptic and Herbal soaps such as Dettol, Lifebuoy, Margo, and Chandrika were collected from the Cholan super market, Mannargudi, Thiruvarur (Dt), Tamilnadu, India. Isolated organisms

were subjected to sensitivity test using 4 different soaps viz Dettol, Lifebuoy, Margo and Chandrika. The antiseptic soap, Dettol showed better response against the bacterial species when compared to others. Dettol was followed by Margo, Chandrika, and Lifebuoy in controlling all the organisms. Our study indicated that gram positive organisms were highly controlled by Dettol due to the presence of highly reactive chemical substances in their formulations. Though the usage of herbal soaps produce lesser effect, it is better than the chemical soaps which affects the skin of the individuals.

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