

Efficacy of Different Soaps available in Kathmandu, Nepal

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Abstract

The main objective of this research was to assess the antibacterial activity of commonly used different soaps. Altogether 8 different types of soaps which were used in our daily life were collected from market of Bagbazar, Kathmandu and processed in the Microbiology laboratory of Padmakanya Multiple Campus from June 2022 to August 2022. Agar well diffusion method was done for studying antibacterial activity of soaps against the bacteria cultures of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella Typhi*, *Pseudomonas aeruginosa*, *Enterobacter spp*, *Proteus mirabilis* and *Shigella dysenteriae* along with ATCC culture of *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922). A cork borer of 7mm diameter was used for preparing wells in MHA plate in which 70µl of soap samples (100mg/1ml) along with positive control (Ofloxacin) and negative control (distill water) were pouring then incubated at 37°C for 24 hrs to 48 hrs. After incubation, clear zone of inhibition was measured in which antiseptic soaps were found to be highly effective for both Gram positive and Gram negative bacteria than other soaps. For total bacterial load reduction (before wash hand and after wash hand with the soaps) from hands of students, antiseptic soaps S₁ and S₂ showed the higher reduction of bacteria which was 91.3% and 95.91% respectively. It was concluded that hand washing with soaps reduced the daily encountered bacteria as well as human pathogenic bacteria.

Keywords: Hand wash, Soaps, test bacteria and Agar well diffusion method.

Introduction

According to UNICEF (2020) and WHO (2009), hand washing is the easiest, most important as well as cheapest method for the prevention of the spread of the COVID

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19. So, World Health Organization (WHO, 2020) recommends either rubbing hand with alcohol-based product or cleaning hands with soap and water regularly for killing microorganisms as well as viruses that may be in our hands. So, from long time, soaps and different cleaning agents are used extensively for different purposes of cleaning (Mwambete and Lyombe, 2011). From generation to generation, it has also been thought that one's personal hygiene can be maintained with washing hands with soap and water. According to health experts, around billions of people of the world faces the challenges due to improper and irregular practices of hand washing (The Economist, 2020).

Generally, soaps are known as disinfectants which are using for cleaning dust, killing of microorganisms, removing stains etc. Common ingredients of soaps are generally fats and oils which are manufacturing by the process of saponification. Antimicrobial substances are those substances which showed antimicrobial activity either by killing microorganism or inhibiting the growth of microorganism which are essential for the prevention of skin infections and many human diseases (Chaudhari, 2016). Based on antimicrobial substances, soaps are manufactured as two types which are antiseptic soap (medicated soap) and non-antiseptic soap. In antiseptic soaps, active ingredients having antibacterial properties are added for enhancing the growth of the microorganisms (Saba et al., 2009). So, antiseptic soaps are very important for reducing 65% to 85% germs from the human skin (Osborne and Grube, 1982). Germicidal ingredients which are incorporated in medicated or antiseptic soaps can eliminate bacteria or show bacteriostatic activity at a specific concentration. However, instead of medicated soaps, many herbal soaps are produced and used for treating in the infection caused by bacteria. This is because herbal soaps also containing active ingredients (Saikia et al., 2006; Solanki et al., 2011).

In the present situation of the Covid-19 pandemic, the importance of hand hygiene has increased significantly. It is important to hand wash to avoid various pathogenic microorganisms. Various soaps have been manufactured and made available in the market which are used in our daily life for different purposes like hand washing, bathing, washing etc. So, besides antiseptic soaps, there are other soaps like herbal soaps, beauty soaps, dish wash soaps etc. in which some ingredients are included which may kill microorganisms. Hence, this study was done to evaluate the antibacterial activities of soaps available in the local market of Kathmandu valley against pathogenic bacteria which are encountered in our daily life.

Materials and Methods

Soaps sample collection: Different types of soaps like herbal soaps (2), antiseptic soap (2), beauty soaps (2), and dish wash soaps (2) which are commonly used by humans in daily life were purchased from local market of Bagbazar, Kathmandu. The content, batch numbers and expiry dates of all soaps were noted. The presence or absences of the manufacturers seal were noted. All the 8 soaps were coded as S₁ to S₈ and processed in the microbiology laboratory of Padmakanya Multiple Campus, Kathmandu, Nepal from June 2022 to August 2022.

Identification of isolates: All pure cultures of pathogenic bacteria were used for the study, which were collected from Med-Micro Research laboratory, Kathmandu. After collection, the pure bacterial cultures of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella Typhi*, *Pseudomonas aeruginosa*, *Enterobacter* spp, *Proteus mirabilis* and *S. dysenteriae* were subcultured on Nutrient Agar (NA) and stored at 4 °C before use in experiments. ATCC bacterial culture of *Staphylococcus aureus* (ATCC: 25923) and *Escherichia coli* (ATCC: 25922) were used for quality control for this study. Before antibacterial activity test, all bacteria used in this study were identified and confirmed by Gram's staining and biochemical tests by using standard identification manual (Cheesbrough, 2006).

Preparation of bacterial cultures: For bacterial inoculums preparation, 3 to 4 colonies of each bacterium from Nutrient Agar plate were transferred to the Nutrient broth and incubated for 4 hours then their turbidity was visually compared with 0.5 McFarland turbidity standard. This bacterial inoculums preparation was used for antibiotic susceptibility test and agar well diffusion method. Antibiotic susceptibility test was done for each bacterium in which all bacteria were found to be MDR (CLSI, 2020).

Preparation of soap samples: A sterile blade was used to scrap one gram (1gm) of each of the soap and dissolved in 10 ml of sterile distilled water to a give a 100 mg/ml solution. The solution of each soap sample was used for antimicrobial activity of all bacteria used in this study (Abbas et al., 2016).

Agar well diffusion method: Agar well diffusion method was used to determine the antimicrobial activity of soaps for triplet times according to Abbas et al., (2016). The inoculum of each bacteria corresponding to 0.5 McFarland was lawn cultured on Mueller Hinton Agar (MHA) plate and allowed to stand at room temperature for 15 minutes. A cork borer of 7 mm diameter was sterilized and pressed above the inoculated agar plates (MHA) for making a well in the plate. Then, 70µl of soaps

samples (100mg/1ml) along with positive control (Ofloxacin) and negative control (distill water) were pouring in each well then incubated at 37°C in an incubator for 24 hrs to 48 hrs. After incubation, clear zone of inhibition was observed around the wells. Diameter of those inhibition zones (mm) were measured and compared with *S. aureus* (ATCC: 25923) and *E. coli* (ATCC: 25922).

Bacterial counts from hand: Nutrient agar (NA) was used for the count of bacteria from hand of students (8) of microbiology laboratory of Padmakanya Multiple Campus with their written consent. A distill water was used for washing hand with soaps (Ahmad et al., 2014). A sterile cotton swab was used to collect swab from before hand wash and after hand wash with soaps. The hand swab samples obtained were inoculated onto NA plates, then incubated at 37°C for 24hrs and examined for growth of bacteria (Jumaa, 2005).

Percentage reduction in the bacterial load was calculated as

$$\% R = [(BBW - BAW)/BBW] \times 100$$

Where, BBW = total number bacteria before using sanitizer and BAW = total number bacteria after using sanitizer.

Data process: All data were entered in MS Excel and analysis was done.

Result

Table 1. Antibacterial activity of different soaps against Gram positive bacteria and Gram negative bacteria.

Among different soaps of 100 mg/ ml solution, antiseptic soaps (S₁ and S₂) were found to be effective for both Gram's positive bacteria and Gram's negative bacteria.

Tested bacteria	Zone of inhibition (mm) of different soaps							
	Antiseptic soaps		Herbal soaps		Beauty soaps		Dish wash soaps	
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈
Gram positive bacteria								
<i>S. aureus</i> (ATCC 25923)	20	21	13	14	0	13	10	11
<i>S. aureus</i>	11	12	13	12	0	12	18	10
<i>B. cereus</i>	10	10	0	0	0	0	0	0

Gram negative bacteria								
<i>E. coli</i> (ATCC 25922)	7	8	9	8	7	12	9	10
<i>E. coli</i>	12	12	14	15	16	12	0	0
<i>K. pneumoniae</i>	10	10	12	10	10	11	0	0
<i>Enterobacter spp.</i>	12	12	18	16	12	18	12	10
<i>S. Typhi</i>	10	12	0	0	0	0	0	0
<i>S. dysenteriae</i>	12	10	0	0	0	0	0	0
<i>P. aeruginosa</i>	12	12	18	16	12	0	0	0
<i>P. miralis</i>	10	9	0	0	0	0	0	0

Table 2: Bacterial count from before and after hand wash with soaps.

Among 8 soaps, antiseptic soaps (S_1 and S_2) showed the higher reduction of bacteria from hand which was 91.3% and 95.91% respectively.

Types of soaps	Soaps samples	Before hand wash	After hand wash	Reduction (%)
1. Antiseptic soaps	S_1	230	20	91.3%
	S_2	49	2	95.91%
2. Herbal soaps	S_3	52	24	53.8%
	S_4	22	2	90.0%
3. Beauty soaps	S_5	210	98	53.3%
	S_6	213	33	84.5%
4. Dish wash soaps	S_7	43	30	30.23%
	S_8	84	11	84.5%



Fig A: Soaps samples



Fig B: Antibacterial activity of different soaps Fig C: Number of bacteria from unwash and wash hand

Discussion

Before antibacterial activity of different soaps against Gram positive bacteria and Gram negative bacteria, identification was done for all bacteria. Antibiotic susceptibility test was also done for each test bacterium and found all bacteria were Multi Drug Resistance (MDR). So, all bacteria were found to be highly resistance. In agar well diffusion method, the well containing positive control (Ofloxacin) showed zone of inhibition while the negative control (distill water) did not show zone of inhibition for all bacteria.

In this study, among all soaps (2 antiseptic soap, 2 herbal soaps, 2 beauty soaps and 2 dish wash soaps), antiseptic soaps (S_1 and S_2) showed zone of inhibition for all bacteria (*E. coli*, *S. aureus*, *K. pneumonia*, *S. Typhi*, *P. aeruginosa*, *Enterobactor* spp, *P. mirabilis*, *S. dysenteriae* and ATCC bacteria (ATCC 25923: *S. aureus* and ATCC 25922: *E. coli*). So, antiseptic soaps were also found to be highly effective for both Gram positive and Gram negative bacteria. The higher zone inhibition was showed by antiseptic soaps S_1 and S_2 on *S. aureus* (ATCC 25923) which were 20mm and 21mm respectively. Similar studies were done by many researchers which showed antiseptic soaps were very effective than other soaps.

According to Selvamohan and Sandhya, (2012), it was studied that an antibacterial soap is more effective in removing bacteria than a plain soap. Antibacterial soaps contain antimicrobial Triclosan, trichloro carbanilide and P-chloro-in-xyleneol

(PCMX/Chloroxylenol) which are the commonly used in antiseptic soaps. Similarly, Abbas et al., (2016) showed the antimicrobial activity of 3 antiseptic soaps, in which all brands of antiseptic soaps showed inhibitory results. In the study of Saikia et al., (2006) and Solanki et al., (2011), they reported antiseptic soaps are very important because it can clean 65-85% germs from human skin. However, some people consider that the antibacterial portion of soaps is effective against microorganisms and can prevent most communicable diseases, but researchers found that too much use of soaps can be a cause of spreading diseases instead of preventing them (Larson, 1989). So, too much use of antiseptic soaps might result in a resistant strain, and then the person is more prone to opportunistic skin infections (Poole, 2002).

In this study, all non-antiseptic soaps which were herbal soaps (2), beauty soaps (2) and dish wash soaps (2) also showed antimicrobial activity for Gram positive and Gram negative bacteria. This may be due to presence of active ingredients in all soaps which showed antibacterial activity. Herbal soaps contain herbal extracts or plant extracts which contain antibacterial components which showed inhibition zones for Gram positive and Gram negative bacteria. Similarly, in the study of Nadaroglu and Baran (2020), the bactericidal effects of herbal soaps and antiseptic soaps was done and reported that herbal soaps contain plants extracts which showed antibacterial activity. However, in this study, beauty soaps and dish wash soaps also showed antibacterial activity for some Gram positive and Gram negative bacteria. In beauty soaps and dish wash soaps, herb extracts or plant extracts were also found to be added for aroma or fragrance which also showed antibacterial activity.

According to Johnson et al., (2002), bacteria found everywhere in air, soil, water, sewage and on human body also. So, bacteria may transfer to human and are of great importance to health. For cleaning as well as for killing bacteria, soaps can be effective. So, in this study, number of bacteria was also counted beforehand wash and after hand wash with all soaps and distill water. All soaps showed reduction of number of bacteria from hand, however, antiseptic soaps S_1 and S_2 showed higher number of reduction of bacterial which were 91.3% and 95.91% respectively. Non-antiseptic soaps like beauty soaps, herbal soaps and dish wash soaps also showed less reduction of number of bacteria from hand as compared to antiseptic soaps. Antiseptic soaps contain active ingredients which showed higher number of reduction of bacteria whereas non -antiseptic soaps also contain herb extracts and plant extracts which showed lower number of reduction of bacteria from hand.

According to WHO (2009), different hand hygiene methods were compared in

hospital settings by many studies but only few studies have been published on the effect of hand hygiene on bacterial contamination of hands in the community. In some studies, a wide variety of hand cleansing means used in poor settings are effective in reducing the contamination with coliform bacteria on hands (Hoque and Briend, 1991; Hoque et al., 1995). In the study of Hoque et al., (1995), it was reported that soap may be more effective than water in reducing the presence of coliform bacteria on hands. So, hand washing with soaps is very effective for removing microorganisms from hand to prevent from different diseases.

Conclusion

It can be concluded that antibacterial activity of antiseptic soaps was found to be highly effective for both Gram positive bacteria and Gram negative bacteria than non-antiseptic soaps. Hand washing with different soaps showed reduction of number of bacteria from hand. So, hand washing helpful in the prevention of infections caused by different bacteria.

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