

Antibacterial Activity of Liquid Hand Washes Against Daily Encounter Bacteria

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ABSTRACT: Hands are out in the open to a lot of substances which comprises of dirt touching during personal hygiene, raw and contaminated materials. Bacteria are deposited on skin from external source causing variety of infections. Cleaning hands with antibacterial hand washes stops the spread of bacteria or loose transient flora preventing cross infections. 195 samples were used for determination of MIC and MBC of different antibacterial hand washes with inoculations of a variety of bacteria. Phenol was used as control to measure up its activity with liquid soaps. In the list of liquid soaps, safeguard was found with highest efficacy in terms of its minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli* while Johnson and Johnson baby liquid soap had maximum activity against various microorganisms. In order as Johnson and Johnson > Dettol >Safeguard >Lifebuoy >Lux. *Staphylococcus aureus* had growing resistance against various liquid soaps as Safeguard < Johnson and Johnson < Dettol < Lifebuoy < Lux liquid soaps. *Escherichia coli* had lessen in sensitivity against various liquid soaps as Safeguard > Johnson and Johnson > Lifebuoy > Lux > Dettol. The MBC values were found to be two to three times greater than its MIC values. The results prove that liquid soaps have a greater effect on inhibition and removal of bacterial population than plain soaps.

KEYWORDS: Liquid soaps, activity, pathogenic bacteria

I. INRODUCTION

Hands perform the many functions of the human body and are open to a variety of substances which take account of dust, different body fluids, raw and contaminated materials from environment and during personal hygiene¹. Cleaning hands with antibacterial hand washes stops the spread of microbes or loose transient flora thus preventing infections². After washing hand with liquid hand washes there remain a layer on our skin exterior, this layer protects our normal flora of hands ensuing low rate of different nosocomial infections³. The simple act of washing hands with soap can cut the risk of diarrhea by more or less half and respiratory tract infections by a third. This makes hand washing a better option for disease prevention than any single vaccine⁴. Hands that are apparently soiled or potentially tainted with muck or organic material must be washed with liquid soap and water.

The significance of hand washing is more vital when it is allied to health care workers because of possible contagion of bacteria that can be pathogenic or opportunistic⁵. Studies have revealed that liquid soaps contain antimicrobial active ingredients which take away more bacteria as compared to plain soap³. For control of *Staphylococcal* infections in hospitals and other health cares, it has been found that the greatest benefits from hand washing came from the first 20% of washing and very petite additional benefits were gained when hand clean-up rate was increased beyond 35%. Washing with plain soap results in more than triple the rate of bacterial infectious diseases transmitted to food as compared to washing with antibacterial hand washes. Comparing hand washes with alcohol-based solutions and washing with antibacterial for a median time of 30 seconds, each one showed that the alcohol-based hand washes reduced bacterial contamination 26% more than the antibacterial. But liquid soap and water is the more effective than alcohol-based hand rubs for reducing *H1N1 influenza A virus* and *Clostridium difficile* spores from hands⁶. The results of a clinical trial with HIV patients found that liquid soap decreased itching symptoms 100% and infectious wounds and abscess⁷. Modified detergents are added in their formulation that enhances their antibacterial activity. So as to all Pakistani branded liquid soaps like Dettol, Lux, Johnson and Johnson, Lifebuoy and Safeguard have ability to remove 65% to 85% bacterial population, prevailing on human skin⁸. Excellent hand germ-free surface cleaning and disinfection can diminish the possibility of spreading diseases. Bacteria can multiply and grow up in the homes for the most part

in the kitchen, bathroom and laundry areas. The maximum count of bacteria in the kitchen and bathrooms are found in wet and soaked areas around the cloths for wiping and or drying kitchen surfaces and the areas around the bathroom sinks⁹. Liquid hand washes contain a substance triclosan that may represent a latent public health risk in regard to development of concomitant resistance to clinically important antibacterial¹⁰ but the number of studies elucidating the alliance between triclosan assistance and resistance to other antibacterial in clinical isolates has been limited.

II. MATERIAL AND METHODS

This study was conducted in Microbiology Laboratory Pathology Department, Allama Iqbal Medical College Lahore Pakistan.

Sample collection

Sterile cotton wool swab sticks wetted by sinking in normal saline were used to collect samples from different peoples (students, laboratory staff, sweepers and teachers). A sum of 195 samples comprising 75 from unwashed hand, 75 from washed hand with liquid soap and bars and 45 from those people who used gloves were collected. As many area as possible of each hand were swabbed and more than one swab stick was used for each hand. The swab sample was brought to the Microbiology laboratory for further processing.

Table 1 showing nature of samples and its collection from various age groups

Sr. No.	Age	Nature of samples			Total samples
		Unwashed	Washed	Glove user	
1	15-20	25	25	15	65
2	20-30	30	30	15	75
3	30-40	20	20	15	55

Sample processing

Collected samples were processed by swab on nutrient agar. The media was prepared according to the manufacturer instructions and after preparation and sterilization poured into the plates. The plates were then incubated for 24 hours to check the sterility of the media.

Isolation of bacteria

Colonies that were grown on nutrient agar were sub cultured on Blood agar, MacCon key agar and EMB agar for isolation of various bacteria. The plates were incubated for 24 hours at 37 °C. Bacterial colonies that developed on the plates were then identified by colony morphology, microscopy of isolated bacteria and various biochemical tests¹¹.

Determination of MIC by Broth Micro Dilution

Two fold serial dilutions of disinfectants were prepared and subjected against broth culture of bacteria. These two fold dilutions of disinfectants were added in micro titer plate's wells, eight in vertical and twelve horizontal. Each micro tube was filled with 100 µl (0.1ml) without two dilution of hand wash concentration in appropriate wells with the help of micropipette. Then 50 µl culture broths of each selected bacteria were added in each dilution well to inoculate. Then those plates were covered with sterilized cover made up of plastic. These micro titer plates were incubated for 24 hours at temperature of 35 to 37 °C. After incubation these plates were placed under magnifying mirror to compare the growth and inhibition of bacterial growth in each well. Turbidity in wells showed as haze or pellet in the bottom of well¹².

Turbidity analysis method

The growth of the selected bacteria in the broth medium with varied concentration of hand washes were determined by turbidity analysis method. In all the bacterial maximum turbidity was recorded at lower concentration of hand washes except a few whereas the minimum value was recorded at higher concentration of hand washes¹³.

Determination of minimum bactericidal concentration by agar method (MBC)

MBC of hand washes were determined by inoculated 10 µl of the broth incubated with various dilutions, hand washes which were cultured on Muller-Hinton agar plate and incubated for 24 hours at 35 to 37 °C. Then growth was checked by observing bacterial colonies on the plates and compare with the standard broths of selected bacteria without hand washes. The reduction of growth to 99.9% at various dilutions of hand washes in each well of the micro titer plate considered as MBC of hand washes dilutions.

III. RESULT AND DISCUSSION

Bacterial isolated colonies that developed on the culture plates (on nutrient agar, blood agar, maconkey agar, and EMB agar) were counted in number and in percentage on microbial colony counter. The *Escherichia coli* was 28% in unwashed hands while those washed with liquid soaps did not show any growth pattern, in the case of gloves used were 6.67% and those used plane soap 1.02%. Colonies of *Pseudomonas aeruginosa* were counted in unwashed hands 17.33%, it was grow in those hands that were used liquid soap and plane soap 1.02% but glove user's colonies were counted 2.22%. In *Enterobacter* species 10.67% colonies were isolated in unwashed hands and no growth were determined in those who were washed their hands with hand washes, in glove users 4.44% colonies were counted. In unwashed hands 14.67% colonies of *Bacillus subtilis* were counted, in the case of liquid and plane soap and glove users there was no observed isolated bacterial colony. Percentage showed in unwashed hands of *Shigella dysenteriae* 9.33% and for plane soap were 2.05, there is no growth pattern when washed with liquid soap and in the case of glove user colonies were 2.22%. The *Staphylococcus aureus* was 12% in unwashed hands while 1.02% was for isolated colonies counted in washed hands with liquid and plane soap and in glove user was 4.44%. *Staphylococcus epidermidis* was isolated from unwashed hands 8.00% and from glove users 2.22% but there was no isolated colony found in the case of hand washes.

Table 2 Percentage of Isolated Bacteria from unwashed hands, glove users and washed with hand washes.

Sr. No.	Isolated Bacteria	Unwashed		Washed (Liquid Soap)		Washed (Plane Soap)		Glove User	
		NO.	%	NO.	%	NO.	%	NO.	%
1	<i>E. coli</i>	21	28.00	00	00.00	02	1.02	03	6.67
2	<i>P. aeruginosa</i>	13	17.33	02	01.02	01	0.51	01	2.22
3	<i>Enterobacter spp.</i>	08	10.67	00	00.00	00	0.00	02	4.44
4	<i>B. subtilis</i>	11	14.67	04	00.00	00	0.00	00	00.00
5	<i>S. dysenteriae</i>	07	09.33	00	00.00	04	2.05	01	2.22
6	<i>Staph. aureus</i>	09	12.00	02	01.02	02	1.02	02	4.44
7	<i>Staph. epidermidis</i>	06	08.00	00	00.00	00	0.00	01	2.22

E - *Escherichia*, *P*- *Pseudomonas*, *B*- *Bacillus*, *S*- *Shigella*, *Staph* - *Staphylococcus*

These results illustrate that liquid soap significantly depressed the bacterial population than plane soaps. Similar result have deduced by Toshima in 2001¹⁴. Actually the liquid soaps hold ingredients (Iodophor and Triclosan). Triclosan is a bactericidal and appears to act upon several non specific targets. Washing with liquid soap is enough to diminish bacteria. Plane soap is less effective than liquid soap. This was also confirmed by Connie and George from London in the Text Book of diagnostic Microbiology that liquid soaps have a greater effect on inhibition and removal of bacterial population than bars¹¹. The minimum inhibition of concentration detected on different dilutions of selective hand washes like Lux, Safeguard, Lifebuoy, Dettol and Jonson and Jonson. MIC determined in micro titer plate in which well add inoculums of growth bacteria and diluted hand washes. On two dilution bacterial growth were high. Dettol > Lux > Lifebuoy > Jonson > Safeguard > 38096 > 26710 > 25944 > 5892 > 4098. It was observed the low growth on 3rd dilution not minimum. On 4th dilution at 50 ml inoculums and 100 µl disinfectants, 24572 > 17882 > 12286 > 9214 > 8304 tested the concentration of bacterial growth by a turbidity analysis in micro titer plate. On 1ml/100 µl diluted sample was high bacterial concentration of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Shigella dysenteriae*, no growth of *Bacillus subtilis*. When increased the dilution at 10ml/100 µl *Escherichia coli* showed moderate growth as well as in *Pseudomonas aeruginosa* also in *Staphylococcus aureus* and no growth of *Bacillus subtilis*. On 50ml/100 µl showed low bacterial growth of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Growth of *Bacillus subtilis* inhibited. On 4th dilution we got mandatory results. Comparable results were also suggested by Hughes in 1996 that antimicrobial property of hand washes was very useful against some pathogenic organisms such as multi-drug-resistant pathogens such as *Staphylococcus aureus*¹⁵. Antibacterial soaps considered to be more effective than plain soaps our study suggests that liquid hand washes are more efficient against Gram-negative and Gram-positive bacteria than plain soaps¹⁶. The results have revealed that MIC has increased with the passage of time as compared to previous researches.

MBC of hand washes were determined by inoculated 10 µl of the broth incubated with various dilutions hand washes, which were cultured on Muller-Hinton agar plate and incubated for 24 hours at 35 to 37 °C.

Then growth was checked by observing bacterial colonies on the plates and match up to the standard broths of selected bacteria without hand washes.

Table 3 Reduction Percentage of Isolated Bacteria for MBC

SR. No.	Test Organisms	Reduction 15 seconds	Reduction 30 seconds	Reduction 60 seconds
1	<i>E. coli</i>	99.9%	>99.9%	>99.9%
2	<i>P. aeruginosa</i>	99.9%	99.9%	99.9%
3	<i>B. subtilis</i>	99.9%	>99.9%	>99.9%
4	<i>Staph. aureus</i>	99.8%	99.8%	99.8%
5	<i>Staph. epidermidis</i>	99.9%	99.8%	99.8%
6	<i>Enterobacter Spp.</i>	99.9%	99.9%	99.9%

E - *Escherichia*, *P*- *Pseudomonas*, *B*- *Bacillus*, *Staph* - *Staphylococcus*, *S*- *Shigella*

The minimum bactericidal concentration (MBC) is the lowest concentration in µl / ml of samples that results in more than 99.9% killed of the bacteria being tested. The decline of growth to 70% at 50 µl dilution of hand washes in each well of the micro titer plate as MBC of hand washes dilution. I required a 99.9% reduction of growth. Then 100 µl of hand wash at that rate of dilution. MBC were determined 90% reduction of growth again added 1000 µl diluted hand wash in micro titer plate the growth were checked its 99.9% reduction of growth. According to Shahida Hasnain 2009 at Lahore Pakistan, MBC of hand washes were determined by inoculated 10 µl of the broth incubated with various dilutions hand washes¹³. My results are different than Shahida Hasnain because of my determined MBC. Much of the debate regarding antiseptic products has focused on the use of antibacterial hand wash that contain the active ingredient triclosan. Actually triclosan possesses mostly antibacterial properties but also some antifungal and antiviral properties. Minimum inhibitory concentration disinfectants were determined by broth dilution method. Minimum bactericidal concentration (MBC) of liquid soaps was determined by agar method. Phenol was used as control to compare its activity with liquid soaps. The MBC values were found to be two to three times greater than its MIC values. The results confirmed that liquid soaps have a greater effect on inhibition and removal of bacterial population than plain soaps.

IV. CONCLUSION

Most of the people do not even bother to use hand washes in our daily life. Nobody knows the significance of liquid hand washes even these are much better than plain soaps due to their ingredients and effectiveness on our skin of hands and as well as suitable for all type of skin might be that was most sensitive. Mostly hand washes protect us from many daily encounter bacteria.

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