▼ Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan.
Vol.15, No.4, December 2004, pp. 361-368 ISSN 1021-1012

# STUDY OF BACTERICIDAL ACTIVITY OF DIFFERENT SOAPS AGAINST *SALMONELLA TYPHI*

Kalsoom Farzana*, Shazia Batool, Farzana Jabeen and Syed Nisar Hussain Shah
Department of Pharmacy, Bahauddin Zakariya University, Multan.

**Abstract:** A prospective bactericidal activity of various market soaps were performed against Gram-negative bacteria, i.e. *Salmonella typhi*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the NCCLS reference microdilution technique. The minimum inhibitory concentrations (MICs) of Johnson and Johnson baby soap and Dettol soap were 1024 $\mu g\ ml^{-1}$ and 2048 $\mu g\ ml^{-1}$ respectively. Whereas, the MIC value was 3072 $\mu g\ ml^{-1}$ for Safeguard. Phenol and Sufi soap had similar activity against *S. typhi* with MIC of 6144 $\mu g\ ml^{-1}$. Lux and Lifebuoy white had higher inhibitory activity against *Salmonella typhi* with MIC of 12288 $\mu g\ ml^{-1}$, whereas Lifebuoy red had 8192 $\mu g\ ml^{-1}$. The bactericidal activity of these soaps was in decreasing order as Sunlite, Lifebuoy white, Lux, Lifebuoy red, Sufi soap, Phenol, Safeguard, Dettol and Johnson and Johnson baby soap respectively. Moreover, the minimum inhibitory concentration of all soaps does not vary significantly against *S. typhi*. However, the minimum bactericidal concentration was significantly different among all the market soaps.

**Keywords:** Bactericidal activity, inhibition, *Salmonella typhi*, soaps.

## INTRODUCTION

Soaps are the combination of fats and oils (animal or vegetable origin) and salt [Friedman and Wolf 1996]. Dermatological bars or cakes and disinfectants are chemically different from soaps, and contain modified detergents to enhance their use for antibacterial activity. An antibacterial soap can remove 65% - 85% of bacteria from human skin [Osborne and Grube 1982].

Microorganisms carried on the skin of the human body of two distinct populations: resident and transient [Lowbury *et al*. 1964]. Resident microorganisms *such as Propionibacterium acnes*, coagulase-negative staphylococci; members of the *Corynebacterium* and *Acinetobacter* species; and certain members of the family Enterobacteriaceae are considered as permanent inhabitants of the skin [Garner and Favero 1985]. Transient microorganisms are found on and within the epidermal layer of skin, as well as other areas of the body where they do not normally reside. Almost all disease-producing microorganisms belong to this category [Steere and Mallison 1975]. Pathogens that may be present on skin, as transient types include: *Escherichia coli*, *Salmonella spp*., *Shigella* spp., *Clostridium perfringens*, and Hepatitis A virus.

Antibacterial soaps and disinfectants are used as an adjunct to acne treatment, since they contain bacteriostatic agents. When used properly, these washes affect in reduction in *P. acnes* and prevent secondary

infections in acne skin, but they are drying and irritating to most skin [Kuehl *et al*. 2003].

Detergency of soaps and disinfectants is an important factor in removing transient microorganisms from hands. High–detergency products possess leathering and emulsification abilities. Determination of the products; soaps and disinfectants that clean hands sufficiently in easiest and most acceptable manner for food operation [Green 1974].

However, bacterial insusceptibility to disinfectants is of two types, intrinsic and acquired. Intrinsic insusceptibility is a natural property of an organism and is shown by bacterial spores, Mycobacteria, and Gram-negative bacilli. Cellular impermeability is a major factor, and in some cases active efflux pumps play an important role. A special example is that of phenotypic (physiological) adaptation to intrinsic resistance found in bacteria present in biofilms. Acquired resistance arises through mutation or via the acquisition of plasmids or transposons; efflux of biocide is a major mechanism, although plasmid-mediated inactivation has also been shown to occur. An additional aspect that must be considered is the stringent response elicited in bacteria on exposure to inimical agencies. There is a possible linkage between certain biocides and antibiotic resistance under experimental conditions [Russell 2002].

*Salmonellae* are resistant to certain chemicals (e.g., brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria [Brooks *et al*. 1998]. This bacterium is spread by food and polluted water which is the most common source of contamination [Rowland 1961]. Therefore, thorough hand and face washing with antibacterial soaps is necessary to avoid skin problems.

The present study was carried out to evaluate the efficacy of local market soaps (disinfectants) used in Pakistan against a transient bacterium. The data will provide information to drug regulatory authorities and may allow the elimination of less effective market soaps. It will also provide data to help the clinician to decide to be the better soap as a protective agent against pathogens or disease causing organisms.

## MATERIALS AND METHODS

### COLLECTION OF DISINFECTANTS (SOAPS)

Different soaps of common use from market were purchased and their dilutions were made for testing the bactericidal activity of Gram-negative bacteria [NCCLS 1993].

### LIST OF SOAPS USED

Sufi Soap
Safeguard
Lifebuoy (White)
Lifebuoy (Red)

Sunlite
Lux (Green)
Johnson and Johnson
Dettol
Phenol

## CONTROL STRAIN

The following standard isolate was collected from Reckitt & Benckiser Pharmaceutical Industry:

*Salmonella typhi* (NCTC 786)

### Morphological and Biochemical Characterization of the Isolate

Identification, morphological and biochemical characterization of bacterial strain was tested in the laboratory. The presence of colonies was confirmed by the following tests.

Red Bile Agar [Cuppuccino and Sherman 1992]
Eosin Methylene Blue Agar [Cuppuccino and Sherman 1992]
Brilliant Green MacConkey Agar [Collee *et al*. 1999]

## PREPARATION OF INOCULUM

For inoculum preparation Mueller-Hinton broth was made according to manufacturer's instructions and 5 ml of broth medium was dispensed in screw capped test tubes and sterilized by autoclaving at 121°C for 15 minutes. The test tubes were cooled and kept in an incubator for 24 hours at 35°C to check sterility. The isolated strain was inoculated in the sterilized test tube containing the medium, and placed in an incubator overnight at 35°C. The presence of turbidity in broth culture was adjusted according to 0.5 McFarland standards to obtain standardized suspension by adding sterile saline against a white background with contrasting black line. The McFarland 0.5 standard provides turbidity comparable to bacterial suspension containing $1.5 \times 10^8$ C fu $ml^{-1}$ [NCCLS 1993].

## DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MIC) BY BROTH MICRODILUTION METHOD

Both microdilution (Elmer, W. Konemam) method was used to determine the minimum inhibitory concentrations (MIC), or the lowest concentration of disinfectant agent required to inhibit the microorganism. Serial two-fold dilution concentrations of these different soaps were made and tested against *S. typhi* (NCTC 786).

The disinfectants used in this study were the soaps and dilution scheme used for broth microdilution doubling dilution. Polystyrene trays were used which contains 96 wells arranged in 12 vertical, permitting the test of 12 different soaps 8 horizontal rows, each of which was taken through 8 doubling dilutions [NCCLS 1993]. Each microtube plate was prepared by adding 100 µl (0.1 ml) of two fold dilution concentration soaps to the

appropriate wells with micropippette. The 0.05 ml of Mueller-Hinton broth was delivered in each well with micropippette and 0.05 ml of inoculum volume was used to inoculate the plate. When inoculum was added to the wells, 1:2 dilutions of the $10^6$ C fu $ml^{-1}$ ($5x10^4$ C fu per well) and also halves the soaps concentration in each well. Each tray was covered with plastic cover and plastic tape around the plastic cover. The microdilution trays were incubated for 24 hour at 35ºC.

Following overnight incubation at 35ºC the tray is placed on the magnifying mirror and well was examined by looking into the magnifying mirror.

Growth was also determined by comparison with growth control well. Growth was noted as turbidity, a haze or a pellet in the bottom of the well.

## DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION

The minimum bactericidal concentration (MBC) of an antibiotic is the concentration of soap that kills at least 99.9% of a standardized bacterial inoculum. Inoculum of isolate *S. typhi* was sub cultured on nutrient agar plates and incubated overnight at 35°C.

For determination of the MBC, the 10 µl $ml^{-1}$ of broth was cultured on the agar plates, and after overnight incubation, growth was checked by observing bacterial colonies and was compared to the number of C fu $ml^{-1}$ in the original inoculum, The concentration, at which the Petri-plate each isolate showed either no growth or reduction of 99.9% from inoculum, was considered as the MBC of the specific antimicrobial agent.

## RESULTS

The *in vitro* inhibitory and bactericidal activity of various market soaps against *Salmonella typhi* were compared by microdilution technique [NCCLS 1993]. The resulting MIC and MBC were summarized in Table 1 and Figs.1, 2.

According to the results Johnson and Johnson baby soap was found to be most active against *Salmonella typhi* with MIC and MBC were 1024 µg $ml^{-1}$ and 2048 µg $ml^{-1}$ respectively. The effectiveness of the other disinfectants in decreasing order against *Salmonella typhi* were found to be Dettol (2048, 6144 µg $ml^{-1}$), Safeguard (3072, 6144 µg $ml^{-1}$), Phenol (6144, 12288 µg $ml^{-1}$), Sufi soap (6144, 12288 µg $ml^{-1}$), Lifebuoy red (8192, 16384 µg $ml^{-1}$), Lifebuoy white (12288, 24576 µg $ml^{-1}$), Lux (12288, 24576 µg $ml^{-1}$) and Sunlite (16384, 32768 µg $ml^{-1}$) MIC and MBC respectively. Whereas the p-value of these soaps do not vary significantly in their minimum inhibitory concentrations (p-value >0.05). The bactericidal activity of these soaps was in increasing order as, Sunlite, Lifebuoy white, Lux, Lifebuoy red, Sufi soap, Phenol, Safeguard, Dettol, Johnson and Johnson baby soap respectively.

**Table 1:** In Vitro Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of Various Soaps Against *Salmonella typhi.*

| | | MIC | | | MBC | | |
|---|---|---|---|---|---|---|---|
| Sr. No | Soaps Used | Test 1 | Test 2 | Range | Test 1 | Test 2 | Range |
| 1 | Sufi | 8192 | 4096 | 4096-8192 | 8192 | 16384 | 8192-16384 |
| 2 | Safeguard | 4096 | 2048 | 2048-4096 | 8192 | 4096 | 4096-8192 |
| 3 | Lifebuoy white | 16384 | 8192 | 8192-16384 | 16384 | 32768 | 16384-32768 |
| 4 | Lifebuoy Red | 8192 | 8192 | 8192 | 16384 | 16384 | 16384 |
| 5 | Sunlite | 16384 | 16384 | 16384 | 32768 | 32768 | 32768 |
| 6 | Lux | 16384 | 8192 | 8192-16384 | 32768 | 16384 | 16384-32768 |
| 7 | Johnson & Johnson | 1024 | 1024 | 1024 | 2048 | 2048 | 2048 |
| 8 | Dettol | 2048 | 2048 | 2048 | 4096 | 8192 | 4096-8192 |
| 9 | Phenol | 8192 | 4096 | 4096-8192 | 8192 | 16384 | 8192-16384 |

P-value of MIC is > 0.05, P-value of MBC is <0.05

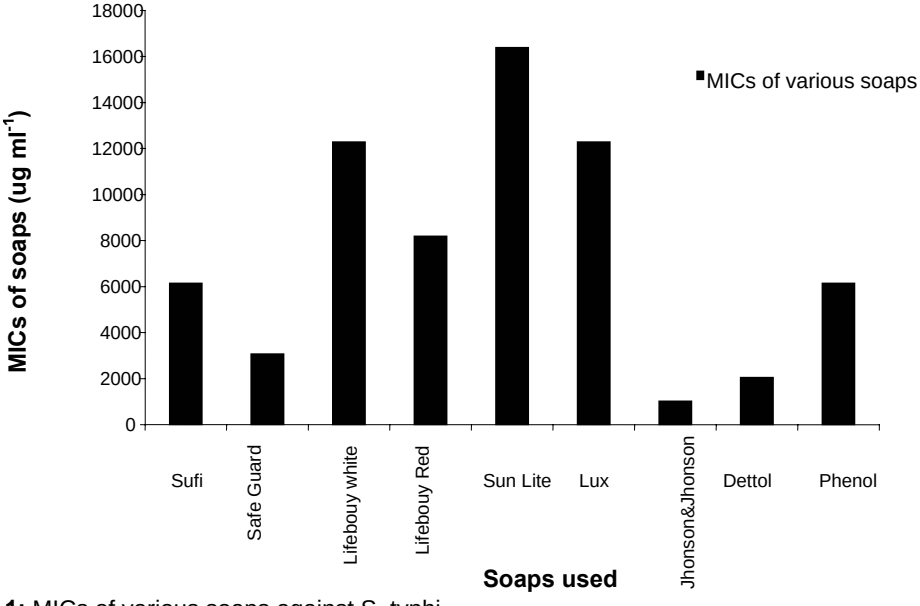


**Fig. 1:** MICs of various soaps against S. typhi.

## DISCUSSION

In this study standard isolate of *Salmonella typhi* was used to test the efficacy of antimicrobial soaps (Safeguard, Dettol, Lifebuoy and Johnson and Johnson baby soap), deodorant soap (Lux), plain soaps (Sufi and Sunlite) and Phenol were compared. Antibacterial soaps had been found to be more effective than plain and deodorant soaps. This study was supported by various workers [Ayliffe *et al*. 1975, Larson *et al*. 1987 and Toshima *et al*. 2001]. This study suggests that antiseptic soaps and detergents were more effective against Gram-negative and Gram-positive bacteria than were plain soaps. Present work showed that plain soaps also possessed antibacterial activity although lesser than that of antibacterial soaps. Garner and Favero [1985] studied the hand washing with plain soaps suspended millions of microorganisms and allowed them to rinse off. This process may be a mechanical removal of micro-

organisms and removes only transient microorganism, that's why Sufi soap seems to possess good activity against *Salmonella typhi*. While antimicrobial containing soaps kills or inhibits the growth of microorganisms (both transient and some resident microorganisms). So Dettol, Safeguard, Johnson & Johnson and Lifebuoy found to be more effective. Study of Garner and Favero [1985] showed that hand washing with plain soap for 15 seconds or less appeared to be sufficient for most routine activities but for invasive procedures within hospitals or health care setting antimicrobial products may be used.

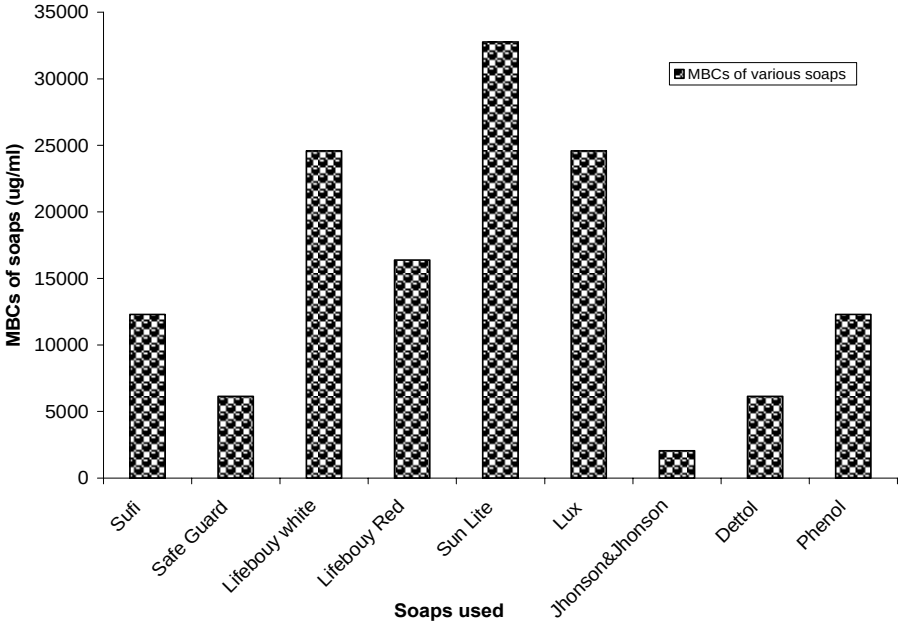


**Fig.2 MBCs of various soaps against *S. typhi***

Most of the research has been focused on hand washing and hand disinfectants for personnel (surgeons, nurses and other health care workers) in health care settings where patients are immune compromised or are at high risk of wound, surgical or burn. Bannan and Judge [1965] indicated that hand washing with bar soap reduced bacterial population *Serratia* $2 \times 10^9$ to $6.2 \times 10^5$ (a 99.97). It was also demonstrated by Osborne and Grube [1982] that, in hand cleansing, a liquid cleanser removed 85% of bacteria while a bar soap was able to remove only 65%. The work reported here suggests that the greater antibacterial activity of Johnson & Johnson and Safeguard is presumably due to the percentage of triclosan.

Tierno [1999] response to the Association for Professionals in Infection Control and Epidemiology (APIC) emphasized the use of antimicrobial household products. Authors point out that triclosan has been used for

approximately 30 years in a number of household products. Triclosan is a broad-spectrum antimicrobial agent that can act bactericidal for fungi, viruses and bacteria. Authors feel that laboratory studies of strain demonstrated tolerance, rather than resistance to triclosan, and do not represent development of resistance. In present study the efficacy of Safeguard, Johnson and Johnson baby soap, Dettol and Lifebuoy soaps may be due to the addition of the additives e.g. triclosan or some other added antibacterial.

The present study suggested that the choice of soap should be that which do not affect the facial tissues i.e., a soap cleanser is important for people with normal skin, as well as effective against disease causing bacteria in a small amount.

**Acknowledgement**

The authors are thankful to the Department of Pharmacy, Bahauddin Zakariya University Multan for providing facilities, chemicals and media for research. The work described here is a part of M. Phil. Thesis by S. Batool.

**References**

Ayliffe, G.A., Babb, J.R., Bridges, K., Lilly, H.A., Lowbury, E.J.L., Varney, J. and Wilkins, M.D. (**1975**) "Comparison of two methods for assessing the removal of total organisms and pathogens from the skin", *J. Hyg. Camb*. 75, 259-274.

Bannan, E.A. and Judge, L.F. (**1965**) "Bacteriological studies relating to hand washing. I. The inability of soap bars to transmit bacteria", *Am. J. Public Health*, 55.

Brooks, G.F., Butel, J.S. and Morse, S.A. (**1998**) "Enteric Gram-Negative Rods (Enterobacteriaceae)", In: Jawetz, Melnick and Adelberg (Eds.), *Medical Microbiology Inter*, 21, 226.

Cappuccino, G.J. and Sherman, N. (**1992**) "Microbiology: A Laboratory Manual", 91(3), 248-251 and 289-294.

Collee, J.P., Fraser, A.G., Marmion, B.P. and Simons, A. (**1999**) "Mackei & MacCarteny Practical Medical Microbiology", *Inter. Student* 14, 400.

Friedman, M. and Wolf, R. (**1996**) "Chemistry of soaps and detergents: various types of commercial products and their ingredients", *Clin. Dermatol.* 14(1), 7-13.

Garner, J.S. and Favero, M.S. (**1985**) "Guidlines for Hand Washing and Hospital Environmental Control" NTIS United States, Department of Commerce, Springfeild, 110-115.

Green, S. (**1974**) "Hand Hygiene in Practice", *Food Manufacture*, 63, 19-20.

Kuehl, B.L., Fyfe, K.S. and Shear, N.H. (**2003**) "Cutaneous Cleansers", *Skin Therapy Letter*, International Article 8, SkinCareGuide.com@ 2002, SkinCareGuide.com Ltd.

Larson, E., Eke, P.I., Wilder, M.P. and Laughon, B.E. (**1987**) "Quantity of soap as a variable in hand washing", *Inf. Cont*. 8, 371-375.

Lowbury, E.J.L., Lilly, H.A. and Bull, J.P. (**1964**) "Disinfection of hands: removal of transient organisms", *Brit. Med. J*. 2, 230-233.

National Committee for Clinical Laboratory Standards (NCCLS), (**1993**) "Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically", 3rd ed., Document M7-A3, Vol. 13, No, 25, *National Committee for Clinical Laboratory Standards* 6, 915-921.

Osborne, R.C. and Grube, J. (**1982**) "Hand disinfection in dental practice", *J. Clin. Prev. Dent.* 4(6), 11-15.

Rowland, H.A.R. (**1961**) "The complication of typhoid fever", *J. Trop. Med. Hyg.*, 64, 143.

Russell, A.D. (**2002**) "Antibiotic and biocide resistance in bacteria: Introduction", *J. Appl. Microbiol. Symp. Suppl*., 92, 1-3.

Steere, A.C. and Mallison, G.F. (**1975**) "Hand washing practices for the prevention of nosocomial infections", *Annals Int. Med*. 83, 683-690.

Tierno, P.M. (**1999**) "Efficacy of triclosan", *Am. J. Infect. Cont.*, 27 (1), 71-72.

Toshima, Y., Ojima, M., Yamada, H., Mori, H., Tonomura, M., Hioki, Y. and Koya, E. (**2001**) "Observation of everyday hand-washing behavior of Japanese, and effect of antibacterial soap", *Int. J. Food Microbiol.*, 68 (1-2), 83-91.