Scientific Research and Essays Vol. 6(16), pp. 3514-3518, 19 August, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.009
ISSN 1992-2248 © 2011 Academic Journals

Full Length Research Paper

Comparative bactericidal activity of various soaps against gram-positive and gram-negative bacteria

K. Farzana[1], S. Batool[2], T. Ismail[3], M. H. H. B Asad[3], F. Rasool[4], S. Khiljee[5] and G. Murtaza[3]*

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.
[2]Nashtar Hospital, Multan, Pakistan.
[3]Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan.
[4]College of Pharmacy, the University of Punjab, Lahore, Pakistan.
[5]Department of Pharmacy, Faculty of Pharmacy and Alternative Medicines, the Islamia University of Bahawalpur, Bahawalpur, Pakistan.

Accepted 10, June 2011

The aim of this study was to check bactericidal activity of eight market soaps from different manufacturers against gram-positive and gram-negative bacteria. Minimum inhibitory concentration disinfectants were determined by broth dilution method. Minimum bactericidal concentration (MBC) of soaps was determined by agar method. Phenol was used as control to compare its activity with soaps. In the list of soaps, along with the standard (Phenol), safeguard was found with highest efficacy in terms of its minimum inhibitory concentration (MIC 256 µg/mL) against *Staphylococcus aureus* and *Escherichia coli*, whereas Johnson and Johnson baby soap (MIC1024 µg/mL) had highest activity against *Salmonella typhi*. *S. typhi* had decreasing sensitivity against various soaps in order as: Johnson and Johnson>Dettol>Safeguard>Phenol>Lifebuoy Red>Lux>Lifebuoy white>Sunlite with MICs values of 1024, 2048, 3072, 6144, 6144, 8192, 12288, 12288 and 16384 µg/mL, respectively. *S. aureus* had increasing resistance against various soaps as: Safeguard<Johnson and Johnson<Sufi soap<Phenol<Sunlite soap<Dettol soap<Lifebuoy red<Lifebuoy white and<Lux soap with MICs values of 256, 1024, 1024, 1024, 2048, 3072, 3072, 3072, and 24576 µg/mL, respectively. *E. coli* had decreasing sensitivity against various soaps as: Safeguard>Johnson and Johnson>Lifebuoy red>Lux>Sufi>Sunlite>Lifebuoy white>Phenol>Dettol soap (MICs 256, 256, 2048, 2048, 3072, 4096, 6144, 8192 and 9216 µg/mL, respectively). The MBC values were found to be two to three times greater than its MIC values. The results confirm that medicinal soaps have a greater effect on inhibition and removal of bacterial population than plain soaps.

Key words: Gram-positive, gram-negative, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Animal or vegetable derived oils, made into salts are known as soaps (Al-Doori et al., 2003). Modified detergents are added in their formulation to enhance their antibacterial activity. Such soaps have tended ability to remove 65 to 85% bacterial population, prevalent on human skin (Larson et al., 2004). There are two sorts of microorganisms present on human skin, resident or transient (Cole et al., 2003). The former includes *Propionibacterium acnes,* member species of *Corynebacterium* and *Acinetobacter*, along with members of the Enterobacteriaceae family (Schmid and Kaplan, 2004). The latter inhabit above the dermal layer of skin and the lesser prevalent sections of human skin for other organisms. Most of the pathogen organisms are from this sort (Collee et al., 1999). *Escherichia coli, Salmonella spp., Shigella spp., Clostridium perfringens* and Hepatitis A virus, are the examples of transient microorganisms.

*Corresponding authors. E-mail: gmdogar356@gmail.com. Tel: 923142082826. Fax: 9299238344.

Due to bacteriostatic action, there use is common as an adjunct for acne treatment. Inspite of *P. acnes* growth inhibition, the certain draw backs include, dryness and irritation (Boyce and Pittet, 2002). The qualities such as lathering and emulsification are significant in view of detergency of soaps. Higher the detergency, greater will be the ability of soap to remove the microorganisms. Whereas, disinfectants play a significant role dealing with transient microorganisms (Connie and George, 2000).

Bacterial susceptibility towards disinfectants is of two types, intrinsic and acquired. Bacterial spores, Mycobacteria, and gram-negative bacilli, are found to raise intrinsic insusceptibility, whereas, mutation with the acquiring of a plasmids or transposons results in acquired resistance (Idemudia and Ajibade, 2010; Garner and Favero, 1985; Jang et al., 2010).

Staphylococcus aureus and *E. coli* are linked with community-acquired infections, which are result of poor hygiene, thus, starting a chain of outbreak beginning with a carrier (Salau and Odeleye, 2007).

Skin carriage of *S. aureus* is associated with outbreak of infection; whereas, it's nasal carriage is less catastrophic (Paulson, 2005).

This project was focused on use of different soaps present in market to conduct a comparative study against transient bacterial species. Thus, allowing clinicians (medical) and beauticians (cosmetic) to refer to such work, in order to optimize the level of sophistication in their respective field.

MATERIALS AND METHODS

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of following soaps were taken and the data obtained was used to conclude the outcomes. The tests were conducted against gram-positive and gram-negative bacteria.

Standard bacteria used

S. aureus ATCC 25923, *E. coli* ATCC 25922 and *S. typhi* NCTC 786.

Soaps used

Sufi soap, Safeguard, Lifebuoy (White), Lifebuoy (Red), Sunlite, Lux (Green), Johnson and Johnson, Dettol and Phenol.

Preparation of inoculum

For inoculum, Mueller-Hinton broth was used and manufacturer's instructions were followed for its preparation. 5 ml screw capped test tubes were taken, and autoclave for their sterilization. After cooling, they were incubated for 24 h at 35°C to ensure the sterility. *S. typhi* (NCTC 786), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were inoculated in the sterilized test tubes containing the medium, and placed in an incubator overnight at 35°C. McFarland standards were followed to monitor any turbidity, while obtaining a standardized suspension, 1.5x108 cfu/mL bacterial suspensions is found to be comparative with McFarland 0.5 standard (National Committee for Clinical Laboratory Standards, 1993).

Determination of minimum inhibitory concentrations (MIC) by broth microdilution

Minimum inhibitory concentration disinfectants were determined by broth dilution method (National Committee for Clinical Laboratory Standards, 1993).Two-fold serial dilutions of disinfectants were made and subjected against broth cultures of these earlier mentioned standard bacteria (*S. typhi* (NCTC 786), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922)). These two-fold dilutions of disinfectants were added in microtitre plate wells eight in vertical and twelve horizontal [13]. Each microtube was filled with 100 µL (0.1 mL) with two-fold dilution of soap concentrations in appropriate wells with the help of micropipette. Then 50 µL culture broth of each standard bacteria was added in each dilution well to inoculate. Then these plates were covered with sterilized cover made of plastic, these microtitre plates were incubated for 24 h at a temperature of 35 to 37°C. After incubation then these plates were placed on 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 well.

Determination of minimum bactericidal concentration (MBC) by agar method

Minimum bactericidal concentration (MBC) of soaps was determined by inoculating 10 µL of the broth from incubated broth with various dilutions of soaps, which was cultured on Mueller-Hinton agar plate and incubated for 24 h, at temperature of 35 to 37°C. Then growth was checked by observing bacterial colonies on the plates and compared with the standard broths of *S. aureus* (ATCC 25923), *S. typhi* (NCTC 786) and *E. coli* (ATCC 25922) without soap. The reduction of growth to 99.9% at various dilutions of soaps in each well of the microtitre plate considered as MBC of soap dilution (National Committee for Clinical Laboratory Standards, 1993).

Statistical analysis

The comparison of various parameters was made to elaborate the significant difference using
software, SPSS version 13.0. The level of significance was set at 0.05.

RESULTS

The minimum inhibitory concentration and minimum bactericidal concentration of different daily used soaps against the gram-positive and gram-negative were compared by microdilution technique (Tables 1 and 2). The results of Safeguard soap were most effective against *S. aureus* and *E. coli* with MIC = 256 µg/mL and MBC = 512 µg/mL. The efficacy values of MIC and MBC of these disinfectant (soaps) were significantly ($p<0.05$) different from each other as: Johnson and Johnson (1024 and 2048 µg/mL), Dettol (3072 and 6144 µg/mL), Lifebuoy white (3072 and 6144 µg/mL), Lifebuoy red (3072 and 6144 µg/mL), Phenol (1024 and 2048 µg/mL), sofi soap (1024 and 2048 µg/mL), Sunlite soap (2048 and

Table 1. Comparison of minimum inhibitory concentrations of various soaps against gram-positive and gram-negative bacteria (µg/mL).

Soaps used	*S. typhi*	*S. aureus*	*E. coli*	P-value
Sufi	6144	1024	3072	0.4000 [b]
Safeguard	3072	256	256	0.0667 [b]
Lifebuoy white	12288	3072	6144	0.2000 [b]
Lifebuoy red	8192	3072	2048	0.0667 [b]
Sun lite	16384	2048	4096	0.2000 [b]
Lux	12288	24576	2048	0.5333 [b]
Johnson and Johnson	1024	1024	2560	0.5333 [b]
Dettol	2048	3072	9216	0.5333 [b]
Phenol	6144	1024	8192	0.8000 [b]

[b] = Non Significant

Table 2. Comparison of minimum bactericidal concentrations of various soaps against gram-positive and gram-negative bacteria (µg/mL).

Soaps used	*S. typhi*	*S. aureus*	*E. coli*	P-value
Sufi	12288	2048	2048	0.2000 [b]
Safeguard	6144	512	512	0.000 [a]
Lifebuoy white	24576	6144	12288	0.2000 [b]
Lifebuoy red	16384	6144	4096	0.0667 [b]
Sun lite	32768	24576	8192	0.0667 [b]
Lux	24576	32768	24576	0.5333 [b]
Johnson and Johnson	2048	2048	3072	0.5333 [b]
Dettol	6144	6144	10240	0.9333 [b]
Phenol	12288	2048	16384	0.8000 [b]

[a] = Significant; [b] = Non Significant.

24576 µg/mL) and Lux (24576 and 32768 µg/mL), respectively.

The bactericidal activity of these soaps was significantly ($p<0.05$) different in increasing order as, Lux, Sunlite, Sufi, Phenol, Lifebuoy white, Lifebuoy red, Dettol, Johnson and Johnson and Safeguard respectively. The effectiveness of other disinfectants in decreasing order against *E. coli* were Lifebuoy red (2048 and 4096 µg/mL), Johnson and Johnson (2560 and 3072 µg/mL), Sufi (3072 and 4096 µg/mL), Sunlite (4096 and 8192 µg/mL), Lifebuoy white (6144 and 12288 µg/mL), Phenol (8192 and 16384 µg/mL), Dettol (9216 and 10240 µg/mL) and Lux (2048 and 24576 µg/mL) MIC and MBC, respectively. The bactericidal activity of these soaps was in increasing order as: Lux, Dettol, Phenol, Lifebuoy white, Sunlite, Sufi, Johnson and Johnson, Lifebuoy red and Safeguard.

According to present results, Johnson and Johnson baby soap was found to be most active against *S. typhi* and *S. aureus* with MIC and MBC of 1024 and 2048 µg/mL, respectively. The susceptibility of the other disinfectants against *S. typhi* was significantly ($p<0.05$) different in decreasing order as: Dettol with MIC and MBC (2048 and 6144 µg/mL), Safeguard (3072 and 6144 µg/mL), Phenol (6144 and 12288 µg/mL), Sufi soap (6144 and 12288 µg/mL), Lifebuoy red (8192 and 16384 µg/mL), Lifebuoy white (12288 and 24576 µg/mL), Lux (12288 and 24576 µg/mL) and Sunlite (16384 and 32768 µg/mL). 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 (Tables 1 and 2).

DISCUSSION

In this study, standard isolates *S. aureus*, *E. coli*, and *S. typhi* were tested against antimicrobial soaps (Safeguard, Dettol, Lifebuoy, Johnson and Johnson baby soap), deodorant soap (Lux), plain soaps (Sufi soap and Sunlite) and disinfectant (Phenol). While their efficacies as antibacterial soaps were compared against *S. aureus* and *E. coli,* Safeguard was found to be more effective. Its MIC was 256 µg/mL against *S. aureus* and *E. coli*. Triclosan played significant role in this activity of Safeguard soap. Such action of the ingredient was also indicated in a previous study (Steinmann et al., 2010).

In agar plate dilution study Bamber and Neel observed the minimum inhibitory concentration of triclosan effective concentration against 186 isolates of MRSA and MSSA, among those 14 isolates had MIC concentration less than one part per million, in addition when EDTA was added, it had greater antibacterial activity.

The present study of Dettol soap was found to be comparable with previous study (Russell and Hugo, 2000), as it was proved effective against *S. aureus* and *S. tyhi* having range of MIC of 3072 and 2048 µg/mL, respectively, probably this activity was due to addition of chloroxynol as it enhanced the activity of Dettol in soap. Dankert and co-workers worked on *Pseudomonas aueroginosa* with lesser efficacy of Dettol because of cultural and environmental conditions to lesser extent towards *S. aureus*. This variation made it helpful in explaining certain irrational errors in retrospect regarding the activity of chloroxylenol containing products. It was also pointed out that the addition of EDTA to these products brought about improvement in these products, when checked *in-vivo*. Phenol efficacy is as the same in Dettol soap when tested against *E. coli* and *S. typhi* due to the added chloroxylenol because its good bactericidal activity. Therefore Dettol soap exhibited rapid bactericidal activity against *S. typhi* and *S. aureus* as compared to Phenol. Russell and Hugo pointed out that this activity is reduced markedly because when the Phenol is used in various dilutions.

Antibacterial activity of Johnson and Johnson, Safeguard soap and Dettol soap were in similar range of affectivity. While Safeguard was most effective against *S. aureus*, followed by Johnson and Johnson soap and Dettol soap, respectively. Both Lifebuoy white and Lifebuoy red were found to have similar antibacterial activity against various isolates. Johnson and Johnson had higher efficacy against *S. typhi* than *S. aureus* and *E. coli.*

Previously done research proved antibacterial soaps to be more effective than plain soaps and deodorant soaps, as shown in various studies (Russell, 2002; Todd et al., 2007; Toshima et al., 2001). A previous research showed that soaps without bacterial agents managed to reduce total coliform to 95%, while the soaps which had antibacterial agents were further active against coliform. Thus, the plain soaps managed to show antibacterial activity, though, lesser than antibacterial soaps. Current study can also be supported in view of antibacterial activity in a previous work (Toshima et al., 2001). Reference to mechanical removal of microbes, the work showed the hand washing with plain soaps to be effective, by the process of suspending millions of microbes and assisting in rinsing them off. Hand washing with antibacterial soaps with their bactericidal and bacteriostatic activity were referred to as acting with chemical removal of transient and some resident microorganism. Study proved the 15 s or less hand washing with a plain soap is sufficient during routine activity.

In hospitals, where pathogenic microorganisms are prevalent, the use of antimicrobial products is rational. Though, acquisition of resistance may be due to transfer of resistance genes between genetic element, within microbes and among microbes. Another facet that must be measured is the stringent response brought forth in bacteria on exposure to detrimental agents (Russell, 2002).

The detergency study of soaps used for hand washing is an important factor in removing transient microorganisms from hands, and is influenced by type and amount of soil and mineral content of the water. A soap product or liquid detergent with high detergency is necessary to remove a large amount of fat, protein or other types of organic soil that bind transient microflora.

Conclusion

The results confirm that medicinal soaps have a greater effect on inhibition and removal of bacterial population than plain soaps. In view of hand hygiene, the selection of soaps does become even more critical. Factors which may be noted to have significance in this selection include, the area of occupation e.g. hospital, clinical, house etc., the incidence of skin infection in the locality, exposure to microbes, etc. Though, the plain soaps can be used in most situations but workers in hospitals should use antibacterial soaps.

REFERENCES

Al-Doori Z, Morrison D, Edwards G, Gemmell C (2003). Susceptibility of MRSA to triclosan. *J. Antimicrob. Chemother.*, 51: 185-186.

Larson E, Lin SX, Gomez-Duarte C, Della-Latta P (2004). Effect of antibacterial home cleaning and handwashing products on infectious disease symptoms. Ann. Int. Med., 140: 321-329.

Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, Newell MS (2003). Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *J. Appl. Microbiol.*, 95: 664-676.

Schmid MB, Kaplan N (2004). Reduced triclosan susceptibility in methicillin-resistant Staphylococcus epidermidis. *Antimicrob. Agents Chemother*, 48: 1397-1399.

Collee JP, Fraser AG, Marmion BP, Mackei SA, MacCarteny (1999). Practical Medical Microbiology. Collee JP, Fraser AG, Marmion BP, Simons A. Livingstone. Edinburg, United Kingdom.

Boyce JM, Pittet D (2002). Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Infect. Control Hosp. Epidemiol., 23: S3-S40.

Connie RM, George M (2000). In Text Book of Diagnostic Microbiology, Connie RM, George M. 6^{th} Ed. WB Saunder. London, United Kingdom, p. 327.

Garner JS, Favero MS (1985). Guidlines for hand washing and hospital environmental control NTIS. United States Department of Commerce. Springfeild, 3: 110.

Jang JH, Wu S, Kirzner D, Moore C, Tong A, McCreight L, Stewart R, Green K, McGeer A (2010). Physicians and hand hygiene practice: a focus group study. The J. Hospital Inf., 76: 87-90.

Salau AO, Odeleye OM (2007). Antimicrobial activity of Mucuna pruriens on selected bacteria. *Afr. J. Biotechnol.* 6: 2091-2092.

Paulson DS (2005). Importance of vertebrate viruses for choosing hand antiseptics with virucidal efficacy. *Am. J. Infect. Control.,* 33: 435-436.

National Committee for Clinical Laboratory Standards (1993). Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically, 3rd Ed. Document M7-A3, National Committee for Clinical Laboratory Standards 6, 13(25): 915.

Steinmann J, Becker B, Bischoff B, Paulmann D, Friesland M, Pietschmann T, Steinmann J, Steinmann E (2010). Virucidal activity of 2 alcohol-based formulations proposed as hand rubs by the World Health Organization. Am. J. Infect. Control., 38: 66-68.

Russell AD, Hugo WB (2000) Chemical disinfectants, antiseptics and preservatives. In Pharmaceutical Microbiology. Russell AD, Hugo WB. 6th Edn. Blackwell Science, Wales, United Kingdom, p. 210

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

Russell AD (2002). Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J. Appl. Microbiol. Symp. Suppl,* 92: 121-135.

Todd ECD, Greig JD, Bartleson CA, Michaels BS (2007). Outbreaks where food workers have been implicated in the spread of foodbome disease. Part 2. Description of outbreaks by size, severity, and settings. J. Food Prot., 70: 1975-1993.

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