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

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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<Lux soap with MICs values of 256, 1024, 1024, 2048, 2048, 3072, 3072, 3072, and 24576 µg/mL, respectively. E. coli had decreasing sensitivity against various soaps as: Safeguard>Johnson and Johnson>Lux soap with MICs values of 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.
Due to bacteriostatic action, there is use common as an adjunct for acne treatment. In spite of *P. acnes* growth inhibition, the certain drawbacks 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, its 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 microtiter plate wells eight in vertical and twelve horizontal [13]. Each microtubes 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 microtiter 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 microtiter 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).

<table>
<thead>
<tr>
<th>Soaps used</th>
<th>S. typhi</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufi</td>
<td>6144</td>
<td>1024</td>
<td>3072</td>
<td>0.4000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Safeguard</td>
<td>3072</td>
<td>256</td>
<td>256</td>
<td>0.0667&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lifebuoy white</td>
<td>12288</td>
<td>3072</td>
<td>6144</td>
<td>0.2000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lifebuoy red</td>
<td>8192</td>
<td>3072</td>
<td>2048</td>
<td>0.0667&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sunlite</td>
<td>16384</td>
<td>2048</td>
<td>4096</td>
<td>0.2000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lux</td>
<td>12288</td>
<td>24576</td>
<td>2048</td>
<td>0.5333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Johnson and Johnson</td>
<td>1024</td>
<td>1024</td>
<td>2560</td>
<td>0.5333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dettol</td>
<td>2048</td>
<td>3072</td>
<td>9216</td>
<td>0.5333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenol</td>
<td>6144</td>
<td>1024</td>
<td>8192</td>
<td>0.8000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Non Significant

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

<table>
<thead>
<tr>
<th>Soaps used</th>
<th>S. typhi</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufi</td>
<td>12288</td>
<td>2048</td>
<td>2048</td>
<td>0.2000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Safeguard</td>
<td>6144</td>
<td>512</td>
<td>512</td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lifebuoy white</td>
<td>24576</td>
<td>6144</td>
<td>12288</td>
<td>0.2000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lifebuoy red</td>
<td>16384</td>
<td>6144</td>
<td>4096</td>
<td>0.0667&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sunlite</td>
<td>32768</td>
<td>24576</td>
<td>8192</td>
<td>0.0667&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lux</td>
<td>24576</td>
<td>32768</td>
<td>24576</td>
<td>0.5333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Johnson and Johnson</td>
<td>2048</td>
<td>2048</td>
<td>3072</td>
<td>0.5333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dettol</td>
<td>6144</td>
<td>6144</td>
<td>10240</td>
<td>0.9333&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenol</td>
<td>12288</td>
<td>2048</td>
<td>16384</td>
<td>0.8000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Significant; <sup>b</sup> = 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. typhi* having range of MIC of 3072 and 2048 µg/mL, respectively, probably this activity was due to addition of chloroxylenol as it enhanced the activity of Dettol in soap. Dankert and co-workers worked on *Pseudomonas aeruginosa* 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**


Jang JH, Wu S, Kirzner D, Moore C, Tong A, McCreight L, Stewart R,