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Antimikrobno djelovanje različitih antibakterijskih i običnih tekućih sapuna

Vildana Hadžić, Anesa Jerković-Mujkić, Renata Bešta-Gajević, Snježana Hodžić, Darja Husejnagić, Emina Mešikić, Amela Hercegovac

SAŽETAK: Antimikrobni sapuni se upotrebljavaju za redukciju tranzitornih patogena i kolonizirajuće mikrobiote. S obzirom da postoji veliki broj sredstava koji se koriste za pranje ruku, cilj ovog rada bio je ispitati antimikrobna svojstva različitih običnih i antibakterijskih tekućih sapuna. U istraživanju je učestovalo ukupno 150 ispitanika; 75 muškog i 75 ženskog spola. Korišteni su tekući sapuni različitih proizvođača. Antimikrobno djelovanje tekućih sapuna testirano je metodom otiska prstiju dominantne i nedominantne ruke na ploče sa TSA agarom. Ukupan broj bakterija nakon perioda inkubacije izražen je kao CFU. Faktor redukcije broja mikroorganizama je određivan na osnovu razlike logaritamske vrijednosti srednjeg broja CFU prije i nakon pranja ruku. Statistička obrada podataka izvršena je primjenom t-testa i testa korelacije. Utvrđeno je da postoji statistički signifikantna razlika između brojnosti bakterija dominantne i nedominantne ruke ne samo prije, već i nakon korištenja tekućih sapuna. Pripadnice ženskog spola imale su prosječno 2.46% više bakterija na rukama nego pripadnici muškog spola, što nije bilo od statističkog značaja. Faktor redukcije broja bakterija bio je statistički značajan kod svih ispitanih antibakterijskih i običnih tekućih sapuna, osim kod antibakterijskog sapuna LS 3. Postoji izrazita negativna korelacija između pH vrijednosti sapuna i njegovog antimikrobnog djelovanja. Tečni sapuni pokazali su podjednak efekat u redukciji broja mikroorganizama na dominantnoj i nedominantnoj ruci kod pripadnika oba spola, što je potvrđeno i statističkom analizom podataka. Sapuni sa nižom pH vrijednošću pokazali su jaču antibakterijsku aktivnost u odnosu na sapune sa višom pH vrijednošću.

Ključne riječi: *antimikrobno djelovanje, dominantna i nedominatna ruka, spol, pH vrijednost, faktor redukcije*

The Antimicrobial Activity of Various Antibacterial and Plain Liquid Soaps

ABSTRACT: The antimicrobial soaps are used for the reduction of transitory pathogens and colonizing microbiota. The aim of this study was to investigate the antimicrobial properties of various ordinary and antibacterial liquid soaps. The study participated a total of 150 examinees, 75 male and 75 female. Antimicrobial activity of liquid soaps tested method of fingerprint dominant and non-dominant hands on plates with TSA agar. The total number of bacteria after incubation period is expressed as CFU. Reduction factor, the number of microorganisms is determined based on the differences of logarithmic values of the average number of CFU before and after hand washing. Statistical analysis was performed using t-test and correlation. It was found that there is a statistically significant difference between the number of bacteria dominant and non-dominant hands not only before but also after the use of liquid soaps. The female gender had an average of 2.46% more bacteria on their hands than members of the male gender, which was not of statistical significance. Reduction factor, the number of bacteria was statistically significant at all tested antibacterial and ordinary liquid soap, except for antibacterial soap LS 3. There is a significant negative correlation between pH soap and its antimicrobial activity. Liquid soaps showed the same effect in reducing the number of microorganisms on the values of dominant hand in both genders, which is confirmed by the statistical analysis of data. Soaps with lower pH showed stronger antibacterial activity compared to soap with a higher pH value.

Keywords: *antimicrobial activity, dominant and non-dominant hand, gender, pH value, factor of reduction*

INTRODUCTION

Hand hygiene represents procedures for the purpose of removing the visible dirt and reduce transient microbiota without necessary impact on the resi microbiota skin. The causal link between contaminated hands and transmission of various diseases has be well documented (Sattar, Tetro, & Springthorpe, 1999). Regular maintenance of hand hygiene is particularly important for employees in health institutions. Washing hands with soap is widely accepted practice in preventing the spread of infections (Jumaa, 2005; Larson, 1995; Zapka, Campbell, & Maxwell, 2011).

It is simple, but the most effective and cheapest way of preventing the spread of pathogens, including resistant strains. The World Health Organization-WHO (World Health Organization [WHO], 2009) and the Center for Disease Control and Prevention CDC (Boyce & Pittet, 2002) have developed a number of strategies to improve hand hygiene of healthcare workers.

choosing soap particular attention should be paid to its composition and pH value. It is important to use soaps that do not damage the skin, especially in people who often have to wash their hands and those who are susceptible to skin allergies. Soaps which are used for hand washing can be categorized as normal (toilet) and antibacterial soaps. The antimicrobial soaps contain active substances which inhibit the growth of microorganisms or they are completely removed from the tissue. Examples of such agents are alcohols, chlorexidine gluconate, chlorine derivatives, iodine, hloreksilenol, quaternary ammonium compounds and triclosan. Washing hands with soap is more convenient and correctly hygienic. However, more research has linked the use of contaminated liquid soap to the spread of infection in health care facilities (Archibald et al., 1997; Parasakthi, Jamuna, Iyer, & Palasubramaniam, 2000; Weber, Rutala, & Sickbert-Bennett, 2007).

To avoid contamination, the CDC recommendation (Boyce & Pittet, 2002) is to use dispensers for liquid soaps that are filled with disposable sealed vials, and if there are ordinary dispensers it is necessary to empty them completely and clean them before refilling liquid soap. Considering that there is a large number of resources that are used for hand washing, the aim of this paper is to examine the antimicrobial effects of various antibacterial and ordinary liquid soaps.

MATERIAL I METHODS

Examinees

The study included a total of 150 volunteers, and 75 male and 75 female. For each subject data were collected on gender and the dominant hand. Dominant hand is the one that is used more often and that is faster and more precise when performing manual tasks.

Liquid soaps

Liquid soaps of different manufacturers have been tested,which are available in our market. From a total of five soaps, two were common, and three antibacterial. Data on the composition and origin of the product are shown in Table 1. In determining the pH of the soap pH meter was used from manufacturer Phywe 13702.3 (Germany).

Taking samples

Samples for microbiological analysis were collected by the fingerprint plate with Tryptone Soya Agar a (TSA M290, HiMedia Laboratories, India). According to the default scheme examinees left prints of left and right hands fingertips on the separate plates, both before and after hand washing.

Each type of soap was tested on 30 different people (15 men and 15 women). Washing hands with suitable liquid soap volume of 5 mL lasted exactly three minutes, after which the hands were thoroughly rinsed with running water and dried with paper towel. In that way total of 600 agar plates with the fingerprints were collected and left in incubation at 37 ° C for 24 hours.

Determination of the number of bacteria

After the incubation period, the total number of bacteria was determined by counting colonies in TSA agar. Winning values are expressed as the number of CFU (colony forming units) of the fingers of the examinees' hands.

Calculation of the reduction factor

Hygienic washing efficiency with hand soap was determined by reduction of total count of microorganisms, which represents the logarithmic value of the average number of CFU before and after washing. The resulting value was indicated as the reduction factor (RF).

Statistical analysis of data

Statistical analysis was performed using t-test and correlation. T-test was used to examine whether there is a statistically significant difference in the concentration of bacteria on the hands between the dominant and non-dominant hands, as well as the gender of the examinees.

Table 1. The chemical composition of the tested liquid soaps

Index	Chemical composition	The country of origin	Volume
LS 1	Aqua, SLS, Cocamidopropyl Betaine, Sodium Chloride, PEG-7 Glyceril Cocoate, Coco Glucoside, Glyceryl Oleate, Propylene Glycol, Olea europea Leaf Extract, Citrus Medica Limmonum Oil, Parfum, Citronellol, D-Limonene, Linalool, CI 47005, CI 42051	Slovenia	300 ml
LS 2	Aqua, Cocamidopropyl Betaine, SLS, Glycerin, PEG-7 Glyceril Cocoate, Helianthus Annus Seed Oil, Glycerin Glucoside, Sodium Chloride, Citric Acid, PEG-40 Hydrogenated Castor Oil, Benzophenone-4, PEG-120 Methyl Glucose Di, Sodium Benzoate, Sodium Salicylat, Linalool, Citronellol, Parfum, CI 10316, CI 42090.	Germany	250 ml
LS 3	Aqua, SDS, Chlorexidine Dicluconate, Sodium Chloride, Cocamidopropyl Betaine, Laureth-3, DMDM Hydantoin, Cocamidopropyl PG Domonium Chloride Phosphate, Glycerin, Parfum, Hexyl Cinnamal, Limonene, Linalool, Methylisothiazolinone, Magnesium Chloride, Magnesium Nitrate, CI 42090, CI 19140.	Croatia	400 ml
LS 4	Aqua, Sodium C12-13 Pareth Sulfate, SLS, Cocamidopropyl Betaine, Lactic Acid, Cocamide MEA, Parfume, Sodium Salycilate, Sodium Benzoate, Sodium Hydroxide, Glycerin, Tetrasodium EDTA, Citric Acid, Poloxamer 124, Benzyl Salicylate, Buthylphenyl Methylpropional, Hexyl Cinnamal, CI 19140, CI 14720.	Italy	300 ml
LS5	Aqua, SLS, Cocamidopropyl Betaine, Cocamide DEA, Glycerin, Sodium Chloride, Parfume, Sodium Benzoate, Potassium Sorbate, Tetrasodium EDTA, Grape seed oil glycereth-8 esters, Benzophenone-4, Citric Acid, Benzyl Salicylate, Linalool, CI 14720.	Germany	400 ml

Correlation test examined interdependence of pH value of used soap and reduction of the number of microorganisms on hands of examinees of both gender.

RESULTS

The total number of microorganisms on TSA surfaces is determined by counting the number of living microorganisms that form colonies (CFU).

Data on the average number of CFU on the fingers of unwashed hands of examinees of both genders are shown in Table 2.

This table shows that in examinees of both genders there are more bacteria on the dominant compared to the non-dominant hand.

For easy visual spotting of ascertained differences in the number of bacteria on the dominant and nondominant hand, provides a summary view of average CFU value for all examinees (Figure 1A). The number of bacteria on the dominant hand was on average higher by 27.5% compared to the non-dominant hand of examinees. The number of bacteria on the dominant hand was on average higher by 27.5% compared to the non-dominant hand of examinees.

The determined difference in the number of bacteria between the dominant and non-dominant hand is statistically significant (p <0 .00001), both for male and for female examinees. Taking into account the gender of the participants, members of the female gender had an average of 2:46% more bacteria on their hands than members of the male gender. (Figure 1B).

Table 2. The average value of CFU ± SD on the fingers of unwashed hands of volunteers

Gender / Hand	Male (N=75)	Female (N=75)
Dominant	115.35 ±29.41	117.85± 30.91
Non-dominant	90.17 ± 22.81	92.72 ± 24.60

However, it was no difference in the number of bacteria on hands between the genders is not of statistical significance (p = 0.232012).

Taking into account the gender of the participants, members of the female gender had an average of 2.46% more bacteria on their hands than members of the male gender. (Figure 1B). However, determined difference in the number of bacteria on hands between the genders is not of statistical significance (p = 0.232012).

The test results of antibacterial activity of the tested liquid soap on 150 patients after three minutes of hand washing are shown in Table 3.

As it can be seen in the table below, all of the tested soaps showed antimicrobial activity

A. Average value CFU Average value CFU dominant hand non-dominant hand

B. Average value CFU on Average value CFU on men's hands women's hands

Figure 1. The average number of CFU on the fingers of examinees in relation to: A) Hand dominance, B) The gender of examinees

Table 3. Average value CFU on the fingers of examinees before and after the hand washing with a liquid soap

Liquid soap	Dominant hand (CFU)		Non-dominant hand (CFU)		Reduction of number MO (%)	
	Before washing	After washing	Before washing	After washing	Dominant hand	Non-dominant hand
LS1	110.74	33.37	80.37	23.00	69.87	71.45
LS2	106.37	25.17	83.20	18.37	76.34	77.92
LS3	131.00	125.70	109.84	103.63	4.05	5.65
LS4	122.44	15.60	90.14	9.87	87.26	89.05
LS5	112.47	69.87	93.70	59.94	37.88	36.03

It was found that there is a difference in the number of bacteria between the dominant and non-dominant hands not only before but also after the use of liquid soap. The greatest reduction of the number of microorganisms was detected upon application of soap LS4 (87.26% of dominant and 89.05% of non-dominant hand), while the lowest concentration of bacteria on the hands was established after application of soap LS3 (4.5% on dominant and 5.65% on non-dominant hand). To determine the reduction factor of micro-organisms after use of liquid soap the log10 of average values CFU of the fingers of both hands of examinees were used before and after hand washing (Table 4).

Table 4. Review of pH soap value and factor of reduction (RF)

Soap	pH	RF
LS1	5.43	0.53
LS2	4.40	0.64
LS3	5.84	0.02
LS 4	3.51	0.92
LS 5	6.73	0.20

It may be noted that there is a causal link between the reduction in the number of bacteria after hand washing and the pH value of the used liquid soap. The fall in pH soap follows the increase in the reduction factor (Table 4). Soap LS4, wherein the lowest measured pH value is 3.51, showed the highest degree of reduction of the bacterial count of 0.92, that is the best antimicrobial activity In contrast, in soap LS3 and LS5 with highest values of pH 5.84 and 6.73 is the lowest recorded reduction of the number of microorganisms (0.02 and 0.20). The results (Table 4) were used to calculate the correlation coefficient. The value of correlation coefficient indicates that -0.884059 correlation between the pH of the used soap and th reduction factor of micro-organisms is negative and distinct (Figure 2).

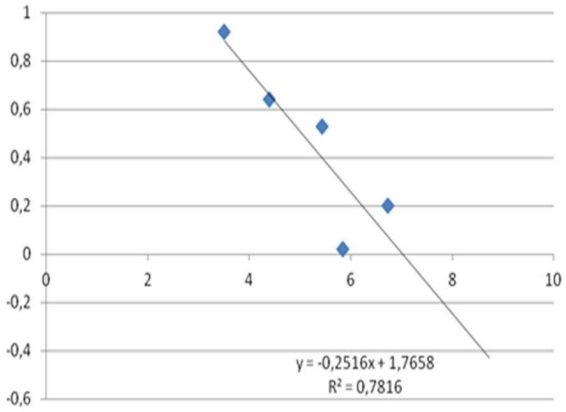

Figure 2. Graphic display of correlation between pH value of tested soaps and factors of reduction in number of bacteria

DISCUSSION

On the skin of hands there are about 150 different species of bacteria (Fierer, Hamady, Lauber, & Knight, 2008), most of which belongs to the phylum Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes (Edmonds-Wilson, Nurinova, Zapka, Fietrer & Wilson, 2015). On the same persons different communities of micro-organisms are present on the left and right hand, with only 17% of species of bacteria common for both arms (Fierer et al., 2008). The arms are a significant vector for interpersonal and intra transfer of microorganisms (Lax & Smith, 2014). Therefore, washing hands with soap is necessary not only because of the physical removal of foreign material, but also pathogenic microorganisms from the skin surface.
this study, the antibacterial activity of five different liquid soap on hands of male and female examinees. The results obtained show that the average number of bacteria is much higher in the dominant hand in relation to the non-dominant hand not only before but also after washing hands with soap. By comparing the number of bacteria on the dominant and nondominant hand, a statistically significant difference ($p < 0.00001$) in subjects of both genders have been determined, which coincides with the data (De Alwis, Pakirisamy, San, & Xiaofen, 2012). The results we obtained were to be expected, because the dominant hand is significantly more used than non-dominant hand. So it is quite understandable that on the dominant hand there is a larger number of microorganisms which mainly come from the environment. In addition to the dominance of a hand, we investigated the influence of the gender of the examinees to the number of bacteria on the hands. Women had a slightly higher average number of bacteria (2.46%) on the fingers than men, but determined difference was not statistically significant ($p = 0.232012$). Previous studies (Fierer et al., 2008) indicate that the diversity of microbial communities significantly is higher in the hands of women than men. The causes of these differences between the genders are not precisely defined, but the factors that may influence: the amount of perspiration and sebum, pH value of the skin, thickness of skin, production of hormone, and use of cosmetic preparations (Dao & Kazin, 2007; Kim et al., 2006; Roth & James, 1988). In this research, the strongest antimicrobial activity exhibited by the soap LS4 which is declared as an antibacterial. Distinct antibacterial activity of soaps is likely to be the result of lactic acid, which is only part of this soap (O'Shaughnessy, O'Maley, & Corbett 1991) and EDTA (Kulthanan, Maneeprasopchoke, Varothai, & Nuchkull, 2014). Both active substances belong to the group of permeabilizers. The next soap according to the hygienic efficiency was plain liquid soap LS2. In its structure, this soap contains essential oils, castor oil and sunflower seeds which are attributed to the antibacterial properties (Alakomi et al., 2000; Brul & Coote, 1999). Soap LS2 contains linalool and that has been shown to enhance the antimicrobial effects and synergistic effects of essential oils (Herman, Tambor, & Herman, 2016). In contrast, antibacterial soap LS3 had the lowest antimicrobial effects of all tested soap. Although the composition of the soap of stated component possessing antimicrobial capacity as limonene, (Dan, Shibura, Sethuraman & George, 2005) SDS (Elramady, Aly, Rossitto, Crook, & Cullor, 2013) and linalool (Herman et

al., 2016), we believe that the low concentration of these active substances is likely to cause its poor effectiveness. Soaps with a lower measured pH valu caused greater percentage reduction of bacteria compared to soap with a slightly higher pH. The most effective LS4 soap had the lowest pH value (3.51), while the lowest antibacterial activity was showed by soaps LS3 and LS5 with the highest pH values of 5.84 and 6.73. There has been determined a statistically significant negative correlation (-0.884059) between the pH of soap and their disinfection capacity. It should be noted that the frequent use of acid soaps can lead to irritation and dryness of the skin. The advantage of using antibacterial soap compared to ordinary soaps is subject of scientific debate. Although antimicrobial soaps are more effective in reducing the number of microorganisms compared to ordinary, yet there is not enough scientific evidence that these soaps are more effective in preventing infections (Fuls et al., 2008). If antibacterial soaps are used daily and in a long term , there is a question of potentially negative effects of their active ingredients on human health, the environment, or the possibility of development of bacterial resistance (Saheed et al., 2012; FDA, 2016). Since in our study it was demonstrated that antibacterial and ordinary liquid soaps showed antimicrobial activity, we can agree with the opinion of (Fuls et al., 2008), that the reduction of microorganisms on hands apart from the active ingredients of soap contribute its quantity, chemical composition, and the duration and frequency of washing. Therefore, the recommendation of the US Agency for Food and Drug Administration - FDA from 2016 (FDA, 2006) that for maintenance of hand hygiene ordinary soaps are used.

CONCLUSION

Based on the research results, we can conclude that there is a statistically significant difference in the prevalence of bacteria between the dominant and non-dominant hands of examinees. Women on average have 2.46% more bacteria on their hands than men, with what ascertained difference was not statistically significant. There has been a statistically significant negative correlation between the pH of the soap and its antimicrobial activity . Since the antibacterial and ordinary liquid soaps showed similar disinfectant action, and taking into consideration the recent literature data, we recommend the use of ordinary soaps for eve use.

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INFORMACIJE O AUTORIMA

Vildana Hadžić
Hospital for Respiratory diseases and TBC, 72 270 Travnik
e-mail: hadzicvildana@yahoo.com

Anesa Jerković Mujkić
University of Sarajevo, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35
e-mail: anesajm@gmail.com

Renata Bešta Gajević
Faculty of Science, Univesity of Sarajevo, Zmaja od Bosne 33 -35
e-mail: renata_besta@yahoo.comUstanova, adresa

Snježana Hodžić

Darja Husejnagić

Emina Mešikić

Amela Hercegovac