International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 4, 2019, pp.-1540-1543.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

COMPARATIVE ANALYSIS OF ANTIMICROBIAL EFFICACY OF TWO POPULAR HAND SANITIZERS SOLD IN INDIA

HARUMAVALI KAUR[1], MANISHA JAIN[1], KAMNA SAINI*[2] AND DIPTI SINGLA[1]

[1]Department of Food Science, MCM DAV College for Women, Panjab University, Chandigarh, 160036, Panjab, India
[2]Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, 201206, Chaudhary Charan Singh University, Meerut, 250001, Uttar Pradesh, India
*Corresponding Author: Email - kamna.sainii@gmail.com

Received: January 14, 2019; Revised: April 16, 2019; Accepted: April 17, 2019; Published: April 30, 2019

Abstract- Hand hygiene is considered to be of prime importance to resist various hygiene related diseases like typhoid, cholera, jaundice etc. This study was aimed to evaluate the antimicrobial efficacy of Dettol and Lifebuoy hand sanitizers among college students. Ten volunteers without any clinical signs of infection were included in the study. Sterile cotton swab sticks were used to take swabs from both hands, both before and after the use of hand sanitizers and after incubation colony forming units count were taken to calculate the percentage reduction in bacterial count. Transient micro flora on hand was isolated and characterised by gram staining and biochemical tests. Antibacterial efficacy of both the hand sanitizers was evaluated by agar-well diffusion assay against the identified isolates of transient micro flora. Dettol hand sanitizer showed 87 to 99% bacterial reduction while Lifebuoy showed 77 to 99.9% bacterial reduction. A total of six isolates of transient microflora were identified as *Escherichia coli, Staphylococcus aureus, Streptococcus lactis, Bacillus cereus, Alcaligenes faecalis* and *Pseudomonas aeruginosa* on the basis of results of Gram staining and biochemical tests (IMViC). Zone of inhibition of 1 to 9mm was observed for Dettol while 0.7 to 4mm was observed for Lifebuoy in agar-well diffusion assay. These results indicate that both the hand sanitizers were effective in their antibacterial properties and can be used as an alternative approach to hand hygiene by washing.

Keywords- *Hand sanitizer, Hygiene, Antibacterial, Hand microflora*

Citation: Harumavali Kaur, *et al*., (2019) Comparative Analysis of Antimicrobial Efficacy of Two Popular Hand Sanitizers Sold in India. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 4, pp.-1540-1543.

Introduction

Today, hygiene is collaboration of disease prevention and health promotion. Its importance is universally recognized and evidence based [1]. The key vehicle in transmission of pathogens is physical contact between people and between people and objects. Therefore, the key intervention in disease prevention is effective hand hygiene [2]. It is an important procedure in the healthcare environment, with regular training being given to healthcare workers about hand hygiene procedures [1]. Hospital and community-acquired infections constitute a serious public health problem all over the world [3].As estimated by the Center for Disease Control and Prevention (CDC) approximately 2 million people acquire nosocomial infections each year and approximately 90,000 of these patients die as a result of this infection [4].Hand hygiene has been promoted as the single most important measure in the preventing hospital associated infections by CDC, WHO and many other experts [5,6]. Many studies have reported an association between improvements in hand hygiene and infectious diseases reduction rates in the community, outside of the healthcare environment [2].It is estimated that one million lives could be saved a year by simple hand washing [6] and "hand hygiene" has been addressed by many public health campaigns worldwide with varying success[1]. There are 2 major components of Hand hygiene: (1) Hand washing: Removal of microorganisms with ordinary soap and water, and (2) Hand antisepsis: Removal or destruction of microorganisms using an antimicrobial soap or an alcohol-based hand rubs [7]. Hand washing refers to the act of cleaning hands for the purpose of elimination of dirt, microbes by the application of a non-antimicrobial or antimicrobial soap; and mechanical friction generated by rubbing the hands together for 60 seconds, followed by rinsing with water, and then followed by thorough drying. The alcohol hand-rub procedure involves the use of alcohol rather than water [3].

However, many studies have shown that as many as 80% of individuals retain some pathogenic bacteria on their hands even after hand washing [8]. Hand washing with soap removes the body's own fatty acids from the skin, which may result in cracked skin that provides an entry portal for pathogens, whereas, high-quality hand disinfectants contain additional skin care products, like emollients [2]. Moreover, the application is easy and uncomplicated as it does not require the use of water. Hand sanitizers are alcohol or non-alcohol based preparations which are designed for application to the hands in order to reduce the number of viable microorganisms on the hands [5]. They are also used as supplements to hand washing with soap and water [9]. Alcohol-based hand sanitizers are available in liquid, foam and gel preparations. Main ingredients of hand sanitizers include isopropanol, ethanol, n-propanol or povidone -iodine while the passive ingredients usually include a thickening agent (such as polyacrylic acid for gels), humectants (such as glycerin for liquid rubs) or propylene glycol and essential oils of plants [10]. Currently there is a wide variety of hand sanitizers in the market containing ethyl alcohol with concentration ranging from 62 to 95%. In the past few decades, several studies have been performed to manifest antibacterial effect of hand gel sanitizer in different settings such as extensive care facilities, schools and hospitals [5]. Some of these studies found that not all sanitizers are equally competent in eliminating all germs. While, some studies proclaimed high efficacy of hand sanitizers in cutting down the microbial flora of hand; other studies were unsuccessful to show such efficacy of hand sanitizers. Although 62% alcohol-based sanitizers were commonly used, most alcohol-based hand sanitizers contain around 60 to 85% of alcohol. Many studies recommended that, sanitizers with at least 70% alcohol were capable to destroy 99.9% of the bacteria on hands [11]. However, the efficacy of these sanitizers relies upon the time of rubbing the sanitizer on hand.

For example, rubbing alcohol-based sanitizers for duration of 30 and 25 seconds was reported to destroy 99.99% of bacteria on hand. Hand sanitizers address the barriers to hand hygiene compliance because they require a fraction of the time for effective hand washing [12], they are less damaging to the skin than soap and water [13] and they are more effective in killing many microorganisms [14]. Alcohol-based hand sanitizers have been manifested to be efficient against a wide variety of Gram-positive and Gram-negative bacteria, multi-resistant pathogens, fungi and many viruses[14], they have also been proclaimed to have very poor action against bacterial spores, protozoan oocysts and certain non-enveloped (non-lipophilic) viruses [5].Even though several reports have stated their efficacy, consumers have been cautioned against bogus claims of efficacy by some manufacturers. Hand washing and drying could be difficult in different part of the country where there is no/low access of water this leads to the use of hand sanitizers for proper health hygiene. Hand sanitizers are relatively new in the market and the government regulatory body, National Agency for Food and Drugs Administration and Control has registered a number of commercial hand sanitizers [14]. Therefore, it is crucial to evaluate the efficiency of these products. The study was designed to evaluate the efficacy of Dettol and Liftboy hand sanitizers in reducing the transient flora on the hands.

Materials and Methods

Hand sanitizers: Two popular brands of alcohol-based hand sanitizers were purchased from local retail outlets in Chandigarh [Table-1].

Table-1 *The composition of two alcohol-based hand sanitizers*

Hand Sanitizer	Composition
Dettol	Alcohol denat, aqua, PEG/PPG-17/16 copolymers, Acrylate/c10-30 Akyl Acrylate cross polymer, Tetrahydroxyl propyl Ethylenediamine, perfume, and limonene
Lifebuoy	Ethyl alcohol 95% v/v IP 55%w/w, isopropyl alcohol IP10% w/w, tocopheryl acetate IP 0.05% w/w, perfumed gel base: Q.S to 100% w/w

Media: Nutrient agar, Nutrient broth, Tryptophan broth, Methyl Red Voges-Proskauer (MRVP) broth, Simmons Citrate Agar.
Reagents: Kovac's Reagent, Methyl red Reagent, VP Reagent I, VP Reagent II, Bromothymol Blue as an indicator.
The study was conducted in three phases:
Phase I. Collection and evaluation of samples
Phase II. Isolation and Identification of hand microflora
Phase III. Antibacterial efficacy of hand sanitizers against hand microflora

Phase I: Collection and evaluation of samples

The study was conducted with a total of 10 students without having any clinical signs of dermal abrasion, infection and trauma. Approximately 2 drops of hand sanitizer were used by each student and were asked to rub the hands ensuring complete dryness. Swab samples from the hands of students were collected before and after using hand sanitizer using sterile saline solution. The Swab samples collected were cultured on nutrient agar plates and the plates were incubated at 37°C for 24 hours. After incubation, plates were observed and count of colony forming units (CFU) was recorded. Percentage reduction in bacterial load was calculated by the formula:

$$\%R = [(BLB-BLA)/BLB] * 100$$

Where, BLB = Bacterial load before using hand sanitizer and
BLA = Bacterial load after using hand sanitizer.

Colonies were subsequently picked and transferred to nutrient broth tubes for the isolation of transient micro flora of hand for phase 2 of study.

Phase II: Isolation and Identification of hand micro flora

In total 20 cultures were isolated. Gram staining and biochemical tests (IMViC) were performed for identification of the isolated cultures. The IMViC test stands for the first letter of four tests: Indole Test, Methyl-red and Voges-Proskauer Test and Citrate Utilization Test with the lower 'i' for pronunciation [15].

Indole production test: The indole test is performed by inoculating the culture into tryptophan broth, the indole produced during the reaction is detected by adding Kovac's reagent (Dimethylaminobenzaldehyde) which produce a cherry-red reagent layer which indicates the determination of indole production from microbial catabolism of tryptophan. Development of cherry red colour in the top layer of the tube indicates a positive result for indole production whereas absence of red colour indicates indole negative [16-19].

Methyl-Red and Voges-Proskauer Test: These tests are used to differentiate two major types of facultative anaerobic bacteria that produce large amount of acid and those that produce neutral product acetoin as end product. Both these tests are performed simultaneously as they are physiologically related and use the same medium MR-VP Broth. Positive methyl red test is indicated by the development of red colour after the addition of methyl red reagent. A negative methyl red test is indicated by absence of red colour after the addition of methyl red reagent. A positive VP test is indicated by the development of red brown colour after the addition of VP reagent I and VP reagent II. A negative test is indicated by absence of red brown colour after the addition of VP reagent I and VP reagent II [18-20].

Citrate Utilization Test: It is used to differentiate among enteric bacteria on the basis of their ability to utilize citrate carbon source. It is performed by inoculating the microorganisms on slants of Simmons Citrate Agar, bromothymol blue dye is used as an indicator. Bromothymol blue is green when acidic (pH 6.8 and below) and blue when alkaline (pH 7.6 and higher). A positive citrate result is indicated by the growth and a blue color change. A negative citrate result is indicated by the absence of growth and no color change in the tube [18-19].

Phase III: Antibacterial efficacy of hand sanitizers

Antibacterial efficacy of the two hand sanitizers was determined by agar-well diffusion assay with little modification. The bacterial culture was inoculated on the surface of nutrient agar plate, first in horizontal direction and then in vertical direction to ensure even distribution of cultures on the agar plate using a spreader. Four wells were made in the agar plates, two of them for checking the antibacterial activity of Dettol hand sanitizer and the rest two were for Lifebuoy hand sanitizer. 2 drops of hand sanitizer were poured in the wells. The plates were kept in refrigerator for about 2-3 hours to allow diffusion of hand sanitizer into the agar. After refrigeration plates were incubated at 37°C for 24 hours. After incubation antibacterial efficacy of the two hand sanitizers were determined by measuring the diameter of the clear zones formed around the wells for each hand sanitizer [21-23].

Results

Phase I: Percentage bacterial reduction after the use of hand sanitizers

Phase I of the study showed a significant bacterial reduction after using hand sanitizers [Fig-1]. Reduction for Dettol ranged from 95.66%-98.80% while for Lifebuoy it ranged from 73.07%-99.95% [Table-2]. Dettol showed a mean reduction of 98.05% which was much higher than that of Lifebuoy, 87.10%. These differences were significant in bacterial reduction between Dettol and Lifebuoy samples.

Table-2 *Mean Percentage reduction of viable bacterial loads on hands after using hand sanitizers*

Samples	Percentage Reduction	
	Dettol	Lifebuoy
Sample 1	98.12	77.24
Sample 2	95.66	88.92
Sample 3	98.80	73.07
Sample 4	97.72	99.20
Sample 5	95.69	99.95
Sample 6	97.76	87.50
Sample 7	97.65	86.89
Sample 8	98.75	83.56
Sample 9	96.87	75.48
Sample 10	97.83	99.24
Mean % Reduction	98.05	87.10

Phase II: Isolation and Identification of hand microflora

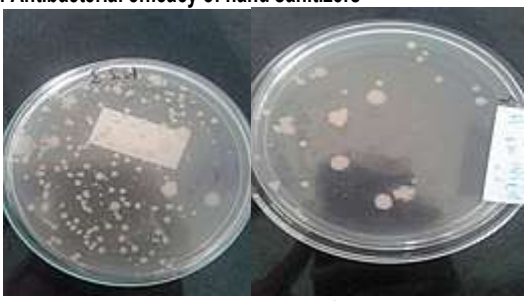
Gram staining of the cultures revealed gram positive as well as gram negative cocci, bacilli arranged singly, in pairs, in clusters or in chains, some spore forming isolates were also observed. On the basis of results of gram staining and biochemical characteristics of isolates several bacterial species were identified as transient hand microflora [Table-3].

Table-3 *Gram character and biochemical characteristics of isolated hand microflora*

Isolate	Gram Character	Indole test	MR test	VP test	Citrate test
Staphylococcus aureus	Coccus +	-	+	-	-
Escherichia coli	Rod -	+	+	-	-
Pseudomonas aeruginosa	Rod -	-	-	-	+
Alcaligenes faecalis	Rod -	-	-	-	-
Streptococcus lactis	Coccus +	-	+	-	-
Bacillus cereus	Rod +	-	-	-	-

Note: (-): Negative reaction, (+): Positive reaction

Phase III: Antibacterial efficacy of hand sanitizers



a b

Fig-1 Bacterial colonies observed from swab samples of hands, before (a) and after (b) the use of hand sanitizer

Table-4 *Diameter of Zone of inhibition (mm) of hand sanitizers against hand microflora*

SN	Test Organism	Zone of inhibition (mm)	
		Dettol	Lifebuoy
1	*Staphylococcus aureus*	1	0.7
2	*Escherichia coli*	8	3
3	*Pseudomonas aeruginosa*	9	3
4	*Alcaligenes faecalis*	0	0
5	*Streptococcus lactis*	5	4
6	*Bacillus cereus*	3	4

Discussion

The use of hand sanitizers has become very well-liked nowadays; hence it is necessary to evaluate the efficacy of these sanitizers. The results of phase I of study showed significant percentage reduction of bacterial load of hands after the use of both the hand sanitizers. As evident from the figure1, significant reduction was observed in CFU count after using hand sanitizer. The results of the present study showed that hand sanitizer effectively reduced bacterial load on hands to a varying degree. These results are in agreement with many studies [24-28]. This reduction in the bacterial load is not just due to alcohol but also due to presence of other components. Several cultures of hand microflora were isolated, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Alcaligenes faecalis, Streptococcus lactis* and *Bacillus cereus*. Present study also throws light on the antibacterial activity of hand sanitizers in which Dettol was the most broad-spectrum antibacterial agent in which zone of inhibition ranging from 1mm -9mm was observed against isolated bacterial cultures. However, 0.7- 4mm zone of inhibition was observed against bacterial cultures with Lifebuoy. Dettol showed maximum zone of inhibition against Escherichia coli (8mm) and Pseudomonas aeruginosa (9mm). No antibacterial activity was observed against Alcaligenes faecalis for both Dettol and Lifebuoy. No skin irritation was observed after use of both the hand sanitizer products and both contained a perfumed gel base and thus proved to be odorless with no dryness after use.

Application of research: This study revealed the transient microflora on the hands and showed the efficacy of Dettol (87 to 99%) and Lifebuoy (77 to 99.9%) in reducing count after their use as hand sanitizer.

Research Category: Hygiene Microbiology

Abbreviations:

CFU: Colony forming units
CDC: Center for Disease Control and Prevention
MRVP: Methyl Red Voges-Proskauer

Acknowledgement / Funding: Authors are thankful to Department of Food Science, MCM DAV College for Women, Panjab University, Chandigarh, 160036, Panjab, India. Authors are also thankful to Institute of Applied Medicines and Research, Ghaziabad, 201206, Chaudhary Charan Singh University, Meerut, 250001, Uttar Pradesh, India.

*** Major Advisor or Chairperson of research: Dr Kamna Saini**
University: Chaudhary Charan Singh University, Meerut, 250001, Uttar Pradesh
Research project name or number: Clinical study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Chandigarh

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Babeluk R., Jutz S., Mertlitz S., Matiasek J., Klaus C. (2014) PLoS One, 9(11), e111969.
[2] Aiello A.E., Coulborn R.M., Perez V., Larson E.L. (2008) American Journal of Public Health, 98(8), 1372–1381.
[3] Hassan A.O., Hassan R.O., Muhibi M.A., Adebimpe W.O. (2012) African journal of microbiology research, 6(24),5162-5167.
[4] Zerr D.M., Garrison M.M., Allpress A.L., Heath J., Christakis D.A. (2005) Pediatrics, 115(4),387-392.
[5] John M., Boyce M.D., Didier Pittet M.D. (2002), American Journal of Infection Control, 30(8), S1–S46.
[6] WHO (2009) Infect Control Hosp Epidemiol, 30(7), 611-622.
[7] Oke M.A., Bello A.B., Odebisi M.B., Ahmed El-Imam A.M., Kazeem M.O. (2013) Ife Journal of Science, 15(1),111-117.
[8] Tambekar D.H., Shirsat S.D., Suradkar S.B., Rajankar P.N., Banginwar Y.S., Continental J. (2007) Biomedical Sciences, (1), 6–10.
[9] Mondal S., Kolhapure. (2004) The Antiseptic,101(2),55-57.
[10] Boyce J.M., Pittet D. (October 25, 2002). Morbidity and Mortality Weekly Report. 51, 1–44.
[11] Bauer A.W., Kirby W.M., Sherris J.C., Turck M. (1966) American Journal of Clinical Pathology, 45(4),493-496.
[12] Mody L., McNeil S.A., Sun R., Bradley S.F., Kauffman C.A. (2003) Infect Control Hosp Epidemiol, 24(3),165-171.
[13] Boyce J.M., Kelliher S., Vallande N. (2000) Infect Control Hosp Epidemiol, 21(7), 442-448.
[14] Larson E.L., Aiello A.E., Bastyr J. (2001) Crit Care Med, 29(5), 944-951.

[15] Vashist H., Sharma D., Gupta A. (2013). Innovare Journal of Life Science, 1(1), 1-7.
[16] Miller J.M., Wright J.W. (1982) Journal of Clinical Microbiology, 15(4), 589-592.
[17] UK Standards for Microbiology Investigations Bacteriology (2014) Test Procedures, 19(3), 1-14
[18] Attri L.K., Narang H. (2014) Research Journal of Pharmaceutical, Biological and Chemical Sciences 5(1), 698-706
[19] Sawian P., Nongkynrih K.J., Anand U., Charan A.A. (2018) Journal of Pharmacognosy and Phytochemistry, 7(1), 395-397.
[20] Werkman C.H. (1930) Journal of Bacteriology, 20, 121–125.
[21] Balouiri M., Sadiki M., Ibnsouda S.K. (2016) Journal of Pharmaceutical Analysis, 6(2), 71–79.
[22] Magaldi S., Essayag S.M., Hartungde C. (2004) International Journal of Infectious Disease, 8(1), 39–45.
[23] Valgas C., DeSouza S.M., Smânia E.F.A. (2007) Brazilian Journal of Microbiology, 38, 369–380.
[24] Paulson D. S., Fendler E. J., Dolan M. J. & Williams R. A. (1999) American journal of infection control, 27(4), 332-338.
[25] Pickering A. J., Davis J. & Boehm A. B. (2011Journal of water and health, 9(3), 429-433.
[26] Oke M. A., Bello A. B., Odebisi M. B., El-Imam A. A., & Kazeem M. O. (2013) Ife journal of science, 15(1), 111-117.
[27] Guilhermetti M., Wiirzler L. M., Facio, B. C., da Silva Furlan M., Meschial W. C., Tognim M. B. & Cardoso C. L. (2010) Journal of Hospital Infection, 74(3), 219-224.
[28] do Prado, M. F., Coelho A. C. C., de Brito J. P. B., Ferreira D. O., Junior A. W., da Silva Menecucci C. & Tognim M. C. B. (2012) Letters in applied microbiology, 54(6), 564-567.