Assessment of Commercial Handwashes for Antimicrobial Activity and Maintenance of Hand Hygiene

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Abstract

Effective hand hygiene is essential for reducing the transmission of infectious diseases in both community and healthcare environments. Although commercial handwashes often claim antibacterial activity, their effectiveness varies based on formulation and user practices. This study assessed the antibacterial efficacy of eight commercially available handwashes (HW1 – HW8) against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas* sp., *Bacillus subtilis*, and *Candida albicans* using the agar well diffusion method, with zones of inhibition measured to evaluate antimicrobial performance. Among the tested products, HW8, HW5, and HW4 demonstrated the highest antimicrobial activity against all test organisms, while HW7 showed the least activity. To evaluate the effectiveness of handwashing in reducing microbial load, optical density (OD at 600 nm) measurements were taken from samples collected from the palms of 20 volunteers before and after washing with HW8. Results indicated a notable reduction in microbial load following handwashing, with percentage reductions ranging from 28.98% to 66.67%. The highest reductions were recorded for Volunteer 6 (66.67%), Volunteer 16 (65.98%), and Volunteer 10 (63.78%), reflecting efficient removal of microbial contaminants. These findings underscore the importance of evidence-based selection of hand hygiene products to ensure effective pathogen removal, thereby supporting infection prevention practices while maintaining hand hygiene standards.

Keywords: Antibacterial activity, hand hygiene, handwashes, Pathogenic bacteria, Volunteers

Introduction

Hand hygiene is a basic measure to prevent the transmission of infectious diseases in the community and healthcare environment [1]. Hands are the principal vehicle for pathogens, be it bacteria, viruses or fungi, causing everything from mild skin infections to serious systemic diseases. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have emphasized that washing your hands with soap and water is one of the easiest methods to minimize and prevent microbial contamination and in controlling the transmission of infectious diseases [2]. Given their ease of use, accessibility, and advertisements claiming antibacterial properties, commercial handwashes have an important role to play in hand hygiene [3]. Within the context of the coronavirus pandemic the demand and use of handwashes has increased around the globe, resulting in a plethora of handwash products containing a diversity of active antimicrobial agents from triclosan and chlorhexidine to herbal extracts [4,5]. The effectiveness of these handwashes varies based on the formulation, concentrations of active ingredients, and use and approach by the consumer; however, that requires an actual scientific evaluation of their antibacterial activity [6]. Assessing the antibacterial efficacy of commercial handwashes is crucial to validate their claims and guide public use. Laboratory methods like agar well diffusion and minimum inhibitory concentration (MIC) assays are frequently used to determine their effectiveness against pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa [7]. These evaluations help in understanding how well these products reduce microbial loads on hands and, consequently, in the prevention of disease transmission.

While the key objective of handwashing is to limit the transfer of pathogens, maintaining skin integrity is equally valuable. Regular use of some types of handwashes, especially those containing harsh chemicals, can lead to drying, irritation, or dermatitis; this, in turn, will limit user adherence to hand hygiene practices [8]. As such, effective hand hygiene products should find a happy medium between antimicrobial efficacy and skin compatibility to facilitate long-term adherence to hand hygiene. Several of the greatest burdens of infectious disease may lack data on the relative effectiveness of commercially available handwashes [9,10,11]. Knowledge of the antibacterial activity of available products in local settings can help improve public health messages and/or guide consumer choices to improve infection control practices, and also help manufacturers produce safer and more effective products that the user will want to comply with and use comfortably for regular handwashing [12].

In light of this information, the current study intends to evaluate the antibacterial activity of selected commercial handwashes, using the agar well diffusion method against a suite of representative bacterial pathogens [13]. This will also consider their effectiveness in maintaining good hand hygiene measures, some considerations such as quality of ingredients and how hand hygiene products may influence skin health [10]. While the accumulation of evidence-based information from the current study may prove valuable for consumers, health professionals, and policymakers concerning selecting and promoting the use of effective hand hygiene products, and ultimately improving infection control and public health measures.

Materials & Methods:

Test Bacterial Samples

The following organisms were used during the study: *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas* sp., *Bacillus subtilis,* and *Candida albicans*. The pathogenic bacterial samples were taken from AMCC (Amity Microbial Collection Center, Jaipur, Rajasthan).

Antimicrobial activity of commercial handwash

Eight commercially available handwashes (coded HW1, HW2, HW3, HW4, HW5, HW6, HW7, HW8) were obtained from local suppliers (Table 1). All products were checked for expiry, kept in a room temperature environment and used undiluted. The antimicrobial efficacy of these handwashes was evaluated using the agar well diffusion assay against a set of six organisms: *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas* sp., *Bacillus subtilis* and *Candida albicans* on Mueller Hinton agar (MHA). MHA was prepared and poured into sterile Petri plates and allowed to solidify. Fresh overnight cultures of the bacteria, to be tested, were grown in nutrient broth and adjusted to a suspension of 0.5 McFarland turbidity standard (~1.5 x 108 CFU/mL) using sterile saline. The prepared bacterial suspension was thoroughly swabbed using a sterile cotton swab, to ensure a confluent lawn of bacterial growth over the surface of the MHA plates. A sterile cork borer (6 mm diameter) was used to punch wells into the plates and 100 µL of each undiluted handwash sample was added to the respective wells using a micropipette. The inoculated plates with loaded wells were incubated in an upright position at 37ºC for 24 hours.

Table 1: Handwashes and their ingredients

S.No.	Handwashes	Ingredients
1	HW1	Rakta chitraka plumbago indica-0.001%, Vanardraka zingiber zerumbet-0.002% Jambira citrus lemon-0.05% Tulsi ocimum sanctum-0.05% Kumari aloe barbadensis-1.0%
2	HW2	Water, Sodium Laureth Sulfate, Sodium Lauryl Sulfate, Cocamidopropyl Betaine, Glycerine, Ammonium Lauryl Sulfate, Sodium Chloride, Laurimide DEA, Perfume DMDM Hydantoin, Disodium EDTA, Peg 40 Hydro Generated Castor Oil, ETH Triclosan, Citric Acid, Ocimum Sanctum (Tulsi Extract), Santalum
3	HW3	Neem (Azadirachta Indica), Tulsi (Ocimum Sanctum), Aqua, Lauric Acid, Myristic Acid, Palmitic Acid, Koh, Dimethicone, Ethylene Glycol Distearate, Diazolidinyl Urea and Ipbc, Sugandhit Dravya, Permitted Colour
4	HW4	Aqua, Ammonium Lauryl Sulfate, Sodium Laureth Sulfate, Glycerin, Sodium Chloride, Cocamide MEA, Citric Acid, Salicylic Acid, Tetrasodium EDTA, Magnesium Nitrate, EDTA, Methylchloroisothiazolinone, Magnesium Chloride, Methylisothiazolinone, Parfum, CI 19140, CI 42090.
5	HW5	Sodium Palmate (Surfactant), Sodium, Palm Kernelate (Surfactant), Aqua (Solvent), Glycerin (Humectant), Parfum (Fragrance), Sodium Lauryl Sulfate (Surfactant), Sodium Chloride (Viscosity Controlling Agent), Limonene (Fragrance)
6	HW6	Aqua, Sodium Laureth Sulfate, Cocamidopropyl Betaine, Disodium Laureth Sulfosuccinate, PEG 400, Cocamide MEA, Sodium Lauroyl Sarcosinate, Sodium Chloride, Fragrance, DMDM Hydantoin, PEG-40 Hydrogenated Castor Oil, Lavendin Oil, Ylang Ylang Oil, Glycerin,

		Propylene Glycol, Tetrasodium EDTA, Benzophenone-4, BHT, Citric Acid, Hexyl Cinnamal, Limonene, Linalool, Geraniol, Citronellol, Citral, CI 60730
7	HW7	Aqua (Water), Sodium Laureth Sulfate, Cocamidopropyl Betaine, Caprylyl/Capryl Glucoside, Coco-Glucoside, Glyceryl Oleate, Phenoxyethanol, Parfum (Fragrance), Sodium Cocoyl Isethionate, Sodium Benzoate, Sodium Chloride, Glyceryl Laurate, Citric Acid, Benzotriazolyl Dodecyl P-Cresol, Disodium EDTA, Linalool, Polysorbate
8	HW8	Sodium Lauryl Sulphate, Guar Gum, Disodium Lauryl Sulfosuccinate, Disodium EDTA, Aloe Vera extract, Fragrance, Cellulose Gum, Silicon Dioxide, Methyl Paraben Neem extract, CI 42090, CI 19140.

Evaluation of Hand Hygiene before and after handwash

To evaluate the effectiveness of commercial handwash (HW8) in maintaining hand hygiene, a before-and-after handwashing test was conducted using the surface swab method and bacterial colony count analysis [14].

Selection of Volunteers

Twenty healthy volunteers (08 male, 12 female), aged 20–50 years, working as housekeeping staff and gardening staff, with no visible skin lesions or recent antibiotic use, were selected after obtaining informed consent (Table 2).

Table 2: List of Volunteers

S.No.	M/F	Age(yrs)	Occupation	Education	Awareness of hand hygiene	Any frequent health issue/condition
1.	M	20	Housekeeping	12^{th}	Soap	No
2.	M	30	Housekeeping	5^{th}	Soap, Mitti	No
3.	F	42	Gardening	-	Soap, Mitti	No

4.	F	40	Gardening	-	Soap/Handwash	No
5.	F	35	Gardening	-	Soap/Handwash	No
6.	F	30	Housekeeping	-	Soap/Handwash	No
7.	F	50	Gardening	-	Soap/Handwash	No
8.	F	18	Housekeeping	12^{th}	Soap/Handwash	No
9.	F	40	Housekeeping	-	Soap/Mitti	No
10.	M	35	Gardening	5^{th}	Soap	No
11.	M	32	Housekeeping	10^{th}	Soap	No
12.	M	44	Gardening	8^{th}	Soap/Mitti	Yes Cold/Cough
13.	M	37	Housekeeping	8^{th}	Soap	No
14.	M	24	Housekeeping	-	Soap	No
15.	F	35	Housekeeping	-	Soap	Yes Cold/Cough
16.	F	35	Gardening	-	Soap	No
17.	F	35	Gardening	-	Soap	No
18.	M	23	Housekeeping	10^{th}	Soap	No
19.	F	22	Housekeeping	12^{th}	Soap	No
20.	F	21	Housekeeping	10^{th}	Soap	No

Sample Collection Before Handwashing

Volunteers were instructed to do their normal activities for a minimum of 30 minutes to allow for natural hand contamination. After that, sterile swabs moistened with sterile saline were then used to sample the palm and fingers on their dominant hand, with both swab samples using consistent pressure throughout the swabbing process. Immediately after swabbing, they were placed into 10 mL of sterile peptone water were vortexed to ensure that the bacteria came loose from the swab. The samples were then left to incubate at 37 °C for 24 hours, while the optical density was measured from each sample at 600 nm using a spectrophotometer.

Handwashing Procedure

Each volunteer washed their hands with 5 mL of the assigned commercial handwash (HW8) under running tap water for 20 seconds using the standard WHO handwashing technique, followed by rinsing and air drying [15].

Sample Collection After Handwashing

After handwashing, samples were then taken from the same area of the hand with sterile saline pre-moistened sterile swabs. The swabs were collected into 10 mL of sterile peptone water and vortexed to release any remaining bacteria (if still present) [16]. The samples were then incubated (for 24 hours at 37°C), and the optical density of each sample was measured at the 600 nm wavelength using a spectrophotometer. The microbial load identified after handwashing was then compared to the values before handwashing. This then measured the effectiveness of the handwash.

Result and discussion

Antimicrobial activity of commercial handwash

The antimicrobial activity of eight commercial hand washes against *E. coli*, *S. aureus*, *Pseudomonas*, *Bacillus*, *Enterococcus*, and *Candida* when determined using the zone of inhibition method, showed considerable differences in effectiveness. HW8 had the highest activity against *S. aureus* (36.67 ± 1.53 mm) and *E. coli* (22.67±1.53 mm), as well as against *Pseudomonas* (21.00 ± 1.00 mm) and *Candida* (24.33 ± 2.08 mm), indicating its broad-spectrum antimicrobial property. HW5 and HW4 also had significant activity against *Enterococcus* (34.67 ± 2.31 mm and 33.67 ± 1.53 mm, respectively), showing they have the potential to reduce Gram-positive bacterial load of hands in healthcare and food-handling contexts. HW3 displayed the highest antimicrobial activity against *Candida* (37.67±1.53 mm), suggesting the antimicrobial formulation may contain effective antifungal agents (Table 3). HW7 had the least antimicrobial activity of all organisms tested, with a minimal zone of inhibition (0-8 mm), demonstrating it may not have sufficient effectiveness for proper hand hygiene.

The differences in potential antimicrobial activity noticed during the study may have been attributed to the varying concentrations and types of active antimicrobial agents in each handwash formulation, including the concentration of triclosan, chlorhexidine, veggie extracts or the existence of synergism. The results were in line with previous reports which shows commercial handwashes differ in their ability to reduce the microbial load from handwashing and also reflects the necessity of commercial handwash samples that are effective, so that any subsequent hand hygiene will not increase the probability of transmission of an infectious agent [17, 18, 19].

These results highlight the potential of certain commercial handwashes to deliver an efficient hand hygiene through reductions in bacterial and fungal contamination (specifically HW8, HW5, HW4).

Table 3: Antimicrobial activity of commercial handwash against common microorganisms

Handwash	*E. coli*	*S. aureus*	*Pseudomonas*	*Bacillus*	*Enterococcus*	*Candida*
Zone of inhibition (in mm)						
	Mean±S.d	**Mean±S.d**	**Mean±S.d**	**Mean±S.d**	**Mean±S.d**	**Mean±S.d**
HW1	10.67±0.58	14.67 ± 1.53	25.00 ± 1.00	7.67 ± 0.58	26.00 ± 1.00	20.33 ± 1.53
HW2	6.33±0.58	21.00 ± 1.00	6.67 ± 0.58	4.00 ± 0.00	16.67 ± 1.53	21.33 ± 1.53
HW3	16.00±1.00	10.00 ± 2.00	15.33 ± 0.58	4.33 ± 0.58	8.67 ± 2.08	37.67±1.53
HW4	14.67±0.58	10.33 ± 0.58	16.67 ± 1.53	4.33 ± 0.58	33.67 ± 1.53	19.00 ± 1.00
HW5	17.67±0.58	20.00 ± 1.00	19.67 ± 0.58	4.67 ± 1.53	34.67 ± 2.31	16.67 ± 1.53
HW6	15.33±1.15	16.67 ± 1.53	7.00 ± 1.00	2.33 ± 0.58	10.33 ± 0.58	33.33±1.53
HW7	6.67±1.53	2.00 ± 1.00	4.33 ± 0.58	6.33 ± 1.53	1.00 ± 1.00	4.00 ± 0.00
HW8	22.67±1.53	36.67 ± 1.53	21.00 ± 1.00	1.67 ± 0.58	6.33 ± 0.58	24.33 ± 2.08

Evaluation of Hand Hygiene before and after handwash

The efficiency of handwashing in decreasing microbial load was evaluated by comparing the optical density (OD at 600 nm) of samples obtained from the palms of volunteer's pre-handwashing (HW8 handwash) and post-handwashing. The results clearly showed a decrease in microbial load, post-handwashing for all 20 volunteers, with the percentage decrease ranging from 28.98% to 66.67%. The greatest reduction was seen in Volunteer 6 (66.67%), with Volunteer 16 (65.98%) and Volunteer 10 (63.78%) showing that efficient removal of microbial contaminants was achieved with HW8 handwash. However, there was also a decrease in the microbial load from Volunteer 3 (28.98%) and Volunteer 2 (34.10%), indicating there was variability in the effectiveness of the handwash treatment, which may reflect differences in an individual's handwashing technique, thoroughness, or adherence to recommended hand washing time (Table 4).

On average, the microbial load significantly reduced post-handwashing treatment, implying the effectiveness of handwashing practices on reducing microbial burden on the hands (Fig 1). The findings also support prior research on the significance and importance of hand hygiene, purposefully interrupting the transfer of pathogenic germs and reducing infection risk [20, 21].

The variation in percentage reduction among volunteers illustrates the need for promoting correct handwashing techniques around proper time and scrubbing across all hand surfaces with effective handwash formulations. Offering educational intervention on hand hygiene practices as a standard practice may further improve handwashing efficacy in community and healthcare settings. Overall, the data support the prominent role of handwashing in mitigating hand contamination, warranting its ongoing promotion as a simple and effective action to promote personal and public health.

Table 4: Microbial load in the palms of volunteers

Volunteers	Before Handwash (OD at 600 nm)	After Handwash (OD at 600 nm)	% Reduction
1	0.311	0.195	37.30
2	0.525	0.346	34.10
3	0.452	0.321	28.98
4	0.313	0.121	61.34
5	0.254	0.112	55.91
6	0.456	0.152	66.67
7	0.213	0.123	42.25
8	0.723	0.321	55.60
9	0.452	0.215	52.43
10	0.392	0.142	63.78
11	0.465	0.272	41.51
12	0.212	0.108	49.06
13	0.402	0.213	47.01
14	0.812	0.312	61.58
15	0.712	0.372	47.75
16	0.923	0.314	65.98
17	0.842	0.375	55.46
18	0.854	0.414	51.52
19	0.391	0.192	50.90
20	0.192	0.089	53.65

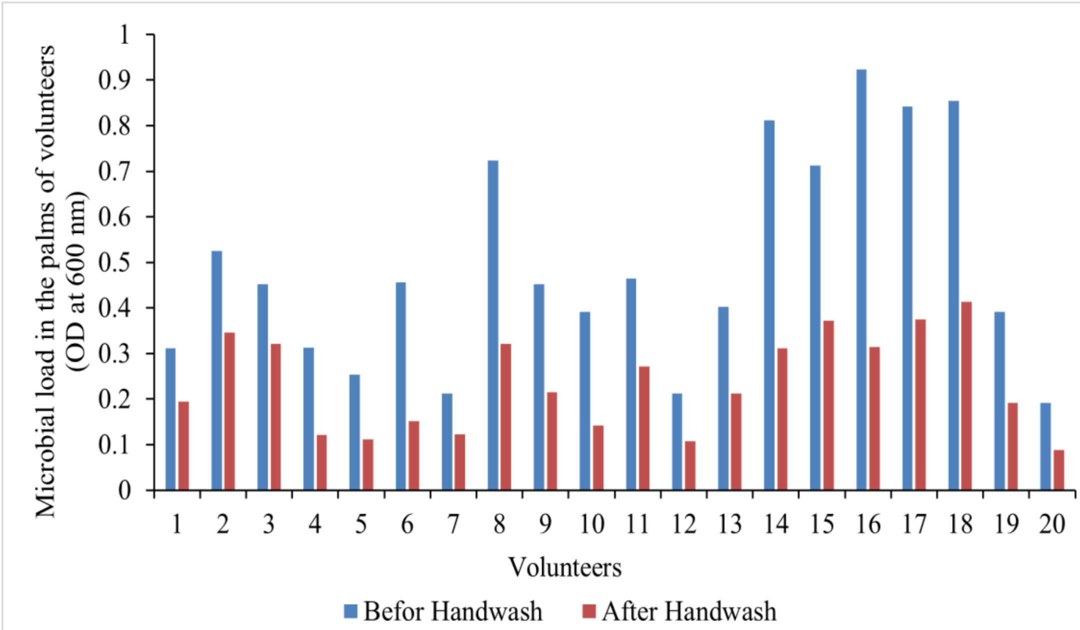

Fig 1: Microbial load in the palms of volunteers (OD at 600 nm)

Conclusion

This study demonstrated that commercial handwashes varied in antimicrobial effectiveness against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas* sp., *Bacillus subtilis*, and *Candida albicans*. HW8, HW5, and HW4 had significant broad-spectrum antimicrobial efficacy, while HW7 was ineffective. Assessment used the microbial load before and after hand washing showed significant decreases, suggesting that handwashing is effective at limiting the transfer of pathogens. The variability in both antimicrobial efficacy and microbial load reductions shows the importance of creating effective handwashing formulations and using proper handwashing techniques to maximize hand hygiene practices. Collectively, the data emphasize that hand hygiene continues to be an important, practical infection prevention practice in healthcare and community settings. Further studies that include the formulations components, user compliance, and duration of efficacy will support the ability to provide evidence-based guidelines to support that effective hand hygiene practices.

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Declarations

Conflict of interest: The authors declare no conflict of interest.

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