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AN ASSESSMENT OF ANTIMICROBIAL EFFICACY OF LIQUID SOAP AND ALCOHOL-BASED HAND SANITIZER ON REGULAR HAND MICROBIOME

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ABSTRACT

Background: Transmission of microbial infections is most commonly through hands coming in contact with contaminated surfaces in our daily routine. Hence the importance of hand hygiene cannot be overlooked, particularly in the ongoing COVID 19 pandemic. This study was undertaken to evaluate the antimicrobial efficacy of liquid soap and hand sanitizer of two popular brands, Dettol and Lifebuoy, against hand microflora. **Materials & Methods:** Six subjects performed their routine activities without washing their hands for six hours. Handprints of the subjects were taken on trypticase soy agar plates, before and after the application of liquid soap/sanitizer for visual assessment of reduction in microflora. The potency of liquid soaps and hand sanitizers was evaluated by the agar well diffusion method using selected isolates from the agar handprints. Two of these isolates were identified by the Vitek technique. **Results and Conclusion:** Remarkable reduction in the microflora was visually observed in the handprints after the application of liquid soaps of both brands. The sanitizers of both brands were found to be more effective in reducing the microbial load when the hands were not visibly dirty. Two of the isolates were identified as *Pantoea spp*. and *Kocuria kristinae*. Thus washing hands with soap and water should be preferred whenever possible.

KEYWORDS: Antimicrobial efficacy, hand hygiene, alcohol based hand sanitizer, liquid soap, hand microbiome.

INTRODUCTION

Community-acquired infections are escalating and pose a serious public health problem worldwide. Hands are considered to be the primary route for transmitting a number of microbes and infections to individuals.[1] The focus on personal as well as hand hygiene to prevent many communicable diseases is gaining importance especially now because of the current Covid 19 pandemic.

Hand washing is an age-old practice. It is defined as a vigorous, brief rubbing together of all surfaces of lathered hands, followed by rinsing under a stream of water. Hand washing suspends microorganisms and mechanically removes them by rinsing with water.[2] With the advancements in handwashing techniques, new products like detergent soaps, liquid soaps, and alcohol-based hand rubs or hand sanitizers, or hand-cleaning reagents like chlorhexidine, hexachlorophene, iodine, and iodophors are increasingly being used [3]. Scientific studies have shown that after washing hands, as many as 80% of individuals retain some pathogenic bacteria on their hands.[4] To overcome the limitations of plain hand washing with soaps, hand sanitizers were introduced claiming to be effective against pathogenic micro-organisms and improving skin condition due to the addition of emollients in it.[5]

Although several studies have compared different hand hygiene methods in hospital settings [6] few studies have been published on the effect of hand hygiene on bacterial contamination of hands in the general community routine. Moreover, most of these studies have used standard bacterial cultures or pathogenic microorganisms to assess the antimicrobial efficacy of the soaps and sanitizers whereas we have used bacterial isolates from the handprints of subjects on agar plates in this study. An effort has been made to study the efficacy of hand wash liquid soaps and alcohol-based hand rub or hand sanitizers manufactured by two well-known brands, Dettol and Lifebuoy, to reduce hand microflora as compared to washing hands with tap water alone. A visual comparison of the microflora on hand impressions taken on agar plates before and after application of the hand hygiene products was followed by estimating the antimicrobial activity of these products by the agar well diffusion method using pure cultures isolated from the handprints.

1. MATERIALS AND METHODS

1.1. Materials

1.1.1.Chemicals and media

All chemicals and solvents were of analytical grade and purchased from Merck, Germany and culture media were obtained from Hi-media, Mumbai, India.

1.1.2. Liquid soaps and hand sanitizers

Two well-known brands producing both liquid soaps and hand sanitizers were selected for this study and bought locally. The chemical composition is as follows:

1.1.3. Dettol liquid soap[7]

Aqua, Ammonium Lauryl Sulfate, Sodium Lauryl Sulfate, Glycol Stearate, Parfum, Cocamide MEA, Glycerin, Propylene Glycol, Salicylic Acid, Tetrasodium EDTA, Citric Acid, Sodium Chloride, Methylchloroisothiazolinone, Methylisothiazolinone, Citral, CI 11710, CI 12085

1.1.4. Lifebuoy liquid soap[8]

Water, myristic acid, lauric acid potassium hydroxide, potassium chloride, palmitic acid sodium lauryl sulfate, glycol distearate perfume, propyl betaine, hydroxypropyl methylcellulose, glycerin, tetrasodium EDTA. bht, silver oxide, pentasodium pentetate, etidronic acid vp/va copolymer, dmdm hydantoin, thymol, terpineol, benzyl acetate geraniol, coumarin, butylphenyl methylpropional CI 45100

1.1.5. Dettol sanitizer[9]

Alcohol (Denatured) eq to absolute alcohol 72.34% v/v, water, PEG/PPG-17/6 Copolymer, Propylene Glycol, Acrylates/C10-30, alkyl acrylate Crosspolymer, Tetrahydroxypropylethylenediamine, chamomile extract, perfume, ponceau SX.

1.1.6. Lifebuoy sanitizer[10]

Ethyl alcohol 95%V/v, I.P 62% W/W, Isopropyl alcohol I.P 3% W/W, Niacinamide I.P 1% W/W in perfumed gel base Q.S to 100% W/W

1.2. Methods

1.2.1. Sample collection/ processing for agar handprints

- Six undergraduate students were selected as volunteers for the study. They performed all the routine activities without washing their hands for six hours (approximately) on the day of the experiment.
- Some of the volunteers had visible dirt on their hands at the end of 6 hours and this data was recorded separately.
- The prints of untreated hands were taken on TSA plates by gently lacing the fingers and palms on two separate TSA surfaces and served as control.
- Thereafter, the volunteers were subjected to three hand treatment protocols: they used either plain tap water, liquid soap (Dettol and Lifebuoy) and water or hand sanitizers of these two brands to clean their hands. The cleaning process was carried out for 20 seconds. Cleaned hands were air-dried and then the prints of treated hands were taken on prepared TSA plates by placing the fingers and palms gently on two separate TSA surfaces.
- While taking the prints of treated and untreated hands due care was taken to keep the orientation of the petri plates constant.
- All the Petri plates were observed after incubation at 37^0C for 24 hours.
- The experimental and control plates were examined and compared to assess a reduction in the microbial colonies after the three treatments.
- The microbial cultures which persisted even after treatment were termed as resistant and the cultures which were eliminated by the treatment were termed as sensitive.

1.2.2. Isolation of bacteria from handprints

Well isolated colonies of resistant as well as sensitive bacteria from the handprints were carefully selected and pure cultures were obtained by the streak plate method. The morphology and gram character of the isolates was determined. Two of these isolates were identified by the Vitek technique. Stock cultures of all the isolates were maintained on nutrient agar plates and slants at 4^0C.

1.2.3. Antibacterial activity

These pure cultures were used as test organisms to determine the antibacterial activity of the liquid soaps and hand sanitizers of both the brands by the agar well diffusion method using 50µl of the respective liquid soaps and hand sanitizers.

2. RESULTS AND DISCUSSION

The prevalence of community infections is majorly attributed to the lack of proper hand hygiene practices among the general public. This study, which reiterates the observation that simple hand washing using soap and water drastically reduces the microbial population and thereby the spread of infectious diseases, gains special significance in the context of the current COVID 19 pandemic.

The handprints of subjects on TSA before and after washing hands with plain water are shown in figure 1.

Figure 1: Effect of washing with water on hand microbiome.

The handprints of subjects on TSA before and after washing hands with liquid soap manufactured by both Dettol and Lifebuoy and water are shown in figure 2.

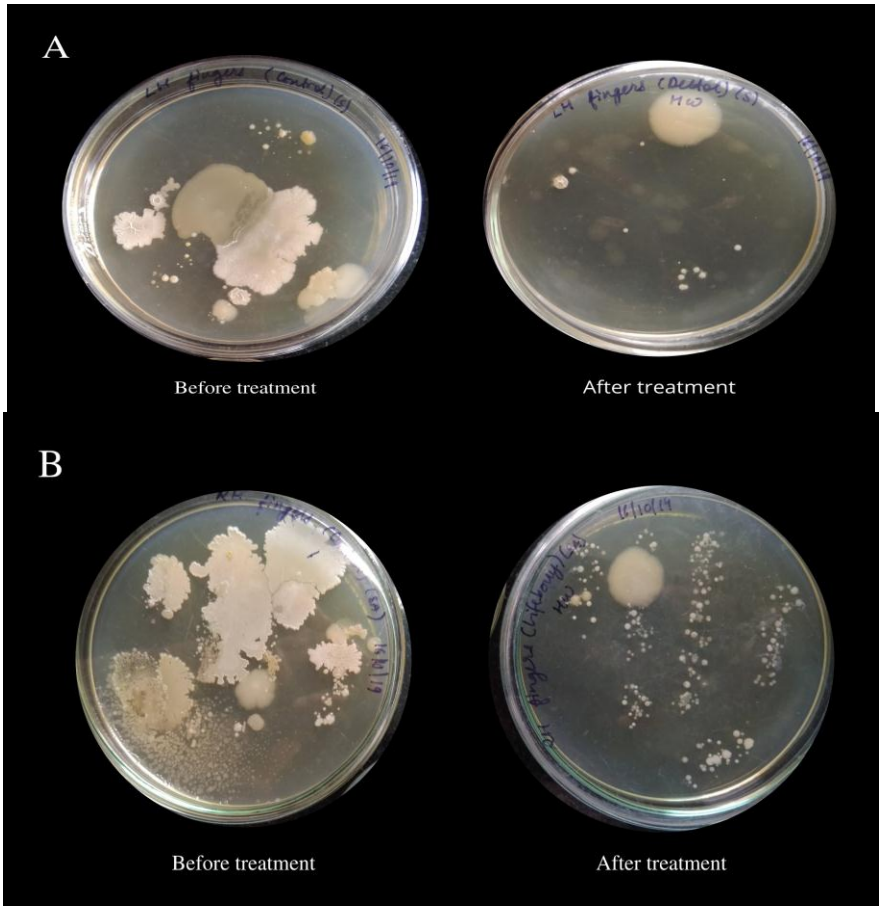

Figure 2: Effect of washing hands with (A) Dettol liquid soap; and (B) Lifebuoy liquid soap on hand microbiome.

A remarkable reduction in the microbial load can be observed after the treatments. Handwashing with soap holds dirt as well as microbial flora in suspension due to the surfactant activity of the soap and mechanically removes them by rinsing with water. The antimicrobial activity of soaps is mainly due to disrupting the cell membrane and proteins in the microbes and is usually sustained on the skin to reduce the number of microbial

flora even after the hand wash is complete.[11] However, after treatment with alcohol-based hand sanitizers manufactured by both the brands, viz. Dettol and Lifebuoy, the handprint on TSA did not exhibit much reduction in the number of colonies when the hands were visibly dirty after being unwashed for six hours (figure 3).

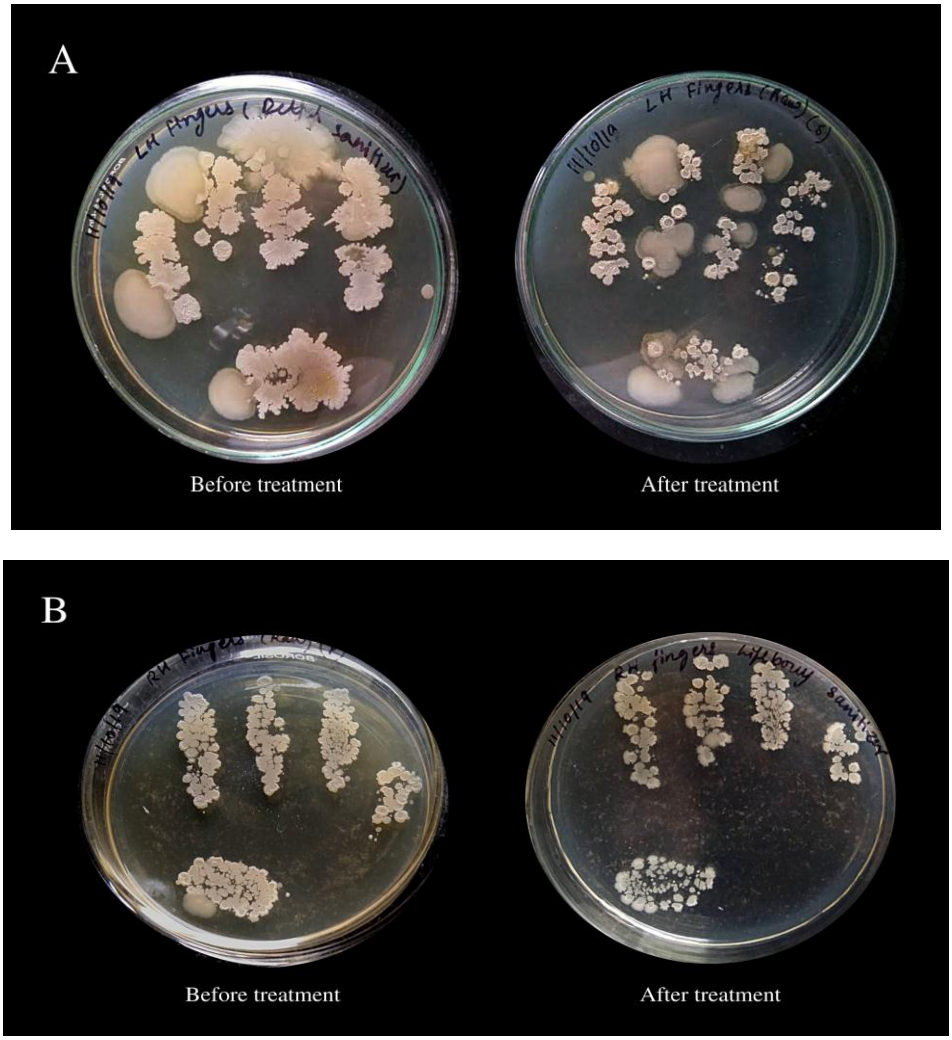

Figure 3: Effect of (A) Dettol sanitizer; and (B) Lifebuoy sanitizer treatment on hand microbiome in presence of visible dirt.

The sanitizers were found to be more effective when the hands were devoid of visible dirt (figure 4).

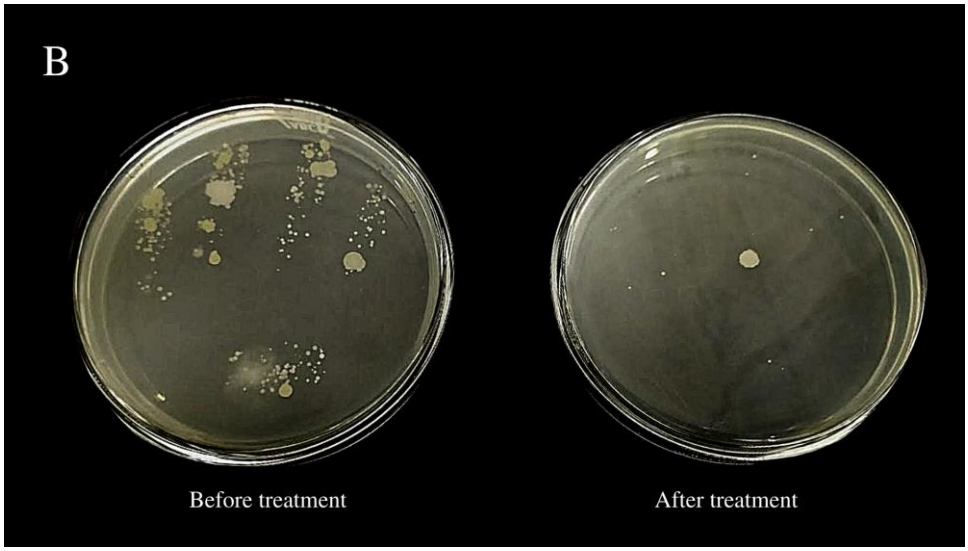

Figure 4: Effect of (A) Dettol sanitizer; and (B) Lifebuoy sanitizer treatment on hand microbiome in absence of visible dirt.

This observation is in agreement with Davis *et al* [12] who have reported that the use of an antibacterial liquid soap was more effective than alcohol-based agents for reducing coliforms on hands. The alcohol-based sanitizers were also found to be less effective as compared to liquid soaps against viruses by several authors.[13]

Bacteria were found to be the most prevalent microorganisms, and very few fungal cultures were observed in the handprints even after the extension of the incubation period to allow the slower growing fungi to proliferate. This fact has been documented in an extensive review on the hand microbiome by Edmonds-Wilson *et al*.[14] The time span before washing hands is also known to impact the microbial profile.

Figure 5: Comparison between liquid soap and sanitizer of both the brands.

An attempt was made to isolate pure cultures from the TSA plates with microbial handprints of treated as well as untreated hands and thirteen isolates were obtained (figure 6).

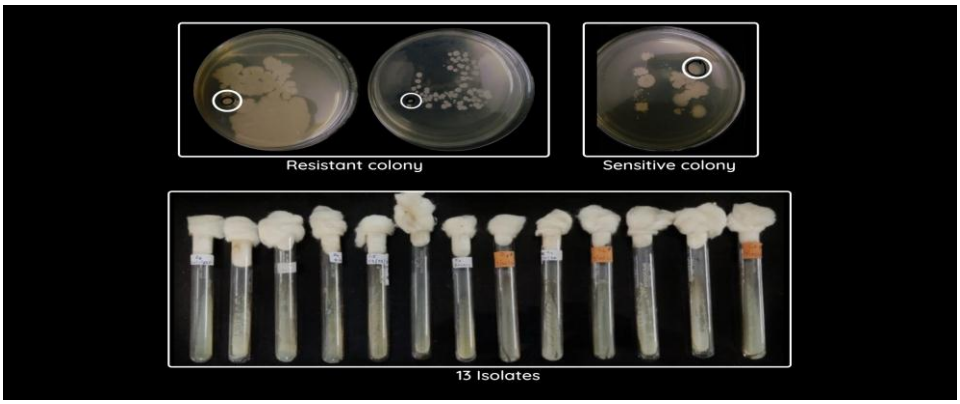

Figure 6: Pure culture isolated from the hand microbiome.

The results of their morphological and gram staining characterization have been presented in Table 1.

Table 1: Morphological characterization of bacterial isolates from hand microbiome.

Sr. no	Isolate	Gram Character	Morphology	
			Shape	Arrangement
1	C1	Positive	Cocci	In groups of 2/3/4
2	C2	Positive	Coccobacilli	In clusters, Endospore forming
3	C3	Positive	Small bacilli	Random arrangement
4	C4	Positive	Long bacilli	In chain, Endospore forming
5	C5	Positive	Cocci (small rods)	In cluster, Endospore forming
6	Z1	Positive	Cocci	Random & in cluster
7	Z2	Positive	Bacilli	In chain, Endospore forming
8	Z3	Positive	Small coccobacilli	Random arrangement
9	Z4	Negative	Cocci	Linear, Majorly diplo or in clusters
10	Z5	Negative	Bacilli	Random arrangement
11	Z6	Positive	Coccobacilli	Random & scattered
12	Z7	Positive	Cocci	Mostly diplo but also in clusters
13	Z8	Positive	Cocci	Random arrangement

These cultures included gram-positive endospore-forming *Bacillus spp.*, gram-positive *Staphylococci*, gram-positive small cocci, gram-negative short rods and gram-positive non-endospore-forming rods. Gram-negative cocci were also observed and they could be *Neisseria spp*. Apart from meningococci and gonococci, the remaining *Neisseria* species are not commonly associated with disease and are considered as commensals within the normal microbiota of the human and animal nasopharynx.[4] Although clinical case reports suggest that they can behave as opportunistic pathogens, commensals are believed to compete for niches with pathogens and thus provide protection from colonization and invasion by virulent pathogens.[15]

Two of the isolates were identified as *Pantoea spp*. and *Kocuria kristinae* and both are ubiquitous in nature. (figure 7).

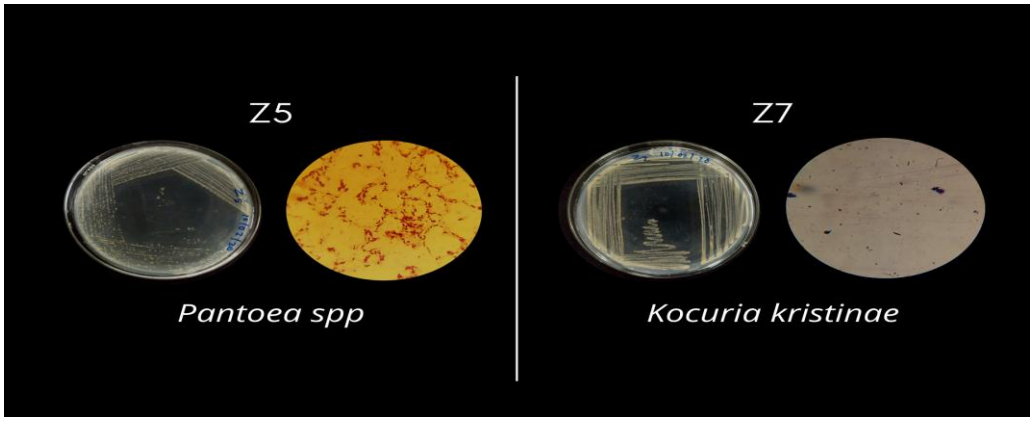

Figure 7: Pure cultures of *Pantoea spp* and *Kocuria kristinae*

They are known to be opportunistic pathogens causing nosocomial infections, mostly in immunocompromised individuals. *Kocuria kristinae*, an opportunistic catheter-associated urinary pathogen.[16] was found to be highly sensitive to Dettol liquid soap showing a wide zone of growth inhibition but moderately resistant to Dettol hand sanitizer showing only a growth retardation zone. However, *Pantoea spp.*, causing skin and open wound infections.[17] was sensitive to Dettol liquid soap but resistant to the sanitizer of this brand.

All thirteen isolates were used as test organisms to study the antimicrobial efficacy of the liquid soaps and sanitizers by the agar well diffusion method (figure 8) and the results are presented in Tables 2 and 3.

Figure 8: Antimicrobial activity of liquid soap and sanitizer (Dettol and Lifebuoy).

Table 2: Antimicrobial activity of Dettol liquid soap and hand sanitizer.

Sr. no	Isolate	Dettol Liquid Soap	Dettol Hand Sanitizer		
		Zone of Inhibition (mm)	Zone of Retardation (mm)	Zone of Inhibition (mm)	Zone of Retardation (mm)
1	C1	28 ± 1	_	_	_
		24 ± 2	_	_	12 ± 2
2	C2	24 ± 1	27 ± 1	_	_
3	C3	34 ± 2	_	_	_
		_	21 ± 2	_	21 ± 2
4	Z2	25 ± 1	25 ± 1	_	_
5	Z3	25 ± 1	_	_	_
6	Z5	25 ± 2	_	_	_
7	Z7	28 ± 2	_	_	11 ± 1

Table 3: Antimicrobial activity of Lifebuoy liquid soap and hand sanitizer.

Sr.no	Isolate	Lifebuoy Liquid Soap	Lifebuoy Hand Sanitizer		
		Zone of Inhibition (mm)	Zone of Retardation (mm)	Zone of Inhibition (mm)	Zone of Retardation (mm)
1	C4	35 ± 0	_	17 ± 3	_
2	C5	21 ± 1	25 ± 1	13 ± 2	_
3	Z1	_	16 ± 1	10 ± 1	12 ± 1
4	Z4	_	_	10 ± 1	_
		25 ± 2	_	25 ± 2	_
5	Z6	23 ± 2	_	12 ± 1	_
6	Z8	_	18 ± 1	12 ± 1	_

The cultures isolated from the handprints of hands treated with Dettol liquid soap and hand sanitizer were used to determine the antibacterial activity of the products of this brand. It was observed that all the isolates were sensitive to Dettol liquid soap with wide zones of inhibition with an average diameter of 28 mm and isolates C2, C3 and Z2 showing additional zones of growth retardation as well. None of the isolates was sensitive to the hand sanitizer of this brand. The cultures isolated from the handprints of hands treated with Lifebuoy liquid soaps and hand sanitizers were used to determine the antibacterial activity of the products of

Lifebuoy. Although three isolates were found to be resistant to Lifebuoy liquid soap, all the isolates were sensitive to the sanitizer of this brand. However, the zones of inhibition were much wider in the presence of liquid soaps of both the brands, indicating a greater antibacterial potency as compared to the hand sanitizers. This observation was in perfect agreement with the visually observed extent of reduction in the microflora in the handprints of hands cleaned with liquid soap *vis a vas* hand sanitizers. Thus Dettol hand sanitizer containing 72% ethanol was found to be less effective than Lifebuoy hand sanitizer which contains 95% ethanol. Alcohol exerts antimicrobial activity by causing protein denaturation, disruption of tissue membranes, and dissolution of several lipids.[13] Microorganisms not in direct contact with the alcohol remain unaffected and the effect is soon lost as the alcohol vaporizes. Moreover, these agents do not remove soil or organic material and hence sanitizers were not found effective when hands are visibly soiled.[18]

It is well established that the use of these hand hygiene products is known to alter the hand microbiome of individuals.[14] Although several studies have established the benefits of handwashing and/or use of alcohol-based hand rubs for controlling/reducing the spread of infections[1] other studies have also shown that frequently washed hands of healthcare workers are colonized with more pathogenic bacteria than those who wash less frequently [14] Thus, there are many variable factors, both intrinsic and extrinsic, that influence the microbial profile of hands[14] It is possible that a routine examination of the hand microbiome can be a valuable indication of our health and/or environmental status enabling us to undertake appropriate preventive health care measures.

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