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Comparative assessment of antimicrobial efficacy of different hand sanitizers: An *in vitro* study

Vardhaman Mulchand Jain[1], Gundabaktha Nagappa Karibasappa[1], Arun Suresh Dodamani[1], Vishwakarma K. Prashanth[1], Gaurao Vasant Mali[1]

[1]Department of Public Health Dentistry, ACPM Dental College, Dhule, Maharashtra, India

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Address for correspondence:
Dr. Vardhaman Mulchand Jain,
D-1, Dongare Maharaj Nagar, Opposite Cotton Market, Parola Road,
Dhule - 424 001,
Maharashtra, India.
E-mail: dr.vardhaman.jain@gmail.com

ABSTRACT

Background: To evaluate the antimicrobial efficacy of four different hand sanitizers against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis* as well as to assess and compare the antimicrobial effectiveness among four different hand sanitizers.

Materials and Methods: The present study is an *in vitro* study to evaluate antimicrobial efficacy of Dettol, Lifebuoy, PureHands, and Sterillium hand sanitizers against clinical isolates of the aforementioned test organisms. The well variant of agar disk diffusion test using Mueller-Hinton agar was used for evaluating the antimicrobial efficacy of hand sanitizers. McFarland 0.5 turbidity standard was taken as reference to adjust the turbidity of bacterial suspensions. Fifty microliters of the hand sanitizer was introduced into each of the 4 wells while the 5th well incorporated with sterile water served as a control. This was done for all the test organisms and plates were incubated in an incubator for 24 h at 37°C. After incubation, antimicrobial effectiveness was determined using digital caliper (mm) by measuring the zone of inhibition.

Results: The mean diameters of zones of inhibition (in mm) observed in Group A (Sterillium), Group B (PureHands), Group C (Lifebuoy), and Group D (Dettol) were 22 ± 6, 7.5 ± 0.5, 9.5 ± 1.5, and 8 ± 1, respectively. Maximum inhibition was found with Group A against all the tested organisms. Data were statistically analyzed using analysis of variance, followed by *post hoc* test for group-wise comparisons. The difference in the values of different sanitizers was statistically significant at $P < 0.001$.

Conclusion: Sterillium was the most effective hand sanitizer to maintain the hand hygiene.

Key Words: Anti infective agent, hand sanitizers, hygiene, organisms, test

INTRODUCTION

Hospital and community-acquired infections are escalating and pose a serious public health problem worldwide.[1] Hands are considered to be the primary route for transmitting microbes and infections to the individuals.[2] Personal as well as hand hygiene is important to prevent many communicable diseases. The word "hygiene" is derived from the ancient Greek goddess "Hygeia" that means "goddess of healing." The importance of hygiene is universally recognized and evidence-based. It is well known

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that hand hygiene is crucial to prevent and minimize healthcare-associated infections.[3] The Centers for Disease Control and Prevention, the World Health Organization, and many other health experts promote hand hygiene as the single most important measure in the prevention of hospital-acquired infections. Several studies have shown the importance of proper hand hygiene in reducing the incidence of nosocomial infections.[4-8] It is estimated that at any one time, more than 1.4 million people worldwide are suffering from infections acquired in hospitals. These nosocomial infections are also, in most cases, the result of poor hand hygiene.[9]

At present, washing hands with appropriate soap followed by applying hand antiseptics are two important hand hygiene method in clinical practice. Hand sanitizers significantly increase the chance of maintaining the hands clean and aseptic.

Traditionally, microbes habitation on hands is divided into resident and transient floras. Involved resident floras are commonly *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus faecalis* that colonize the deeper skin layers and are resistant to mechanical removal. The transient floras consists of *S. aureus, Escherichia coli*, and *Pseudomonas aeruginosa* that colonize the superficial layers of skin in a short period of time.[10] Therefore, we selected these organisms to determine their susceptibility to different hand sanitizers tested in this study.

Scientific studies have shown that after hand washing, as many as 80% of individuals retain some pathogenic bacteria on their hands.[11] Hand washing removes body's own fatty acids from the skin, which may result in cracked skin that provides an entry portal for pathogens.[12,13] To overcome the limitations of plain hand washing, hand sanitizers were introduced claiming to be effective against those pathogenic micro-organisms as well as to improve skin condition due to the addition of emollients in it.[14]

Hand sanitizers were also effective in reducing gastrointestinal illnesses in households,[15] respiratory tract infections, and skin infections,[16] in curbing absentee rates in elementary schools,[17] and in reducing illnesses in university dormitories.[18] Furthermore, to reduce infections in healthcare settings, alcohol-based hand sanitizers are recommended as a component of hand hygiene.[19]

Many hand sanitizers are available in the market with varying degree of effectiveness that are registered in the National Agency for Food and Drugs Administration and Control. Moreover, in outreach programs, screening procedures in day-to-day practice, water scarcity areas, and bed-side and chair-side clinical examination, hand sanitizers could be an alternative to achieve asepsis. However, clinicians and common man face the dilemma while choosing the best among the lot.

Some products marketed to the public as antimicrobial hand sanitizers are not effective in reducing bacterial counts on hands. In fact, despite a label claim of reducing "germs and harmful bacteria" by 99.9%, some studies have observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing.[20] Hence, there still exists a need for verification of these claims by the regulatory authorities for the enforcement of good-quality measures. To overcome this ambiguity, the present study was carried out to assess the antimicrobial effectiveness of four different hand sanitizers against the test organisms.

MATERIALS AND METHODS

The present study is an *in vitro* study conducted at the Department of Microbiology, Government Medical College, Dhule, Maharashtra, India. Ethical clearance for the study was obtained from the Institutional Ethical Review Committee. Four different brands of hand sanitizers were selected out of many available in the market based on their popularity and maximum usage in Dhule City. Selected hand sanitizers to test their antimicrobial efficacy were Sterillium (Bode Chemie, Hartmann Group, Germany), PureHands (Himalaya Drugs Company, India), Dettol (Reckitt Benckiser, UK), and Lifebuoy (Hindustan Unilever Pvt. Ltd., India) [Figure 1]. Recently manufactured and packed sanitizers have been purchased based on their popularity from the local retail outlet. The study was conducted over a period of 10 days. The composition of various hand sanitizers is shown in Table 1.

The culture media used in the present study were Mueller-Hinton agar for agar diffusion method while nutrient broth and nutrient agar medium for bacterial isolate preservation. The clinical isolates of *S. aureus, S. epidermidis, E. faecalis, E. coli,* and *P. aeruginosa* were obtained from the culture plates of the respective

micro-organisms preserved on the nutrient agar slants and were stored at 4°C in the Department of Microbiology, Government Medical College, Dhule, Maharashtra, India.

Preparation of McFarland standards and standardization of test organisms

Bacterial suspensions varied in the turbidity and could cause potential bias in the result, to overcome this and standardize the microbial testing; McFarland standards was taken as a reference to adjust the turbidity of bacterial suspensions. The McFarland 0.5 turbidity standard was prepared by adding 0.5 ml of 1.175% w/v barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution to 99.5 ml of 15 w/v sulfuric acid (H_2SO_4).[21] This was mixed well and then aliquoted into test tubes identical to the ones used in preparing inoculum suspensions of the test organisms. The accuracy of the density of the standard was verified using a spectrophotometer. The absorbance of the 0.5 McFarland standards at wavelength of 625 nm was 0.08–0.10. The tubes were stored in a well-sealed container in the dark at room temperature until when required.[22]

A sterile loop was used to pick a loopful of inoculum from a pure culture of the test organism. This was then transferred and suspended into a tube containing sterile normal saline (NaCl 8.5 g, distilled water 1 L). The tube was compared with the turbidity standard, and the density of the organism was adjusted by adding more bacteria or sterile saline until standardization was attained.[23]

Agar diffusion test (well variant) to determine susceptibility of test organisms to hand sanitizers

Disk agar diffusion technique described by Bauer *et al*. and Valgas *et al*. was used for the evaluation of antimicrobial efficacy of hand sanitizers.[24,25] Sterile Mueller-Hinton agar plates were inoculated with standardized test organisms [Figure 2]. A sterile cotton swab was dipped into a test tube containing the inoculum and was rotated properly to allow maximum contact. Excess inoculum was removed by pressing and rotating the swab firmly against the inside wall of the tube above the liquid level. The swab was then streaked over the surface of the medium three times while rotating the plate at 60° angle after each application. The swab was also passed around the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed.

With the aid of a sterile 6 mm cork borer, 4 equally spaced holes were bored in the agar plate with a fifth hole in the center of the plate. The agar plugs were discarded using a sterile needle [Figure 3]. Fifty microliters of the hand sanitizer was then introduced into each of the 4 wells while the central well was filled with an equal volume of sterile water to serve as control [Figure 4]. This was done for all the test organisms and hand sanitizers. The plates were incubated for 24 h at 37°C in an upright position. They were then examined for zones of inhibition which indicated the degree of susceptibility or resistance of the test organism to the antibacterial agent [Figure 5]. The point of abrupt diminution growth which

Table 1: Hand sanitizers used in the study and their Ingredients

Hand Sanitizer	Ingredients
Sterillium	Propan-2-ol, Propan-1-ol, Mecetronium ethyl sulfate, Glycerol, Tetradecan-1-ol, fragrances, Patent blue V, Purified water
PureHands	Hrivera, Coriander, Lime, Ushira, Neem
Dettol	Denatured Alcohol- 69.4% w/w, Water PEG/PPG-17/6 copolymer, Propylene glycol, Acrylate/C10-30 alkyl acrylate, cross polymer, Tetrahydroxpropyl ethylenediamine, Perfume.
Lifebuoy	Ethyl alcohol 95% v/v IP 55% w/w, Isopropyl alcohol 10% IP w/w, Tocopheryl acetate IP 0.05% w/w, Perfumed gel base: qs to 100% w/w

Figure 1: Different hand sanitizers used in the study.

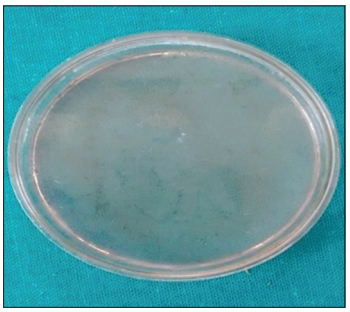


Figure 2: Sterilized Mueller-Hinton agar plates inoculated with standardized test organisms.

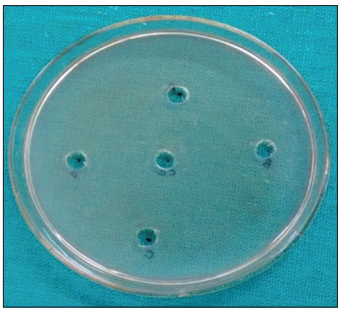


Figure 3: Four equally spaced holes in the agar plate with the 5th hole in the center.

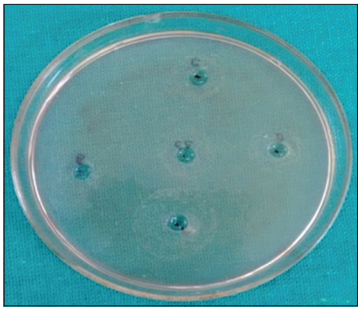
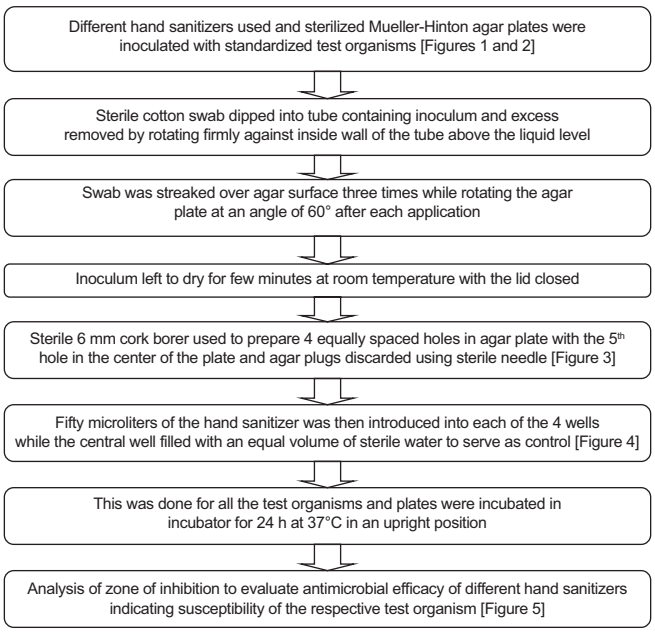


Figure 4: Fifty microliters of the hand sanitizer used was introduced into each of the 4 wells and central well filled with an equal volume of sterile water.

corresponds to the complete growth inhibition was taken as the zone edge.[24] The test was carried out five times, and the average of all readings was taken as the zone of inhibition in each case. Inhibition zones were measured with the aid of a digital caliper (mm).

Schematic representation of agar diffusion test (well variant) to determine susceptibility of test organisms to hand sanitizers



Statistical analysis

Data were statistically analyzed with analysis of variance followed by *post hoc* test for group-wise comparisons. All statistical procedures were performed using Statistical Package for Social Sciences (SPSS) version 21.0 software (IBM, Armonk, NY, USA). The data exhibited a normal and homogeneous distribution; thus, zone of inhibition (in mm) was analyzed using the mean of all the readings obtained, and the difference in the values of different hand sanitizers was statistically significant at $P < 0.001$.

RESULTS

Hand sanitizers were effective against all the test organisms. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the particular test organism. Maximum inhibition (in mm) was seen in Group A (Sterillium), i.e., 27 ± 1.414 and minimum in Group B (pure hands), i.e., 3.5 ± 4.95 against *S. aureus*. The difference in the values of the different sanitizers was statistically significant [$P < 0.001$, Table 2].

Group A showed the highest antimicrobial effectiveness followed by Group C, Group D, and Group B respectively, against all the different test organisms used in the study. Group A could inhibit all the bacteria either Gram-positive or Gram-negative effectively, whereas other sanitizers showed a limited action [Graph 1].

When subjected to *post hoc* test analysis, statistically high significant difference was observed against all the bacterial isolates when Group A was compared with any other group. However, there was no statistically significant difference when Group B, Group C, and Group D were compared with each other against all test organisms. The mean difference is statistically significant at the 0.05 level [Tables 3-5].

DISCUSSION

Infection with environmental microbes is increasing alarmingly. Normal human skin always harbors bacteria (10^2 and 10^6 CFU/cm^2). The transfer of bacteria from the hands to food, objects, or people plays an important role in the spread of many communicable diseases.[26] The critical density of micro-organisms on the hands needed for the spread of pathogens remains unknown, and it may depend on the type and duration of contact, the type of micro-organism, the patient's resident flora, and their colonization resistance.[2]

To overcome the negative impact of microbial contamination in health-care settings, hand sanitizers are recommended as an adjunct to plain hand washing.[19] Most commonly and easily available hand sanitizers in the Indian market were selected for the study. Among the four hand sanitizers used in this study, Sterillium, Dettol, and Lifebuoy were alcohol-based and PureHands was herbal, i.e., non-alcohol-based hand sanitizer.

Alcohol was the main active ingredient in alcohol-based hand sanitizer which exerts antimicrobial activity by causing protein denaturation, disruption of tissue membranes, and dissolution of several lipids.[27] Alcohol has increasing effectiveness from 60% to 90% with 1-propanol being most effective followed by 2-propanol and finally by ethanol, whereas Coriander, Lime, and Neem were the active ingredients responsible for antimicrobial activity in PureHands herbal hand sanitizer.

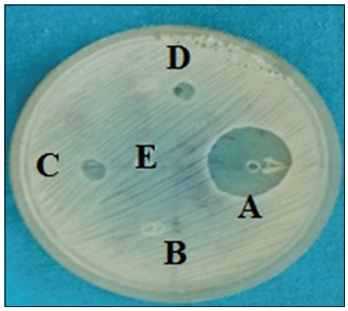
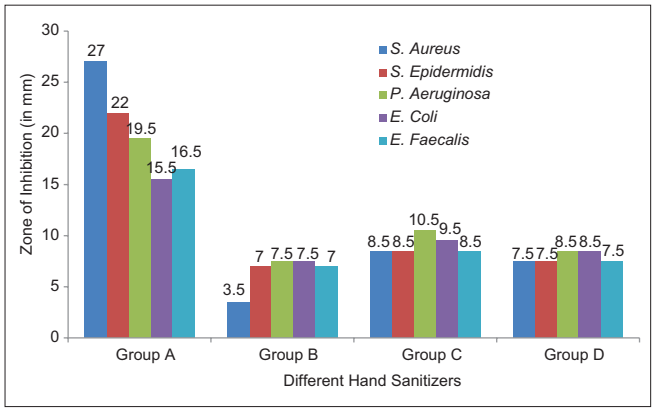


Figure 5: Analysis of zone of inhibition to evaluate antimicrobial efficacy of different hand sanitizers. Labelling on the side of respective zone of inhibition as A, B, C, D etc.



Graph 1: Zone of inhibition (in mm) measured at the end of 24 h of different hand sanitizers against all the test organisms.

Many studies have been conducted to assess the antimicrobial effectiveness of hand sanitizers alone, but very few literature are available to assess the difference between various disinfectants and hand sanitizers. Disinfectants are chemical agents with an immediate and sustained activity which destroys micro-organisms to such a level mandated for hygienic and surgical indications. Sanitizers, on the other hand, are agents with an immediate activity that reduce the number of micro-organisms to a safe level to meet the public health requirements. Disinfectant uses a better form of alcohol (propanol) to achieve more bacterial reduction as compared to sanitizers (ethanol). Both can achieve bacterial reduction on contact (in 15–30 s).

Traditionally, agar diffusion method and agar dilution method are commonly employed for assessment of the antimicrobial activity of any material. In the present study, the Kirby–Bauer method (Agar disk diffusion method) was chosen instead of the agar dilution method. The disadvantage of the agar dilution method is being technique-sensitive and can alter some properties of the sanitizer being tested, and few sanitizers could not be homogeneously dissolved. The advantages of agar disk diffusion method are chemical properties of the sanitizer remains unchanged, an easy and less technique sensitive method.[28,29] It allows direct comparison of all groups of sanitizers, indicating which group has the maximum potential to eliminate that particular test organism.

Sterillium was the most effective disinfectant among all the hand sanitizers against all the bacteria used

Table 2: Zone of inhibition (in mm) measured at the end of 24 h of different hand sanitizers against particular test organism

Test Organism	(Mean±SD)				ANOVA	*P*
	Group A	Group B	Group C	Group D		
S. aureus	27±1.414	3.5±4.95	8.5±0.707	7.5±0.707	F=31.921	0.003
S. epidermidis	22±1.414	7±0.0	8.5±0.707	7.5±0.707	F=138.0	0.001
P. aeruginosa	19.5±0.707	7.5±0.707	10.5±0.707	8.5±0.707	F=120.0	0.001
E. coli	15.5±0.707	7.5±0.707	9.5±0.707	8.5±0.707	F=51.667	0.001
E. faecalis	16.5±0.707	7±0.0	8.5±0.707	7.5±0.707	F=106.11	0.001

Group A-Sterillium; Group B: PureHands; Group C: Dettol; Group D: Lifebuoy ** *P*<0.001, HS

Table 3: Comparison between different groups of Hand sanitizers in relation to the *S. aureus* and *S. epidermidis* by *Post hoc* test

Bacteria	Group	Other groups	Mean Difference	Significance (*P*)
S. aureus	Group A	Group B	23.500*	0.005
		Group C	18.500*	0.013
		Group D	19.500*	0.010
	Group B	Group A	−23.500*	0.005
		Group C	−5.000	0.775
		Group D	−4.000	1.000
	Group C	Group A	−18.500*	0.013
		Group B	5.000	0.775
		Group D	1.000	1.000
	Group D	Group A	−19.500*	0.010
		Group B	4.000	1.000
		Group C	−1.000	1.000
S. epidermidis	Group A	Group B	15.000*	0.000
		Group C	13.500*	0.001
		Group D	14.500*	0.000
	Group B	Group A	−15.000*	0.000
		Group C	−1.500	0.950
		Group D	−0.500	1.000
	Group C	Group A	−13.500*	0.001
		Group B	1.500	0.950
		Group D	1.000	1.000
	Group D	Group A	−14.500*	0.000
		Group B	0.500	1.000
		Group C	−1.000	1.000

*.The mean difference is significant at the 0.05 level

Table 4: Comparison between different groups of Hand sanitizers in relation to the *P. aeruginosa* and *E. coli* by *Post hoc* test

Bacteria	Group	Other groups	Mean Difference	Significance (*P*)
P. aeruginosa	Group A	Group B	12.000*	0.000
		Group C	9.000*	0.001
		Group D	11.000*	0.001
	Group B	Group A	−12.000*	0.000
		Group C	−3.000	0.079
		Group D	−1.000	1.000
	Group C	Group A	−9.000*	0.001
		Group B	3.000	0.079
		Group D	2.000	0.285
	Group D	Group A	−11.000*	0.001
		Group B	1.000	1.000
		Group C	−2.000	0.285
E. coli	Group A	Group B	8.000*	0.002
		Group C	6.000*	0.006
		Group D	7.000*	0.004
	Group B	Group A	−8.000*	0.002
		Group C	−2.000	0.285
		Group D	−1.000	1.000
	Group C	Group A	−6.000*	0.006
		Group B	2.000	0.285
		Group D	1.000	1.000
	Group D	Group A	−7.000*	0.004
		Group B	1.000	1.000
		Group C	−1.000	1.000

*.The mean difference is significant at the 0.05 level

in the present study. This might be attributed to the presence of alcohol (propanol 75%) in liquid form that soaks and penetrates the skin creases and nail folds unlike the gel form sanitizers that glide over and coat the skin. It also contains Mecetronium ethyl sulfate to contribute for residual effect for approximately 3–5 h which is lacking among other hand sanitizers.[30]

The results of the present study are similar to the findings of Reena Rajkumari, where sterillium was more effective against *Candida albicans, E. coli*, and *Klebsiella pneumoniae*.[31] A study conducted by Oke *et al*. revealed that Dettol hand sanitizer was effective only against *P. aeruginosa* whereas it was not effective against *S. aureus* and *E. coli*.[32] Lifebuoy hand sanitizer also showed antimicrobial activity against the tested organisms; however, the exact and valid comparison could not be done with other studies due to lack of scientific literature.

Similar to the present study, several studies reported significantly better antimicrobial efficacy of hand sanitizers, as well as a decrease in nosocomial infection rates as compared to hand washing.[9,33] Furthermore, a study conducted among school children showed significantly high efficacy of hand sanitizers in reducing microflora on hand.[17,34]

A study conducted by Mondal and Kolhapure showed that PureHands, the herbal hand sanitizer, was effective against *E. coli, Proteus mirabilis, Shigella sonnei, S. aureus*, and *S. epidermidis*.[2] The present study also showed antimicrobial efficacy of PureHands against tested organisms; however, it was the least effective among all the hand sanitizers which may be probably due to low antimicrobial potency of Coriander, Lime, and Neem present in it. Further studies are required to find the exact cause of least effectiveness of PureHands herbal hand sanitizer against the tested organisms.

CONCLUSION

Sterillium possessed maximum antimicrobial effect against all the Gram-positive as well as Gram-negative bacteria used in the study, followed by Dettol, Lifebuoy, and PureHands respectively. Despite the claims of efficacy and 99.9% bacterial reduction by hand sanitizer manufacturers, there still exists a need for verification of

Table 5: Comparison between different groups of Hand sanitizers in relation to *E. faecalis* by *Post hoc* test

Bacteria	Group	Other groups	Mean Difference	Significance (*P*)
E. faecalis	Group A	Group B	9.500*	0.001
		Group C	8.000*	0.001
		Group D	9.000*	0.001
	Group B	Group A	−9.500*	0.001
		Group C	−1.500	0.423
		Group D	−0.500	1.000
	Group C	Group A	−8.000*	0.001
		Group B	1.500	0.423
		Group D	1.000	1.000
	Group D	Group A	−9.000*	0.001
		Group B	0.500	1.000
		Group C	−1.000	1.000

*.The mean difference is significant at the 0.05 level

these claims by regulatory bodies and higher authorities for the enforcement of good-quality measures.

Dental public health significance

Hand sanitizers are more effective than plain soap and water alone in preventing transmission of bacteria from the hands of individuals. They play a significant role and could be an effective alternative to hand washing to achieve asepsis for all the health-care professionals in outreach program, water scarcity areas, and in routine clinical practice. Hence, stressing proper hand hygiene is an important first-line defense against the spread of multiple infectious diseases.

Recommendation

The present study has its own limitations – as only the antimicrobial efficacy of different hand sanitizers was assessed. Further studies are required to assess the exact quantity and duration of application of hand sanitizer or disinfectant.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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