

Comparative assessment of antimicrobial efficacy of different hand sanitizers an in-vitro study in Bareilly

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Abstract— Background: Hand washing is recognized as a key element to prevent the spread of infectious disease. Hand sanitation is the act of cleansing hands with sanitizers to ensure proper hand hygiene.

Aim of the study: To evaluate the antimicrobial efficacy of fifteen different brands of hand sanitizers.

Methodology: The susceptibility test was performed by agar well diffusion method on *Klebsiella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Enterococcus faecalis* and *Shigella* sp.

Results: Finest results were shown by Jungle magic, Instant germs, Nomarks hand sanitizer and Sterillium. Jungle magic shows the best results among all the sanitizers, that inhibited growth of the following species; *Klebsiella* sp. 10.6mm, *E. coli* 10.4mm, *Staphylococcus aureus* 19mm, *Proteus* sp. and *salmonella* sp. shows zone of inhibition by 14mm, *Enterococcus* 17mm and *Shigella* sp. 18mm.

Conclusion: It is universally recognized that hand hygiene is the best and most cost effective way to prevent infections and illness. The efficiency of sanitizer depends on the concentration and grade of its active ingredient. Despite claims that hand wash manufacturers are effective and reduce bacteria by 99.9%, these claims still require legal and parental review to follow good quality measures.

Index Terms: Hand sanitizer, susceptibility, hand hygiene.

INTRODUCTION

Hands serve as the primary source of germ transmission. Hand is considered the most contagious part of the body. Infections that were acquired while receiving healthcare have increased thousands of death rates worldwide.¹The word "hygiene" comes from the ancient Greek goddess "Hygea" which means "goddess of healing". The importance of

hygiene is widely recognized and is based on evidence.²Several studies have shown that hand hygiene is important in reducing the incidence of nosocomial infections.^{3,4,5,6,7}

The WHO has come up as a global effort with the launch of "SAVE LIVES: Clean Your Hands" campaign in 2009 for infection control as well as to assure patient safety and to reduce healthcare associated infections. The World Health Organization (WHO) has recommended this as the preferred hand washing method, especially in hospitals and clinics.^{3,8}Health-care staff or others should be in a position to properly practice hand hygiene.⁹Hand hygiene, according to the Centers for Disease Control (CDC), includes hand washing with soap and water, antiseptic hand washes, antiseptic hand rubs including alcohol-based hand sanitizers (ABHS), foams or gels, and surgical hand antiseptics.^{10,11}

Hand washing is recognized as a key factor in preventing the spread of infectious diseases. Hand hygiene cleans your hands with hand sanitizers to ensure proper hand hygiene. Hand sanitizer is another way to clean your hands. Because of their accessibility, lack of water and time, and proven effectiveness in lowering microbial load, hand sanitizers are becoming more popular as a disinfectant. They come in various forms, mainly in gel or liquid formulations. All disinfectants have active ingredients such as ethanol or isopropanol. The antibacterial properties of disinfectants are based on the active ingredient. In addition, some inactive ingredients such as polyacrylic acid, glycerol, propylene glycol or plant extracts are added to the hand sanitizer.¹²Using alcohol-based hand sanitizers can reduce the risk of infection spreading such as covid in across society. Alcohol is a wide range of

disinfectants that kill bacteria and fungi. The mechanism of action of alcohol against bacteria and viruses is explained by Golin AP, et al.¹³ There is great demand in medical institutions, schools, food processing companies, etc.^{14,15,16}

The antimicrobial property of different hand sanitizers varies, and there is less literature available for the same. Therefore this study was conducted to evaluate the antimicrobial activity of various brands of hand sanitizers available in the local market and to determine which hand sanitizers are proportionally effective.

AIMS AND OBJECTIVES

AIM: To evaluate the antimicrobial efficacy of 15 different hand sanitizers against *Klebsiella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Enterococcus faecalis* and *Shigella* sp.

Objectives:

1. To isolate and identify the different bacterial species using morphological and biochemical approaches.
2. To compare the antibacterial activity of 15 different alcohols- based hand sanitizers against different bacterial species.

MATERIALS AND METHODS

Sample Collection

In Bareilly, India, fifteen hand sanitizers of various brands were purchased from local supermarkets based on their popularity and maximum usage. All the samples were within their expiry date from date of manufacture. Before antimicrobial activity analysis, the batch amount, manufacture date, and product composition were all registered.

The present study was an in-vitro study performed at the Department of Microbiology in Bareilly, India. Ethical clearance for the study was obtained from the Institutional Ethical Review Committee. The 15 selected hand sanitizers were Savlon (ITC Ltd), PureHands (Himalaya Drugs Company, India), Dettol (Reckitt Benckiser, UK), Pure and safe hand sanitizer (Joy), Clean hands spray (Herbal tree), Nomarks hand sanitizer with neem and aloe-vera (Bajaj), Instant germs (CAD Johnson), Tea tree aloe-vera gel (NutriGlow naturals), Safe kind

(Mankind), Surgical spirit, Jungle magic (Pritam International Pvt Ltd.), Lifebuoy (Hindustan Unilever Pvt. Ltd., India), Liquid shield (Abbott), Sterillium (MIL Laboratories Pvt.Ltd.) and Tap water as control (Figure 1). The study was conducted over a period of 10 days. The composition of various hand sanitizers is shown in Table 1.



Figure 1: Different hand sanitizers used in the study

Table 1: Shows the composition of various hand sanitizers

S. No	Sample name	Active ingredient
1.	Savlon	Alcohol (denatured) eq. to Absolute alcohol 72.34%
2.	PureHands	Rectified spirit (Alcohol 60% w/w)
3.	Dettol	Alcohol IP (Denatured eq. to absolute alcohol 72.34% v/v)
4.	Joy (Pure and safe hand sanitizer)	Herbal ingredient and 70% alcohol base
5.	Clean hands spray	Isopropyl Alcohol 70%
6.	Nomarks hand sanitizer with neem and aloe-vera	Isopropyl Alcohol 70%, with neem and aloe-vera
7.	Instant germs	Ethyl alcohol 70% V/V, Isopropyl Alcohol IP 10% v/v
8.	Tea tree aloe-vera gel	Isopropyl Alcohol 70%
9.	Safe kind	Alcohol (denatured) 70% v/v, with neem and aloe vera
10.	Surgical spirit	Methyl salicylate 0.5% v/v (ethyl alcohol 95%)
11.	Jungle magic	Ethanol(95% V/V), 62% v/v, isopropyl alcohol (denatured) 3.3% v/v
12.	Lifebuoy	Alcohol 75% v/v
13.	Liquid shield	Alcohol (denatured) 95%, Eq. to Absolute alcohol 72.34%
14.	Sterillium	Ethyl alcohol (denatured) 70% v/v
15.	Tap water	-

The culture medium used in this study was Mueller-Hinton agar (Figure 2) for the agar diffusion method, and the agar culture medium for the protection of isolated bacteria. The clinical isolates of *Klebsiella* sp. (A), *Escherichia coli* (B), *Pseudomonas aeruginosa* (C), *Staphylococcus aureus* (D), *Proteus* sp. (E),

Salmonella typhii (F), *Enterococcus faecalis* (G) and *Shigella* sp. (H) were obtained from culture plates of suitable microorganisms stored on nutrient agar filters and stored at 4 °C in the Department of Microbiology in Bareilly, India.

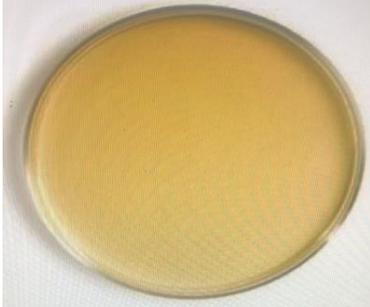


Figure 2: Sterilized Mueller-Hinton agar plates inoculated with standardized test organisms
 Agar diffusion test (well variant) to determine susceptibility of test organisms to hand sanitizers
 The antimicrobial activity of different sanitizers was determined by agar-well diffusion method. Nutrient agar plates were inoculated with 0.5 mL of 24-hour broth culture. Different wells at equidistance were punched in each agar plate using sterile Durham’s tube (figure 3). These wells were then filled with 50 mL different sanitizer dilutions and pyrogen free distilled water (figure 4). The Petri plates were then incubated at 37°C for 24 hours. After the prescribed time of incubation, zones of inhibitions were observed around the wells and the diameters of these inhibition zones were measured (in mm) using graduated scale by Kirby–Bauer method.¹⁷ Only those Petri plates were selected in which uniformly circular zones of inhibition was observed with a confluent lawn of growth (figure 5). Petri plates with tear in agar during punching of holes or those with individual colonies of microorganisms were discarded.

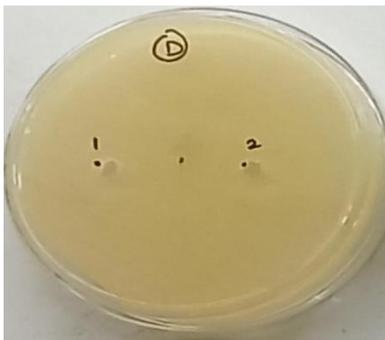


Figure 3: Equally spaced holes in the agar plate

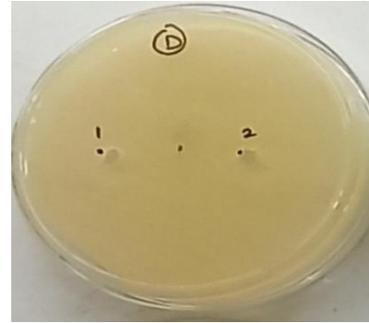


Figure 4: Fifty microliters of the hand sanitizer used was incorporated into every wells

After the evaluation of zones of inhibition, the culture plates were collected in yellow-coded bags and sent to the biomedical waste management company for further disposal, wherein the waste was incinerated and the ashes were buried. The results obtained were tabulated and subjected to statistical analysis.

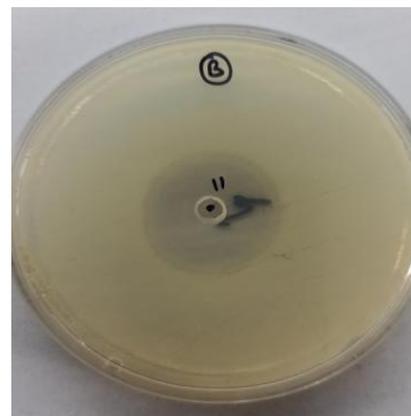
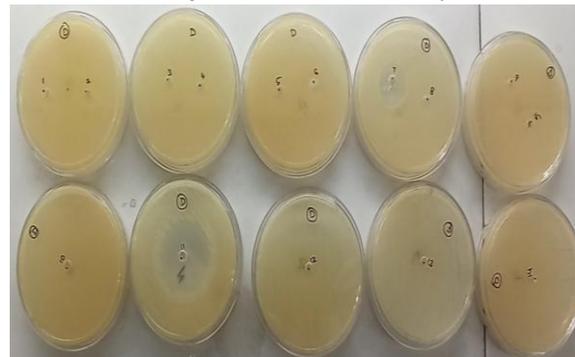
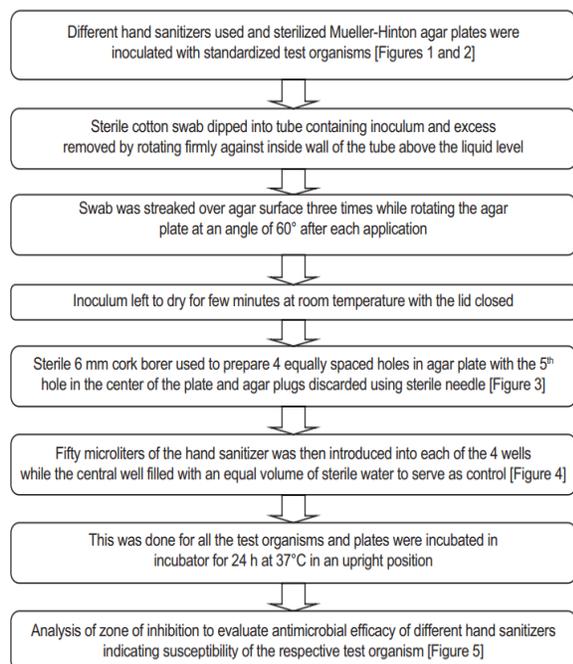


Figure 5: Analysis of zone of inhibition to evaluate antimicrobial efficacy of different hand sanitizers
 Schematic representation of agar diffusion test (well variant) to determine susceptibility of test organisms to hand sanitizers



The antimicrobial susceptibility was indicated by the zone diameter of inhibition in millimeter. The results were compared with standard streptomycin zone of inhibition.

Table 2: Standard Zone of Inhibition of Streptomycin

Zone diameter(mm)	Interpretation
≥ 15	Susceptible
11-14	Intermediate
≤ 10	Resistant

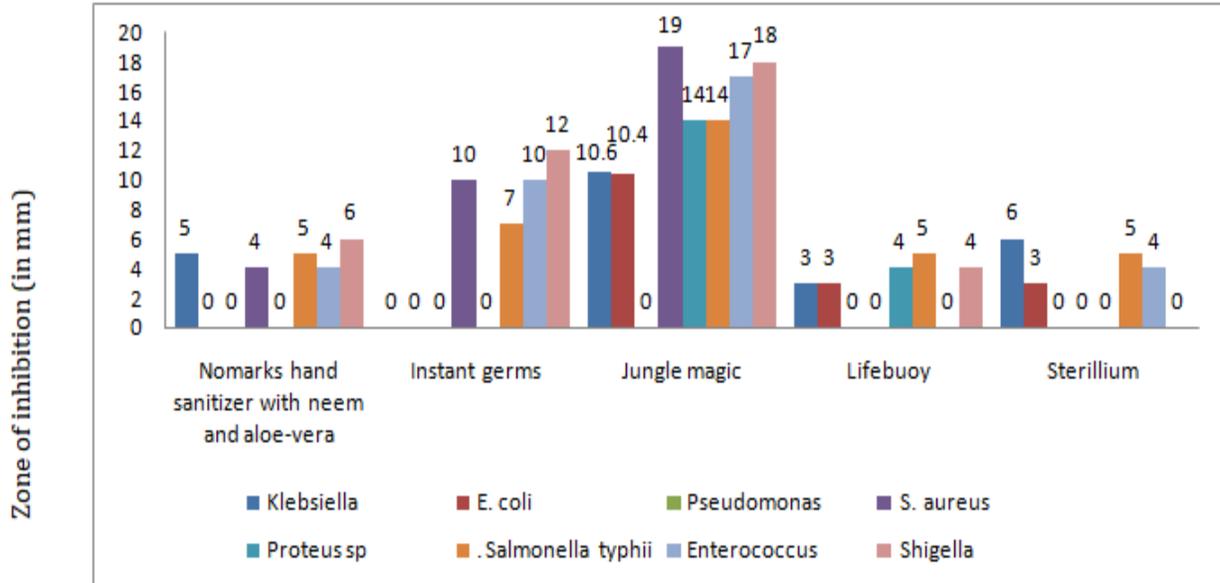
RESULTS

In our study we tested efficacy of different hand sanitizer on eight microbial cultures. Escherichia coli, Klebsiella, Proteus vulgaris, and Pseudomonas aeruginosa are Gram negative bacteria that cause gastrointestinal and nosocomial infections. The low activity of hand sanitizers against Pseudomonas aeruginosa is due to the hardy nature of Pseudomonas, it has been reported to survive and have high resistance to antibiotics. Shigella is also gram negative bacteria responsible for GIT infections (Shigella dysenteries) and Salmonella typhii cause typhoid fever. Among Gram positive bacteria, Staphylococcus aureus can cause gastrointestinal, skin, systemic and nosocomial infections. Enterococcus faecalis can cause gastrointestinal tract infections in humans.

Table 3 and Graph 1 show the antimicrobial susceptibility pattern of hand sanitizers for each organism.

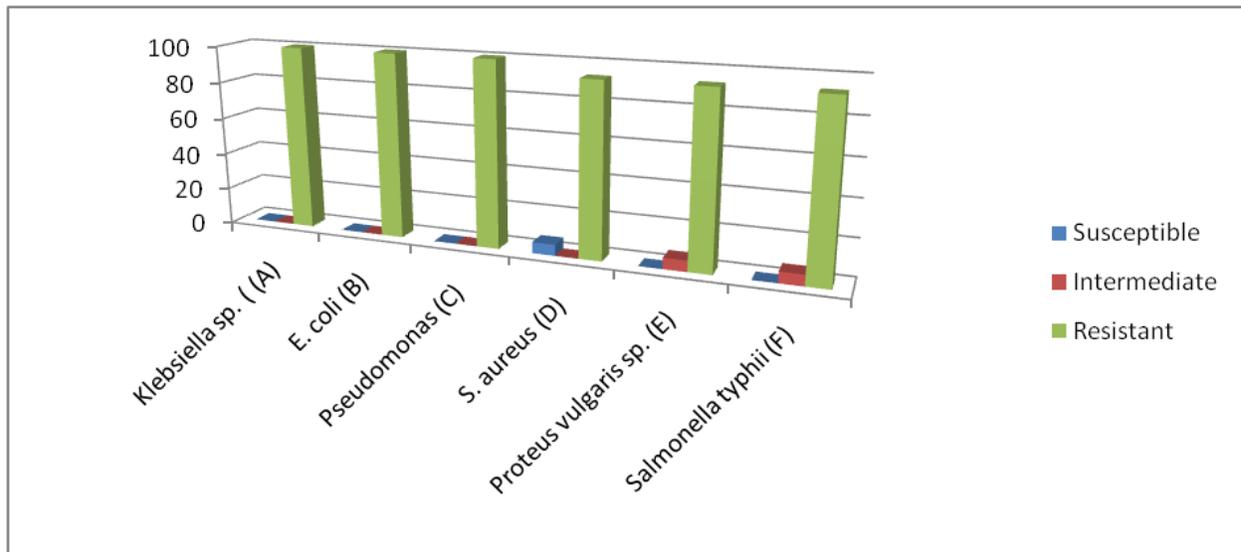
Table 3: shows the zone of inhibition in mm

S. NO	Sample name	Zone diameter of inhibition (mm)							
		Klebsiella sp. (A)	E. coli (B)	Pseudomonas (C)	S. aureus (D)	Proteus vulgaris sp. (E)	Salmonella typhii (F)	Enterococcus (G)	Shigella (H)
1.	Savlon	-	-	-	-	-	-	3mm	-
2.	PureHands	-	4mm	-	-	4mm	-	-	-
3.	Dettol	-	-	-	-	-	-	-	-
4.	Joy hand sanitizer	-	-	-	-	-	-	-	-
5.	Clean hands spray	-	-	-	-	-	-	-	-
6.	Nomarks hand sanitizer	5mm	-	-	4mm	-	5mm	4mm	6mm
7.	Instant germs	-	-	-	10 mm	-	7mm	10mm	12mm
8.	Tea tree aloe-vera gel	-	-	-	-	-	-	-	-
9.	Safe kind	3mm	-	-	-	-	-	-	-
10.	Surgical spirit	-	-	-	-	4mm	-	7mm	-
11.	Jungle magic	10.6mm	10.4mm	-	19mm	14mm	14mm	17mm	18mm
12.	Lifebuoy	3mm	3mm	-	-	4mm	5mm	-	4mm
13.	Liquid shield	-	3mm	-	-	-	5mm	-	-
14.	Sterillium	6mm	3mm	-	-	-	5mm	4mm	-
15.	Tap water	-	-	-	-	-	-	-	-



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

The overall percentage of resistance and susceptibility was also calculated as shown in Graph2. Pseudomonas aeruginosa was 100% resistant to all the hand sanitizers. Pseudomonas is an emerging threat due to its biofilm forming ability and antibiotic resistance.



Graph 2: Susceptibility pattern of microorganisms against different hand sanitizers.

DISCUSSION

Infection with environmental microbes is increasing alarmingly. Normal human skin always harbors bacteria (10^2 and 10^6 CFU/cm²). Bacterial transmission from hands to food, objects, or people is a significant factor in the spread of many

communicable diseases.¹⁸ Although, human skin contains two forms of normal flora, one that is already present on skin and is known as resident flora, such as Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus faecalis, and the other that is introduced on skin from the external environment and is known as transient flora, which

involves *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.^{19,20}

Alcohol is the main active ingredient in alcohol-based hand sanitizers, exhibiting antibacterial activity, causing protein denaturation, tissue membrane destruction and some lipid dissolution.²¹ Alcohol has an increasing effectiveness from 60% to 90%, with 1 propanol being the most effective, followed by 2 propanol, and finally ethanol, while the active ingredients responsible for antimicrobial activity in PureHands, Joy, Safe kind and nomarks hand sanitizer were coriander, lime, aloe-vera, and neem. They achieve bacterial reduction on contact (in 15-30s).

The results of this study contradict RajkumariBR, et al²² findings that Sterillium is more effective against *Candida albicans*, *E. coli*, and *Klebsiella pneumoniae*. Oke MA, et al²³ found that Dettol hand sanitizer was only effective against *P. aeruginosa*, and not against *S. aureus* or *E. coli*, according to their research. The similar findings of Tambekar, et al²⁴ showed that *Staphylococcus aureus*, *Streptococcus*, and *Pseudomonas* were all resistant to Dettol sanitizer. However, the results of this study contradict those of Kimura et al.¹⁸, who found that Dettol hand sanitizer was successful against *S. aureus* and *S. epidermidis*, with zones of inhibition of 5 mm for each of them. The Lifebuoy hand sanitizer also showed antimicrobial activity against the species tested; however, due to a lack of scientific literature, an exact and accurate comparison with other studies could not be made. Furthermore, a study of schoolchildren revealed that hand sanitizers have a high efficacy in minimizing microflora on the hands.^{18,25}

PureHands, a herbal hand sanitizer, was proven to be effective against *E. coli*, *Proteus mirabilis*, *Shigella* sp., *S. aureus*, and *S. epidermidis* in a study conducted by Mondal, et al¹ PureHands had antimicrobial effectiveness against the species tested in this sample, but it was the least effective hand sanitizer, which may be attributed to the poor antimicrobial potency of the Coriander, Lime, and Neem in it. Further research is necessary to confirm the exact cause of PureHands herbal hand sanitizer's ineffectiveness against the species studied.

Best results were shown by Jungle magic, Instant germs, Nomarks hand sanitizer and Sterillium. Jungle magic shows the best results among all the sanitizers,

that inhibited growth of the following species; *Klebsiella* sp. 10.6mm, *E. coli* 10.4mm, *Staphylococcus aureus* 19mm, *Proteus* sp. and *salmonella* sp. shows zone of inhibition by 14mm, *Enterococcus* 17mm and *Shigella* sp. 18mm. There was no literature available present about the Jungle magic hand sanitizers.

Limitations of sanitizers²⁶

- The alcohol content of the sanitizer should be at least 60%. When using alcohol, the percentage of alcohol should be between 60 and 95 percent. The ingredient may be ethyl alcohol, isopropanol, or ethanol, which are all acceptable. Every sanitizer is not manufactured equally; hence, it should be checked before purchasing.
- To ensure that the sanitizer's efficacy or sufficient effectiveness, it should be used on hands that are free of dust, dirt, blood, or lubrication.
- Hand sanitizers are not a replacement for hand washing; rather, they are a complementary habit that is much more efficient when combined with soap and water.

CONCLUSION

The effectiveness of hand sanitizers is determined by the active ingredient, which must be present in the correct concentration. Any sanitizer is ineffective at killing the pathogens. As a result, knowing how to choose appropriate alcohol-based hand sanitizers is critical for reducing infection transmission, especially when dealing with colleagues in hospitals, clinical laboratories, and among schoolchildren. also, The most important factor in reducing contamination of your phone is routine and proper hand washing, your phone can be disinfected with an alcohol disinfectant (using 70% isopropyl alcohol).²⁷ Despite claims that hand wash manufacturers are effective and reduce bacteria by 99.9%, these claims still require legal and parental review to follow good quality measures.

Dental public health significance

They play an important role and can be an effective alternative to hand washing to achieve sterility for all healthcare professionals participating in outreach programs in areas where water is scarce and in routine clinical practice. As a result, emphasizing

good hand hygiene is an effective first line of protection in the fight against the spread of infectious diseases.

Recommendation

The current study has its own limitations, as it only looked at the antimicrobial efficacy of various hand sanitizers. Further research is needed to determine the exact amount and duration of hand sanitizer or disinfectant application.

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