

Original Research Article

Inhibitory effects of different hand sanitizers against the resident microflora of skin

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ABSTRACT

Background: Practice of hand washing is very important to eliminate the microbial contamination especially during the work in laboratories, hospital and even at home before taking food. Proper use of hand sanitizer can significantly reduce the rate of hospital acquired infection also.

Methods: The current investigation was designated to identify different bacterial species from the upper skin of hands of the laboratory managements through conventional culture methods and the efficacy of the samples (Dettol, Purell and Savlon) against the isolated bacteria through agar well diffusion method and minimum inhibitory concentration (MIC)

Results: Different concentrations such as 25%, 50% and 100% of each of antimicrobial agents showed their antibacterial activity against *Staphylococcus* spp., *Klebsiella* spp., *E. coli* and *Pseudomonas* spp. those were isolated from the hand. 25% of Dettol exhibited 20 mm zone diameter against *Klebsiella* spp. Whereas 25% of Purell and Savlon unveiled 15 mm and 22 mm zone of inhibition against *E. coli* and *Pseudomonas* spp. respectively. In case of 100% sample of Dettol, Purell and Savlon, the highest zone diameter was observed as 55 mm, 50 mm and 45 mm against *Klebsiella* spp., *Staphylococcus* spp., and *E. coli* consecutively. The MIC of Dettol sample was 8 µl against *Staphylococcus* spp., and *Pseudomonas* spp. In case of Purell, the MIC was 128 µl against *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. were inhibited at 4 µl samples.

Conclusions: The in-vitro antibacterial activity of the hand sanitizers was so satisfactory against the isolated bacteria. This finding would be very helpful for the laboratory management in order to minimize the rate of contamination during the research and supervision of the different experiment.

Keywords: Sanitizers, Antibacterial activity, MIC

INTRODUCTION

Most of the germs that cause serious infections in healthcare are spread by people's actions. Hand hygiene is a great way to reduce the transmission of infectious disease particularly in hospital.^{1,2} There are several methods of hand hygiene such as hand washing or sanitizer to kill or eliminate the pathogenic microorganisms present on hands. However, various

organizations such as Centers for Disease Control and Prevention (CDC) and WHO have been published guidelines on appropriate hand hygiene.² Currently, the concept of hand sanitization has been in place right from the start of the hand hygiene campaign by many governmental and non-governmental organization.^{3,4} Previous researchers have been focused on the importance of hand sanitizer as an infection control means particularly against the communicable diseases.^{2,4}

However, in the early 2000s the CDC has been issued a proper guideline which recommended that alcohol-based hand rub (ABHR) should be routinely used for decontaminating hands.⁵ In recent years, the most commonly used hand sanitizers are ABHR which are often composed of alcohol, ethanol, isopropanol or propanol.^{1,6} The recommended concentration range of these sanitizers are 60 to 95%.⁷ For the time being, hand sanitizers not only very effective to minimize the infection rates but also these are very useful alternative source of water where access to water is so limited for hand cleaning.^{6,7} Beside the antibacterial activity, alcohol-based hand sanitizers have been reported as one of the commonly recommended hand hygiene against the diseases outbreaks causes by Ebola-Virus.^{8,9} As described by previous researchers that hand sanitizers have been found as very effective agent in order to eradicate the gastrointestinal infection as well as hospital acquired infection.^{7,10} Eventually, people are now more interested to use hand sanitizer instead of only hand washing due to its better performance against the resident skin flora such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*.⁶ As a result, many companies have now launched varieties of hand sanitizer in the market without verifying the proper concentration and activity of the products which making huge dissatisfaction among the customers.^{11,12} To confirm the efficacy of the hand sanitizer, present study attempted to isolate several inherent microflora from the hand of laboratory stuffs and introduce the effectivity of the three common hand sanitizers (Dettol, Savlon and Purell) against the growth of the microbes.

METHODS

Type of study, sample collection and processing

This is an observational or analytical study where the samples (3 types of hand sanitizer such as Dettol, Purell and Savlon) were randomly collected from different super shop in Dhaka city, Bangladesh, from June 2018 to September 2018 to detect their efficacy against some skin residual micro-flora. All the samples were transported in the microbiology laboratory for the detection of anti-bacterial activity.¹³

Test isolates

Four important bacteria such as *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. were isolated from the surface of the hand of 5 laboratory stuff (laboratory cleaner 1, laboratory cleaner 2, lab assistant 1, lab assistant 2 and research assistant) who worked at microbiological laboratory, Stamford University Bangladesh. The sterile cotton swabs were rubbed on the surface area of the hand and lawn on to the MacConkey agar, mannitol salt agar and cetrimide agar then the plates were incubated at 37 °C for 24 hours.¹⁴

Antibacterial activity of the products through agar well diffusion methods

For the determination of anti-microbial activity, modified agar well diffusion method was employed using MHA plates.¹⁵⁻¹⁷ Suspensions of different bacterial strains such as *E. coli*, *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus aureus* were prepared using normal saline, consisting of 10⁶ cfu/ml with a turbidity equivalent to that of the 0.5 ml McFarland standard. Each suspension was then inoculated on the Muller-Hinton agar (MHA) (Oxoid Ltd., Basingstoke, Hampshire, England) by using Lawn method. 4 wells were created on the plates using a 6 mm cork borer and 0.1 ml of different concentrations (100%, 50% and 25%) of the test substance (Dettol, Purell and Savlon) was added to individual wells. After 24-hour incubation at 37 °C, the zones of inhibition were then measured.

Minimum inhibitory concentration

The MIC of the substances was estimated to identify the least concentration, which had the ability to inhibit the growth of test bacteria.^{18,19} Different concentrations (such as 2 µl, 4 µl, 8 µl, 16 µl, 32 µl, 64 µl, 128 µl, 256 µl, 512 µl, 1024 µl and 2048 µl) of the substances (Dettol, Purell and Savlon) were introduced into the inoculum broth.

Minimum bacteriocidal concentration

To determine the minimum bacteriocidal concentration (MBC) of the test ingredients, the same concentrations of the substances (as indicated in MIC) were used against the same bacterial suspension and evaluated the lowest amount, which can kill the growth of bacteria.⁸

RESULTS

Detection of different bacteria from the hands of laboratory stuffs

Staphylococcus spp., *Klebsiella* spp., *E. coli* and *Pseudomonas* spp. were found from the all 5 laboratory stuffs (laboratory cleaner 1, laboratory cleaner 2, lab assistant 1, lab assistant 2 and research assistant). Among the 5 stuffs the massive microbial growth was found on the samples collected from the laboratory cleaners 1 and 2. The bacterial contamination was in moderate range in the hands of laboratory assistant 1 and 2 but the bacterial contamination was quantified in minimum range in case of research assistant (Table 1).

Antibacterial activity of different hand sanitizers through agar well diffusion methods

All three hand-sanitizer exhibited their anti-bacterial activity against the tested isolates (Table 2). The maximum zones of inhibition were recorded at concentrations of 100% for all the samples. In case of Dettol sample, the zone of inhibition was quantified as 40, 45, 55 and 35 mm against *Staphylococcus* spp., *E.*

coli, *Klebsiella* spp., and *Pseudomonas* spp. consecutively at 100% concentration, at 50% concentration of dettol the zone of inhibition 26, 25, 45 and 25 mm was measured against *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. consecutively and 25% of Dettol showed anti-bacterial activity 10, 8, 20 and 15 mm against *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. consecutively (Table 2). The zone diameter (30-50 mm) was recorded for Purell against the isolates at 100% concentration while the zone diameter 20-35 mm was measured at 50% concentration and the growth of bacteria was killed up to 15 mm at 25% of contractarian. Conversely, 100% Savlon exhibited the zone diameter 35, 45, 40 and 35 mm against the growth of *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. consecutively. 50% of the savlon was able to inhibit the growth of bacteria with zone diameter within the range of 20-38 mm likewise, 25% of savlon showed zone diameter 12, 17, 15 and 22 mm against the *Staphylococcus* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp., consecutively (Table 2). 100% samples of all the disinfectant were found to be more effective to reduce the growth of all the tested bacteria. Among the 3 disinfectant savlon was generated maximum zone diameter at lowest concentration (25%) (Table 2).

Determination of MIC and MBC of different hand sanitizers

Additionally, the result of MIC and MBC of the samples were supported the results of agar well diffusion methods (Table 3). In case of dettol sample, the MIC and MBC was recorded at 8 μ l and 16 μ l respectively against *Staphylococcus* spp., 32 μ l and 64 μ l concentrations were observed as MIC and MBC respectively for *E. coli*, 128 μ l and 256 μ l for *Klebsiella* spp. and 8 μ l and 16 μ l were documented as MIC and MBC for *Pseudomonas* spp. (Table 3). Another hand sanitizer purell showed MIC value at 128 μ l and MBC at 256 μ l against *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. likewise the value MIC and MBC was recorded at 256 μ l and 512 μ l against *E. coli* respectively. Likewise, the 4 μ l and 8 μ l concentration of Savlon were determined as MIC and MBC respectively against *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp (Table 3).

In this study, the lowest concentration of savlon (4 μ l and 8 μ l) was recorded as most effective hand sanitizer against the bacteria found from the surface area of the laboratory.

Table 1: Microbiological quality analysis of hands of different laboratory stuffs.

Sample specification	<i>Staphylococcus</i> spp.	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.
Lab cleaners 1	+++	+++	+++	+++
Lab cleaners 2	+++	+++	+++	+++
Lab assistant 1	++	+	+	+
Lab assistant 2	++	++	+	+
Research assistant	+	0	0	0

+++; High growth rate; ++; Moderate growth rate; +; Low growth rate.

All the experiments were performed three times and one reproducible data

Table 2: Inhibitory effect of different hand sanitizers through agar well diffusion technique.

Zone of inhibition (mm)				
Antibacterial agent	<i>Staphylococcus</i> spp.	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Pseudomonas</i> spp.
Dettol				
25%	10	8	20	15
50%	26	25	45	25
100%	40	45	55	35
NC (BPW)	0	0	0	0
Purell				
25%	8	15	10	15
50%	35	20	35	25
100%	50	40	40	35
NC (BPW)	0	0	0	0
Savlon				
25%	12	17	15	22
50%	20	20	38	28
100%	35	30	50	35
NC (BPW)	0	0	0	0

All the experiments have been performed three times and one reproducible data has given.

Table 3: Detection of MIC of different hand sanitizers against the isolates.

Zone of inhibition								
Antibacterial agent	<i>Staphylococcus spp.</i>		<i>E. coli</i>		<i>Klebsiella spp.</i>		<i>Pseudomonas spp.</i>	
	MIC (µl)	MBC (µl)	MIC (µl)	MBC (µl)	MIC (µl)	MBC (µl)	MIC (µl)	MBC (µl)
Dettol	8	16	32	64	128	256	8	16
Purell	128	256	256	512	128	256	128	256
Savlon	4	8	32	64	4	8	4	8

All the experiments have been performed three times and one reproducible data has given.

DISCUSSION

As reported in many studies that the upper layer of the skin serve huge amount of nutrient for the propagation of different bacteria especially *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Cornnebacterium*, *Streptococcus pyogenes* etc., those are responsible for transmitting several communicable diseases as well as hospital acquired infection.²⁰⁻²³ Beside such diseases outbreaks these bacteria can also be transmitted as contamination during the laboratory experiment.⁵ In order to eliminate the proliferation of such bacteria, use of hand sanitizer or disinfectant is very significant before starting any experiment and taking any food as well.^{22,16-26}

In recent years, the rate of communicable diseases and hospital acquired infection have increased alarmingly which has become a serious public health problem through worldwide.^{27,28} The most common route for transmission of infection or communicable diseases are hands and skin. Thus hand hygiene has become essential to prevent communicable disease and diseases that acquired from health care centre including nosocomial infection.²⁹⁻³⁰ Although, human skin contain two different types of normal flora, one that always presence on skin known as resident flora e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and other that are introduced on skin from external environment, which called transient flora consists *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.²⁴ Analysis of several scientific studies explained that hand washing without sanitizers, does not remove pathogenic microorganisms from hands. Even most of the pathogenic organisms about 80% remain on skin, therefore scientists have introduced different hand sanitizers to improve skin condition as well as to reduce" will be added after different and pathogenic microorganisms such as bacteria, virus, fungi from hand and skin surfaces and in improving skin condition.^{6,22}

Moreover, using hand sanitizers decreased the risk of spreading gastrointestinal and respiratory infection can minimize skin dryness and irritation also in reducing the rate of absentee in schools and college. Direct use of alcohol can cause skin dryness but alcohol based sanitizers are effective to prevent infection in hospitals and also in reducing the load of pathogenic microorganisms from hand.^{31,32}

CONCLUSION

Our study showed that the Dettol, Purell and Savlon have suitable range of antibacterial activity against the isolated bacteria from the hands of laboratory officers. So, as a final point it can be assumed that the hand sanitizers are more effective to eliminate the skin microflora than the hand washing and ordinary water. However, there are several complain in market by the customers regarding the low efficacy of the hand sanitizer. Therefore, the higher authorities and regulatory bodies should justify the client's opinion very positively and ensure the quality of the products.

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