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Evaluation of antimicrobial efficacy of different hand sanitizers available in Thimphu, Bhutan

Vishal Chhetri[1], Sonam Jamtsho[2], Kelzang Wangdi[3], Sonam Gyeltshen[4], Tshering Dorji[5], Amin Ngawang Tashi[6]

[1,3-6]*Royal Centre for Disease Control, Ministry of Health, Thimphu, Bhutan*
[2]*Drug Regulatory Authority, Royal Government of Bhutan, Thimphu, Bhutan*

ABSTRACT

Introduction: Hand washing and the use of alcohol based hand-rub are two effective hand-hygiene procedures. Though the compliance to hand washing was relatively low, the COVID-19 pandemic situation has drastically increased the use of hand sanitizers. Thus, many hand sanitizer brands have entered the market and this study was taken up to assess the microbial efficacy of different types of hand sanitizers available in Thimphu. **Methods:** Ten gel based and ten liquid hand sanitizers were collected from various retail pharmacy shops in Thimphu during first quarter of 2020. Laboratory based experiment was performed using standard organism; Gram Negative Bacteria (GNB) *Escherichia coli* (American Type Culture Collection 25922) and *Pseudomonas aurigenosa* (American Type Culture Collection 27853), Gram Positive Bacteria (GPB) *Staphylococcus aureus* (American Type Culture Collection 25923) and a cocktail of GNB and GPC. The efficacy of hand sanitizer was assessed with the size of the Zone of Inhibition (ZoI). **Results:** The hand sanitizers were found to be effective against all strains of micro-organism but with different size of ZoI. The maximum size of Zone of Inhibition was observed by sample N and Q (ZoI diameter 33.0±0.14mm) for American Type Culture Collection 25923. The Zone of Inhibition of liquid base hand sanitizers was significantly higher than that of gel base (p<0.05). **Conclusions:** The study presents that liquid base alcohol based hand sanitizer is more efficient than the gel form.

Keywords: Antimicrobial; Hand hygiene; Hand sanitizer; Infection control; Zone of inhibition.

INTRODUCTION

The principles of hand washing are based on the work of Semmelweis and hand hygiene is paramount in preventing the transmission of any pathogens and nosocomial infections[1]. Hand washing and the use of alcohol based hand-rub are two fundamentally different hand-hygiene procedures used in everyday life of health workers, the former being considered the best approach by Centre for Disease Control (CDC)[2,3]. Several evidences shows the effectiveness of hand hygiene in the prevention of hospital acquired infections and the World Health Organization (WHO) recommends proper hand washing following 7 steps for at least 20 seconds and the use of hand rubs (alcohol base) to be effective means of preventing respiratory infections; including Corona virus[4-6].

Human skin has normal flora of bacteria; including both transient flora (*Escherichia coli, Pseudomonas aeruginosa*) and resident flora (*Staphylococcus aureus*) up-to 10 log 2 to 10 log 3 CFU/cm^2 [1]. As clinical services require touching of patients, healthcare workers can gradually hoard micro-organisms and transmit them to weak and immuno-compromised patients and there is scientific evidence of epidemic being caused by healthcare workers' ineffective methods of infection control[7,8]. Hence, the importance of hand hygiene has been recognized as an effective method in the prevention of infection.

The compliance to hand washing is relatively low, scarcely up to 40%, even in the critical care units[9,10], therefore the use of alcohol based hand rub has come out as an effective measure[11,12]. Hand sanitizers are presented either in gel or liquid form and available instantly. The major compositions being alcohol as the active component; the antimicrobial activities of alcohols are based on denaturation of surface protein, breaking down of water-based membranes and disruption of protein structures. Glycerol is used as humectants, but other emollients may be used for skin care. Hydrogen peroxide is used to prevent contaminating bacterial spores in the solution and is not an active substance for hand antisepsis. A colorant is added to allow differentiation from other fluids, but it should not add to toxicity, promote allergy, or interfere with antimicrobial properties. Furthermore, hand sanitizers are also found to be effective in curbing the current pandemic[6].

Amidst the COVID-19 pandemic, many brands of hand sanitizers have entered the Bhutanese market and although many

Corresponding author:
Vishal Chhetri
vchhetri@health.gov.bt

of the sanitizers claims to destroy 99% of pathogenic organisms, yet formal assessment has not been conducted in the Bhutanese market. Thus, this study was taken up to assess the antimicrobial efficiency of different types of hand sanitizers available in Thimphu, Bhutan.

METHODS

The study was performed during the COVID-19 pandemic as different brands of hand sanitizers entered to the market. The experiment was performed at Royal Centre for Disease Control (RCDC), Serbithang, Thimphu. The administrative clearance was obtained from Drug Regulatory Authority (DRA) of Bhutan and ethical clearance from Research Ethics Board of Health (Ref. No. REBH/PO/2021/155). The hand sanitizers were collected by Drug Inspectors during their regular quality control inspection. Ten liquid and ten gel forms of hand sanitizers (Figure 1) were collected from various retail pharmacies in Thimphu.

Figure 1. Various types of hand sanitizers used for experiment

Muller Hilton Aagar (MHA) was used as the medium for the assessment of inhibitory action and Zone of Inhibition (ZoI), which was measured in millimeters in diameter (mm)[13]. The isolates of American Type Culture Collection (ATCC) 25922 (*E. coli*), ATCC 25923 (*Staphylococcus aureus*) and ATCC 27853 (*Pseudomonas aeruginosa*) were used to measure the inhibitory action of various sanitizers. These test organisms were obtained from Enteric and Invasive Disease Laboratory (EIDL), RCDC and preserved in Nutrient slant butt.

Preparation of test organism and disk diffusion method

The chosen ATCC strains were sub-cultured in nutrient agar and incubated over 24 hrs. A standard of 0.5 McFarland was taken as the standard to adjust the turbidity of bacterial suspension and avoid bias during the experiment[14]. A sterile saline solution of 0.1% (w/v) was used to prepare the bacterial suspension. A loopful of 24 hours pure culture test organism was taken and suspension adjusted to 0.5 McFarland[15]. A cocktail of microbial was obtained by mixing equal portion of 0.5 McFarland bacterial suspension of ATCC 25922 (Gram Negative Bacteria) and ATCC 25923 (Gram Positive Bacteria)[16].

Though there are several in-vitro methods to determine bacterial susceptibility, the method developed by Bauer et al. was performed using 6 mm agar depth and 90mm petri plates[17]. A sterile cotton swab stick was dipped into a standardized test organism and uniformly plated onto MHA plates. The plates were let to dry inside the safety cabinet for 10-15 minutes. With the use of a sterile 6mm cork borer, four holes were bored in the MHA plates and 100µL of hand sanitizer was introduced into the boreholes, aseptically[18]. Each of the tests was performed in duplicate. Sterile distilled water was used as an internal quality control. The plates were incubated in the upright position for 24 hours at 37°C. The ZoI was recorded with complete inhibition of test organism and measured the diameter in mm.

Data analysis

Analysis of variance (ANOVA) and Post Hoc Multiple comparisons by Duncan test were performed using IBM-SPSS statistics package version 22 (SPSS Inc., Chicago, IL, USA). A probability at $p<0.05$ was considered statistically significant. Data entry was done using Microsoft excel and descriptive information is presented as mean and standard deviation.

RESULTS

As per the label claim all hand sanitizers had active ingredients as either isopropyl alcohol or ethyl alcohol in different concentrations; the other ingredients in the sanitizers are as listed in Table 1. The hand sanitizers were found to be effective against all strains of micro-organism but with different sizes of ZoI. The liquid form of hand sanitizers had remarkably higher range of ZoI as compared to the gel forms. The maximum size of ZoI in all the three test organism was seen with sample codes M and T (liquid form), and minimum with sample code E and J (gel form), is statistically significantly $p<0.05$.

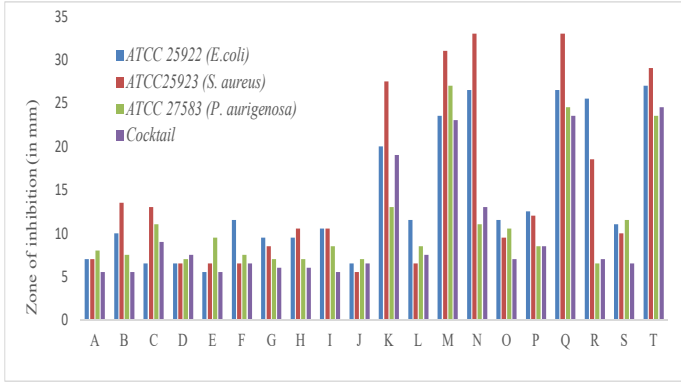

Figure 2. Measurement of zone of inhibition of different hand sanitizers on different test organisms (Code A to J Gel form, Code K to T liquid form)

Table 1. Hand sanitizer code and their ingredients (Sample code A to J in Gel form, sample code K to T in liquid form)

Sample Code	Ingredients	Sample Code	Ingredients
A	Isopropyl alcohol, Aqua, Propylene glycol, Glycerin, Aloe vera extract, Vitamin E oil, Fragrance	**K**	Isopropyl alcohol 70%, chlorhexdine gluconate, purified water, brillant blue
B	Isopropyl alcohol 70%, acqua, cross polymer, apple flavor	**L**	Ethyl alcohol 70%, Chlorhexidine gluconate 2.5%, Emollients and moisturizers
C	Isopropyl alcohol 60%, P.G, Carbomer, T.E.A, glycerin, lemon extract, preservatives and fragrance	**M**	Ethanol 96%, hydrogen peroxide 3%, glycerol, distilled water
D	Coleus vitiveroides 6%, Corandrum satuvum linn 6%, Citrus limom, Burm. Frg. 6%, Vetiveria zizanicides 6%, Azadirachta indica A Juss 6%, Isopropyl alcohol 70%	**N**	2-propanol IP, 1-propanol, Mecetronium ethylsulphate, Brillant blue
E	Isopropyl alcohol 75%, neem extract 5%, alovera 10%, Chlorohexidine Gluconate	**O**	Ethyl alcohol, fragrance, Peg 40 hydrogenated castor oil, Undecylenoyl glycine, Aloe barbadensis, Tocopheryl acetate
F	Isopropyl alcohol 70%, acqua, Acrylate crosspolymer and perfume	**P**	Ethyl alcohol 70%, Carprylyl glycol, Glycerin, Isopropyl myristate, Tocopheryl acetate, Acrylates/C10-30 acrylate crosspolymer, Aminomethyl propanol
G	Isopropyl alcohol 72.34%, PEG/PPG-17/6 Copolymer, propylene Glycol, Acrylates/ C10-30 Alkyl acrylate crosspolymer, Tetrahydro0xypropylethylenediamine, perfume	**Q**	Chlorhexidine gluconate solution IP, Ethyl alcohol, Purified water
H	Ethyl alcohol 70%, Carbomer, Glycerin, Propylene glycol, fragrance, Aloe barbadenis leaf juice, Triethanolamine	**R**	2-propanol 45%, 1-propanol 30%, Macetronium ethyl sulphate 0.2%, Brillant blue
I	Iso-propyl alcohol	**S**	Isopropyl alcohol 70%, purified water
J	Disodium EDTA, Carbopol, Propylene, glycerin, 70% ethanol, Triethanolamine	**T**	Chlorhexidine gloconate 2.5%, 2-propanol IP 70%, Emollient and moisturizer, Brillant blue

Table 2. Zone of inhibition of different gel based hand sanitizers on different standard organisms

Sample code	Zone of Inhibition (mm)			
	ATCC 25922 ***E. coli***	**ATCC 25923** ***Staphylococcus aureus***	**ATCC 28753** ***Pseudomonas aeruginosa***	**Cocktail (mixture of *E. coli* and *S. aureus*)**
A	7.0 ± 0.14^{ab}	7.0 ± 0.14^{b}	8.0 ± 0.14^{a}	5.5 ± 0.07^{a}
B	10.0 ± 0.14^{c}	13.5 ± 0.21^{d}	7.5 ± 0.07^{a}	5.5 ± 0.07^{a}
C	6.5 ± 0.07^{a}	13.0 ± 0.42^{d}	11.0 ± 0.14^{b}	9.0 ± 0.14^{b}
D	6.5 ± 0.07^{a}	6.5 ± 0.07^{bc}	7.0 ± 0.14^{a}	5.5 ± 0.07^{a}
E	5.5 ± 0.07^{a}	6.5 ± 0.07^{bc}	9.5 ± 0.07^{ab}	5.5 ± 0.07^{a}
F	11.5 ± 0.07^{c}	6.5 ± 0.07^{bc}	7.5 ± 0.07^{a}	6.5 ± 0.07^{ab}
G	9.5 ± 0.07^{bc}	8.5 ± 0.07^{bc}	7.0 ± 0.14^{a}	6.0 ± 0.14^{a}
H	9.5 ± 0.07^{bc}	10.5 ± 0.07^{bc}	7.0 ± 0.14^{a}	6.0 ± 0.14^{a}
I	10.5 ± 0.21^{c}	10.5 ± 0.07^{bc}	8.5 ± 0.07^{ab}	5.5 ± 0.07^{a}
J	6.5 ± 0.07^{a}	5.5 ± 0.07^{a}	7.0 ± 0.14^{a}	6.5 ± 0.07^{ab}
p value within group ($p<0.05$)	0.004	0.005	0.081	0.112

**Different lower-case superscripts within a column indicate significant difference ($p<0.05$), represents average size of zone of inhibition of different hand sanitizers to different target organism*

Table 3. Zone of inhibition of different liquid based hand sanitizers on different standard organisms

Sample code	Zone of Inhibition (mm)			
	ATCC 25922 *E. coli*	ATCC 25923 *Staphylococcus aureus*	ATCC 28753 *Pseudomonas aeruginosa*	Cocktail (mixture of *E. coli* and *S. aureus*)
K	20.0±0.18[b]	27.5±0.07[d]	13.0±0.14[c]	19.0±0.14[c]
L	11.5±0.07[a]	6.5±0.07[a]	8.5±0.07[ab]	7.5±0.07[a]
M	23.5±0.21[bc]	31.0±0.14[d]	27.0±0.14[e]	23.0±0.14[c]
N	26.5±0.07[c]	33.0±0.14[d]	11.0±0.14[bc]	13.0±0.14[b]
O	11.5±0.07[a]	9.5±0.07[bc]	10.5±0.07[bc]	7.0±0.14[a]
P	12.5±0.07[a]	12.0±0.14[b]	8.5±0.07[ab]	8.5±0.07[a]
Q	26.5±0.07[c]	33.0±0.14[d]	24.5±0.07[de]	23.5±0.07[c]
R	25.5±0.07[c]	18.5±0.07[c]	6.5±0.07[a]	7.0±0.14[a]
S	11.0±0.14[a]	10.0±0.14[ab]	11.5±0.07[c]	6.5±0.07[a]
T	27.0±0.28[c]	29.0±0.14[d]	23.5±0.21[d]	24.5±0.14[c]
p-value within group ($p<0.05$)	0.000	0.000	0.000	0.000

[*]Different lower-case superscripts within a column indicate significant difference ($p<0.05$), represents average size of zone of inhibition of different hand sanitizers to different target organism

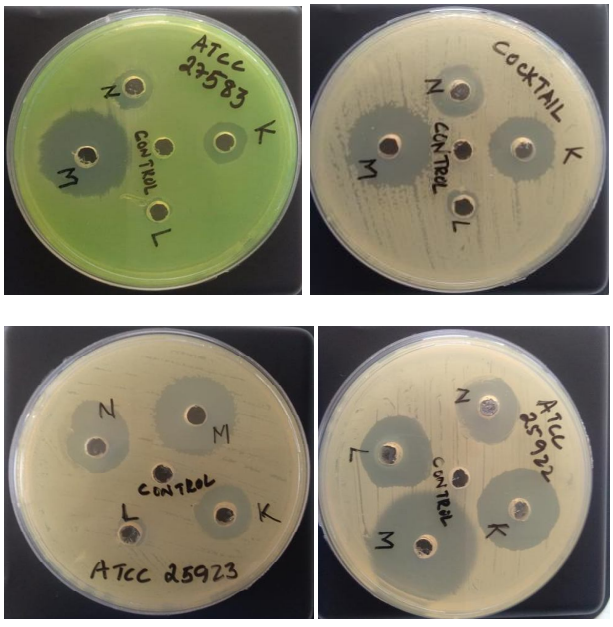

Figure 3. Zone of Inhibition of different hand sanitizer (sample code K, L, M, N) in target organism
(ATCC 27583 (Pseudomonas aurigenosa), ATCC 25923 *(Staphylococcus aureus),* ATCC 25922 *(E. coli) Cocktail* (1:1, ATCC 25922: ATCC25923)

DISCUSSION

The use of alcohol based hand sanitizers increased significantly in Bhutan after the WHO advised it as a means of preventing current COVID-19 pandemic. The goal of hand hygiene using hand sanitizers or water based is to reduce the microbial counts on the skin to prevent cross-transmission of pathogens among patients by healthcare workers and prevent the transmission of viruses within the family and community. Alcohol based hand rubs are effective only if the concentration of alcohol is proportionate. The hand sanitizers in the current study were marked as having 60% - 75% of alcohol, which is found to be effective to destroy microorganisms. The Food and Drug Administration (FDA) recommends a concentration of 60% to 95% ethanol or iso-propanol[19,20]. The use of methanol in hand sanitizer can be hazardous and is therefore not recommended[21] and none of the hand sanitizers included in current study was labeled as methanol based[21].

Although both the gel and liquid forms of alcohol based hand sanitizers claim to be effective, the study result shows that liquid formulations are more effective (Table 2 and 3). These findings are in concordance with the earlier studies conducted by Dharan et al., (2003) and Kramer et al., (2003)[22,23]. The gel form has statistically significant ($p<0.05$) ZoI for ATCC 25922 and ATCC 25923 ($p<0.05$) but insignificant to ZoI for ATCC 28753 and Cocktail strain. On the other hand, the liquid form of sanitizers showed statistically significant ($p<0.05$) ZoI for all the target test organisms ($p<0.05$). It was observed that different brands of hand sanitizers produced higher range of ZoI to either

GPB or GNB and minimum ZoI to cocktail strain. The cocktail of GNB and GPB represents the different types of micro-organisms that can be present in our hand. Though the liquid based hand sanitizers show higher range of ZoI, the efficacy also depends on the appropriate procedures of application. Few liquid sample hand sanitizers (Sample code K, L, Q, T) had added chlorohexidine gluconate but the results were similar to the other alcohol based hand sanitizers, even though chlorohexidine is claimed to be more efficient than alcohol as antimicrobial agent[24].

Among the twenty samples tested in the current study, 90% (*n*=18) of samples were imported products. Only 10% (*n*=2) was were locally produced alcohol based hand sanitizer (Sample code J and K). Sample code J is a gel based and sample code K is a liquid based sanitizer. The Ssample code J had the minimum efficiency on all test organisms and while the sample code M had the significantly higher range of ZoI in all test organisms.

To overcome and reduce the microbial contaminations and hospital associated infections in health-care settings, the use of alcohol based hand sanitizers are recommended along with the facility of hand washing station. Moreover, the current COVID-19 pandemic has further boosted the importance of hand hygiene and escalated the use of hand sanitizers. The main limitation of the current study is the inability to include we could not include all the hand sanitizers that is available in the market. Moreover, the non-availability of standard international guidelines for interpretation of ZoI was a challenge.

CONCLUSIONS

This study shows that all the twenty samples collected from Thimphu demonstrated different sizes of ZoI on target test organisms. The study concluded that liquid based hand sanitizers are more effective than the gel form. Since most of the sanitizers are imported in Bhutan, the concerned authority must evaluate the efficacy of hand sanitizers as some products marketed, despite a label claim of reducing "germs and harmful bacteria inhibition by 99.9%" to the public as antimicrobial hand sanitizers may not demonstrate the claimed efficacy.

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AUTHORS CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

VC: Concept, design, data collection and analysis, manuscript writing and review

SJ: Design, data collection, manuscript writing and literature review

KW: Design, data collection, manuscript writing and literature review

SG: Design, data collection and analysis, manuscript writing and literature review

TD: Design, data collection and analysis, manuscript writing and literature review

ANT: Design, data collection and analysis, manuscript writing and literature review

Author agree to be accountable for all respects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

None

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None