Microbiological Evaluation of Commercial Hand Sanitisers Available in Pakistan Using European Standard and Membrane Filtration Method

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ABSTRACT

Objective: To evaluate the antibacterial efficacy of various commercially available alcohol-based hand sanitisers (ABHS) using European standard (EN 1500) method and perform ABHS testing with membrane filtration method.

Study Design: A Cross-sectional observational study.

Place and Duration of the Study: Quality Control Section of the Microbiology Laboratory, The Aga Khan University Hospital, Karachi, Pakistan, from February to April 2023.

Methodology: Efficacy of 14 commercially and widely accessible hand sanitisers was defined as reducing micro-organism growth. It was determined using the EN 1500 European standard test and membrane filtration method.

Results: Majority (92.8%) ABHS showed a significant bacterial reduction except one ABHS tested with the EN 1500 method. Only six ABHS products were tested through the membrane filtration method because high viscosity of hand sanitisers was causing damage to filter membranes.

Conclusion: Continued vigilance in evaluating hand sanitiser's efficacy through robust testing methods is essential to ensure public health and prevent the dissemination of misleading products that may compromise hand hygiene practices.

Key Words: *Hand sanitisers, European standard, Membrane filtration method, Antibacterial efficacy.*

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INTRODUCTION

In alignment with the WHO's sustainable development goals (SDG), hand hygiene is an important indicator for improving the health of the global community.[1] It is a well-recognised fact that hand hygiene is an effective single intervention that has reduced the disease-burden in communities and hospitals.[2,3] Many studies have shown that lack of hand hygiene is responsible for causing diarrhoea and colds.[4] The importance of hand hygiene is highlighted by the scientific evidence that one million lives could be saved annually by just washing hands.[5,6] Hand hygiene can be done by hand washing with soap or using hand disinfectant. Hand washing has universal low compliance, mainly due to its several limitations.

The most common issue in resource-limited settings is the non-availability of water, soap, sink, and towel/tissue paper. Other limitations are its time requirement and the potential to give rise to skin cracks that can act as a portal of entry for a variety of pathogens.[7,8]

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In the community, simple soap can be used, but in healthcare settings, the use of disinfectant soap is recommended by the Centre of Disease Control (CDC). The use of hand sanitisers is relatively a simple and quick method of hand hygiene. The antiseptic property of hand sanitiser and skin compatibility are the two cardinal attributes of a hand sanitiser.[9] Different disinfectants can be a part of a hand sanitiser product, however, WHO strongly recommends alcohol-based hand sanitisers (ABHS) as the gold standard in healthcare facilities and the community. This is due to their broad antimicrobial spectrum including both enveloped and non-enveloped viruses, quick action, quick evaporation, and cost-effectiveness. Regarding alcohol-type and its percentage in ABHS solution, WHO endorses 60-95% of ethanol or isopropyl alcohol by volume for optimum bactericidal and virucidal activity.[10,11] During the COVID-19 pandemic, many commercial hand sanitiser products were marketed in countries, with no proven efficacy.[12] For example, a study from Johannesburg reported substandard alcohol concentration i.e. <60% in 41% of locally available hand sanitiser brands.[12]

The consumption of suboptimal and under-tested sanitisers may give false security of infection prevention. Therefore, there is a critical need to assess the effectiveness of these sanitisers via standard testing methods such as using EN 1500.[13]

To the best of the authors' knowledge, this is the first-of-its-kind study in Pakistan to microbiologically evaluate commercially

available hand sanitisers. Moreover, the literature search for the present study also showed the use of the membrane filtration method (MFM) for evaluating the efficacy of chemical disinfectants. As MFM is not a standard method and has a complex and time-consuming methodology,[14] therefore, the use of this method was also evaluated.

This study aimed to evaluate the antibacterial efficacy of various commercially available ABHSs using European Standard (EN 1500) and membrane filtration method.

METHODOLOGY

This was a cross-sectional laboratory-based study conducted from February to April 2023, in the Clinical Microbiology Laboratory of The Aga Khan University Hospital. Fourteen samples of ABHS were selected from retail outlets in Karachi Pakistan, based on their market availability. Each sample comprised of unique commercially available brand of hand sanitiser as shown in Table I. Before performing microbiological testing, expiry dates of all the retrieved ABHS samples were checked. All ABHS products contained Ethanol (ethyl alcohol). Notably, variations among 14 products samples were observed, with some containing additional potent constituents such as povidone-iodine, quaternary ammonium compounds (QAC), triclosan, or chlorhexidine. Chemical analysis of the hand sanitisers was not performed. The EN 1500 standard was applied for the testing of reference ABHS along with 14 ABHS testing, and log-reduction was noted. The tested products must be equally effective as the reference product.

The EN 1500[15] is a European standard that evaluates the efficacy of hand sanitisers by comparing them to a reference disinfectant containing 2-propanol, 60% volume per volume [v/v]. This method estimates the efficacy of a hygienic hand rub by measuring the number of viable bacteria using *Escherichia coli* K12 (NTCC 10538) as the test organism that remains on the fingertips after contamination and exposure to the hand rub. By comparing the pre- (Figure 1) and post-values (Figure 2), a ratio known as the reduction-factor ≥2-log is produced, which according to FDA's tentative final monograph (TFM) offers a numerical assessment of ABHS's antimicrobial efficacy.

Initially, using this standard, pre-value testing of the FDA-approved reference control ABHS was performed. For this, volunteers with healthy hands were cleansed with a gentle soap to remove natural transient micro-organisms, and then thoroughly dried with a paper towel. After that, their hands were submerged keeping their fingers spread apart in a 10ml 0.5 McFarland of the pure culture of a non-pathogenic strain of *Escherichia coli* inoculum, up to the mid-carpals, for five seconds. The hands were then allowed to air dry for three minutes. To determine the pre-values of viable bacteria existing on the hands, dried fingertips then rubbed into a petri dish containing 10ml sterile tryptic soy broth (TSB) for 60 seconds. Fifty microlitter (50 µl) of TSB was subsequently poured on MacConkey's agar by easy spiral diluter (Easy Spiral Dilute®) up to three dilutions, that is 1:10, 1:100, and 1:1000. Easy Spiral Dilute® is a 2-in-1 automatic diluter and plater, which allows serial dilutions. It automatically plates on petri dish, with a countable range from 30 to 1 x 10^{12} countable cfu/ml. After incubation at 36 ± 1°C for 18-24 hours, colonies on plates were calculated by exponential mode.[16] The exponential mode is plating on surface with a decreasing surface concentration. To determine the bacterial count in cfu/ml the authors multiplied the number of colonies counted on plate, from edge to centre in exponential mode by inverse of the dilution factor 1:1000. Immediately after pre-value sampling, the test was repeated for post-value. For this, all the steps remained same except that the hands after inoculation with organism were subjected to decontamination with ABHS. This step was performed according to ABHS manufacturer recommendations for hand disinfection. The fingertips were then sampled again on 10ml of TSB containing a chemical neutraliser for post-values. The plates were initially incubated at 36 ± 1°C for 18-24 hours, counted, and followed by an additional 24-hour re-incubation to detect any possible slow-growing colonies. The test was repeated for all 14 ABHSs and the respective reduction factor was calculated.

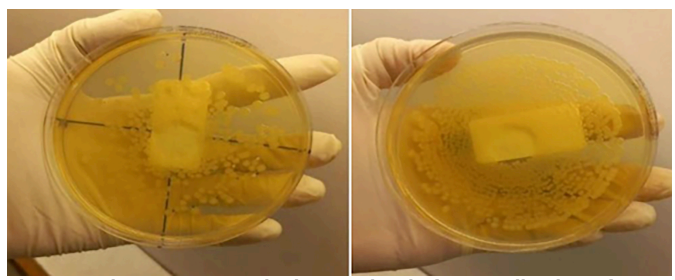

Figure 1: The EN 1500 method pre-value before application of ABHS (representative image) manual counting by easy spiral diluter.

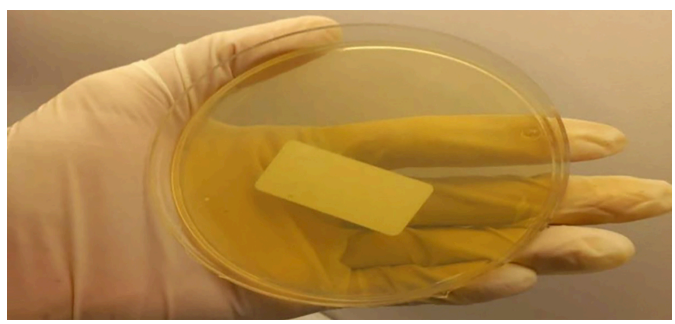

Figure 2: The EN 1500 method post-value after application of ABHS (representative image).

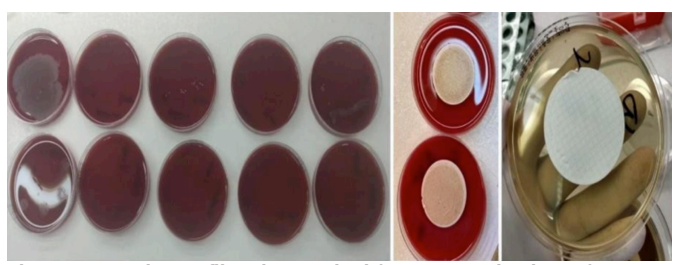

Figure 3: Membrane filtration method (representative image).

In membrane filtration method,[17] three bacterial strains (*Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*), and one fungal (*Candida albicans*) ATCC control strain

were utilised. To get baseline growth, 0.5 McFarland inoculum of each organism was made and set on an agar plate to grow. To test the activity of the ABHS sample, it was inoculated with 0.5ml of one of the ATCC organisms. One minute after organism inoculation, ABHS solution was 1:8 times diluted with normal saline depending upon the viscosity of ABHS. The ABHS solution was then filtered through a membrane using a filter funnel and vacuum system. Finally, filtration membrane was transferred to a solid media plate and incubated at 36 ± 1°C, for 24 hours. Any organisms that managed to survive the disinfectant activity of the tested ABHS would grow on the surface of the membrane (Figure 3).

This was a descriptive observational study. By comparing the pre- (Figure 1) and post-values (Figure 2) which were obtained from the fingers, a ratio known as the reduction-factor was produced. The reduction-factor offers a numerical assessment of ABHS's antimicrobial efficacy. The efficacy criteria of the FDA's tentative final monograph (TFM) are a ≥2-log-reduction. The EN 1500 standard was applied for testing of reference ABHS along with 14 ABHS testing, and log-reduction noted, as shown in Table II.

RESULTS

One ABHS (HS2) comprised of ethanol alone, as its active ingredient. While 13 ABHS samples had combination of ethanol with various other compounds including propylene glycol, triclosan, chlorhexidine (HS9), aloe barbadensis (HS8), and hydrogen peroxide (HS12) as shown in Table I. Ethanol concentrations of ABHS samples either alone or in combination with other compounds, varied between 70-80%.

Using the EN 1500 method, 93% ABHS showed a significant reduction in the bacterial growth, while only one ABHS sample (HS2) did not show any log reduction. The log reduction of the hand sanitisers is shown in Table II. The hand sanitisers that had ethanol combinations with other compounds showed higher log-reduction up to five times as compared to those that had ethanol alone, as shown in Table II.

Only six out of 14 ABHS samples were tested *via* membrane filtration method. Other samples failed to pass through membrane due to their high viscosity of gel content. No growth was found for all four tested organisms in all of the six ABHS samples.

Table I: Composition of hand sanitisers.

Commercial name (Company)	Ethanol %	Other ingredients
Hifazat (Pharmevo)	Ethanol 70% (compound)	De-ionized water, glycerol, hydrogen peroxide, carbomer, neutraliser, and fragrance.
HO (Ho Dental Company)	Ethanol 80% (single formulation)	Glycerol.
Lifebuoy (Unilever)	Ethanol 70% (compound)	Water, glycerin, carbomer, amino methyl propanol, benzophenone-1, aloe barbadensis, propylene glycol, parfum, citronellol, limonene, hexyl cinnamal, butylphenyl methylpropional, linalool, CI 19140, and CI 42090.
Delite (Aromistic Ltd.)	Ethanol 70% (compound)	PG, acrylates/C10-30 alkyl acrylate crosspolymer, perfume, and triethanollamine.
Dettol (Reckitt Benckiser)	Ethanol 70% (compound)	Water, glycerine, acrylin copolymer, triethanolamine, vitamin beads, preservative, colour, and fragrance.
Cool & cool (Cool & Cool)	Ethanol 70% (compound)	Water, carbomer, triethanolamine, glycerin, vitamin E, and fragrance.
Palmolive mint (Colgate)	Ethanol 70% (compound)	Water, fragrance, carbomer, PEG 30 glyceryl cocoate, eucalyptus oil, aminomethyl propanol, vitamin E acetate, tetrasodium EDTA, and CI 60730.
Fa (Schwarzkopf)	Ethanol 70% (compound)	Water, acrylates/C10-30 alkyl acrylate crosspolymer, ethanolamine, hexyl laurate, parfum, polysorbate 80, Hydroxyethyl urea, urea, butylphenyl methylpropional, linalool, citronellol, benzyl alcohol, hexyl cinnamal, benzyl acetate, benzyl salicylate, limonene, and benzophenone-1.
Garnier (Garnier)	Ethanol 80% (compound)	Water, glycerin, and hydrogen peroxide.
Palmolive (Lemon) (Colgate)	Ethanol 70% (compound)	Water, fragrance, carbomer, PEG 30 glyceryl cocoate, eucalyptus oil, aminomethyl propanol, vitamin E acetate, tetrasodium EDTA, and CI 60730.
Carex (Cussons)	Ethanol 70% (compound)	Water, PEG/PPG-17/6 copolymer, propylene glycol, acetates/C10-30Alkyl acetate crosspolymer, tetrahydroxpropyl ethylenediamine, fragrance, and limonene.
HiClean (Medinostic Healthcare)	Ethanol 70% (compound)	Water and carbomer.
Ho (Apple) (Ho Dental Company)	Ethanol 70% (compound)	Water, glycerine, acrylin copolymer, triethanolamine, vitamin beads, preservative, colour, and fragrance.
Purell (GOJO industries)	Ethanol 70% (compound)	Water, isopropyl alcohol, caprylyl glycol, glycerine, isopropyl myristate, tocopheryl acetate, acrylates/c10-30 alkyl acrylate copolymer, aminomethyl propanol, and fragrance.

Table II: Change in colony forming unit/ml before and after ABHS use by the EN 1500 method.

Hand sanitiser no.	Before ABHS use colony forming unit/ml	After ABHS use colony forming unit/ml	Log-reduction	Interpretation
01	12 X 10^5 cfu/ml	10x10^3 cfu/ml	02	Effective
02	44 X 10^4 cfu/ml	14x10^4 cfu/ml	00	Not Effective
03	15 X 10^5 cfu/ml	No growth	05	Effective
04	12 X 10^3 cfu/ml	No growth	03	Effective
05	96 X 10^4 cfu/ml	No growth	04	Effective
06	12 X 10^5 cfu/ml	No growth	05	Effective
07	13 X 10^5 cfu/ml	No growth	05	Effective
08	89 X 10^4 cfu/ml	No growth	04	Effective
09	63 X 10^4 cfu/ml	No growth	04	Effective
10	11 X 10^5 cfu/ml	No growth	05	Effective
11	12 X 10^5 cfu/ml	60x10^2cfu/ml	03	Effective
12	22 X 10^5cfu/ml	16*10^3 cfu/ml	02	Effective
13	13 X 10^5 cfu/ml	No growth	05	Effective
14	44 X 10^4 cfu/ml	No growth	04	Effective

DISCUSSION

Alcohol-based hand sanitisers (ABHSs) are the most widely used hand sanitisers. In the present study, out of 14 ABHSs (HS1-HS14), 13 were effective against tested *E. coli* strain showing a minimum of two log-reduction. Only one (7%) product (i.e. HS2) of commercially available ABHS samples was identified to have no activity. However, as chemical analysis of sample was not a part of the study, it cannot be commented on the product claim. Results of the current study support the antimicrobial activity of 60-95% alcohol recommended by the FDA. Previous researchers have proven that solutions containing >95% concentrations of alcohol are less effective. This is due to the low water content that leads to the failure of microbial protein denaturation. Recently, Cartner *et al*.[18] demonstrated the benefits of an ethanol-containing hand rub by investigating the effects of three different alcohol-based systems on the skin over two weeks. Skin irritation is substantially worse when ABHSs containing n-propanol or isopropanol are used comparing to when ethanol is used. It is important to take these outcomes into account when trying to improve compliance.

In several countries including Pakistan, the ABHSs are sold as over the counter (OTC) medicines. Hence, to deliver the anticipated level of quality, safety, and efficacy, ABHS products must meet the minimal requirements established by standard authorities. The alcohol concentration should be examined as the primary factor ensure substantial antimicrobial activity. Other factors that affect the functionality and acceptability of ABHS include the target pH, viscosity, and hydrogen peroxide level. So, it is necessary to pay attention and exercise control over the product's efficacy and safety.

This study shows various limitations of the membrane filtration method (MFM), therefore it should be avoided for evaluating the antimicrobial effectiveness of ABHS. Firstly, viscous ABHS samples failed to pass the membrane, so they could not be tested by this method. Secondly, micro-organisms may not exhibit equal viability or growth under the conditions needed in MFM. Another significant limitation was the high cost associated with initial investment in equipment including membranes, pumps, and other components. Furthermore, ongoing costs of maintenance, replacement of membranes, and energy consumption can add up over time. The main limitation of the study was that it could not test all commercial ABHSs available OTC in Karachi, as many of them were out of stock at the time of the study. Another limitation was that the efficacy of disinfectants was only estimated against bacteria (even though enveloped viruses such as influenza viruses are destroyed by ABHSs, and inadequate funding limited the capacity to categorise specific strains of bacteria. Finally, the relationship of effective hand decontamination in reducing the transmission of infectious agents has yet to be studied and would require a more complex research design. This study has many strengths. Firstly, it provides important information on the effectiveness of commercially available ABHSs in Pakistan, which can contribute to the regional literature on hand hygiene. The study found that most hand sanitisers (93%) showed a significant reduction in bacterial growth, except for one sanitiser that contained 80% ethanol in single form, which was found to be completely ineffective. This highlights the importance of evaluating the efficacy of hand sanitisers before their use. Secondly, this study also provides information on the different compounds used in hand sanitisers in Pakistan, which can be useful for healthcare professionals and consumers to make informed decisions about their use. Additionally, the study evaluated the effectiveness of hand sanitisers using two different methods, which can provide a useful comparison for other studies in the region. Overall, the study's findings can help improve hand hygiene practices and can reduce the risk of infections in Pakistan.

CONCLUSION

The study underscores the critical importance of evaluating the efficacy of hand sanitisers, particularly considering the vast array of products available in the market. Study findings indicate that the most ABHSs that were tested, demonstrated significant bacterial reduction, in line with WHO recommendations for hand hygiene. However, the presence of one ABHS that did not meet efficacy standards according to the EN 1500 method raises concerns regarding the reliability of certain products. Moving forward, continued vigilance in evaluating hand sanitiser efficacy through robust testing methods is essential to ensure public health and prevent the dissemination of misleading products that may compromise hand hygiene practices.

ETHICAL APPROVAL:
Approval was obtained from the university's Institutional Review Board (IRB) of The Aga Khan University (Approval no. 2023-6997-24008, Dated: 4-March-23).

PATIENTS' CONSENT:
Not applicable.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
AA, SI: Conception, study design, data collection, data analysis, and the critical revision of the manuscript.
AA: Drafting of the manuscript.
Both authors approved the final version of the manuscript to be published.

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