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Original Research Article

Evaluating and comparing the efficacy of alcohol-based hand sanitizers commonly available in India

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ABSTRACT

Context: Alcohol-based hand-rubs (ABHR) are widely used for hand hygiene. The presence of many brands with different formulae and prices complicates product selection.**Aim:** To demonstrate differences in antibacterial efficacy, if any, among ABHRs available in India.**Objectives:** 1) Evaluate and compare six common Indian brands of ABHRs, and also the ABHR formulation recommended by the WHO, against 60% iso-propanol. 2) Compare prices of Indian brands**Methods and Material:** Hands were artificially contaminated with Escherichia coli, ATCC[®] 25922™ strain. Bacterial load on hands was tested before and after ABHR use to assess efficacy. Testing technique was a modification of the EN 1500 method of the European Committee for Standardization.**Statistical analysis used:** ANOVA.**Results:** Three products were as effective as 60% iso-propanol, two were better and one was actually worse. Branded ABHR were five to six times more expensive than 60% isopropanol.**Conclusions:** Several expensive brands of ABHR were no better than 60% iso-propanol. Additional CHG made no difference to ABHR efficacy.**Key Messages:** Four of the six Indian ABHR brands tested were no better than 60% iso-propanol, the reference standard handrub of the European Committee for Standardization. CHG did not demonstrate better antibacterial efficacy in the 30 seconds of hand rubbing provided ABHRs were tested with CHG neutralizing products to eliminate the carry-over effect.© This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Hand hygiene is one of the most effective measures for controlling healthcare-associated infections.¹ Hand hygiene may be achieved by either washing hands with soap and water, or rubbing hands with an alcohol-based handrub (ABHR).

ABHRs came into use in a big way after the year 2000 when Pittet et al. showed in a landmark publication that hand hygiene with ABHRs could reduce healthcare associated infections (HAI).² Subsequently ABHRs were defined as the gold standard of care for hand hygiene in

healthcare settings and hand washing came to be reserved only for particular situations, such as visible soiling of hands and after caring for diarrhoea patients without gloves.³

Alcohol-based sanitizers are preferred because they work faster (20-30 seconds), are less irritating to the skin than soap and water, and do not require the use of paper towels for drying hands afterwards. Hand washing is preferred only when hands are visibly soiled with body fluids or excretions, and after caring for patients with diarrhoea caused by alcohol-resistant pathogens such as Clostridium difficile or norovirus without wearing gloves.

Modern hospitals spend substantial funds on ABHRs. However, the existence of a large number of brands makes it difficult to come to an informed decision on the most cost-effective product.

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The most important differences among different products are as follows:

1. The nature of alcohol - this can be either ethanol or iso-propanol. This is not of great importance because ethanol and iso-propanol are practically equivalent in their activity against vegetative bacteria.⁴
2. The concentration of alcohol. The most effective concentrations are considered to be 70% V/V for ethanol and 60% V/V for iso-propanol, but there is evidence to show that higher concentrations can be more effective, at least under "in-use" conditions.⁵
3. The presence of gelling agents. Alcohol solutions are more effective than gels containing the same concentration of alcohol.⁶ Nevertheless, gels continue to be widely sold because users prefer the softer feel of a gel on their hands.
4. The presence of chlorhexidine gluconate (CHG). Most ABHRs used in studies on the efficacy of hand hygiene in infection control have contained CHG, which is traditionally considered to impart residual activity. However, there is convincing evidence to suggest that the residual activity of CHG in an ABHR is a laboratory artefact, caused by the carry-over of CHG into culture media used to determine bacterial counts.⁷
5. The presence of additional ingredients. Some ABHR brands in India contain herbal ingredients in addition to alcohol and gelling agents. The antimicrobial value of these ingredients remains untested.
6. Price. The most expensive ABHR in the Indian market costs more than twice as much as the cheapest brand.

Therefore, it was decided to study and compare the most widely sold ABHRs in India in terms of

1. Efficacy in reducing the number of test bacteria in artificially contaminated hands under "in use" conditions
2. Residual action of added CHG, if any

2. Materials and Methods

The study material was done on six ABHR brands widely sold in India. The only selection criteria were availability in India, and alcohol-based composition. In addition, we tested 75% iso-propanol made "in house" for use within our hospital according to WHO guidelines.⁸ All products were evaluated against 60% iso-propanol, which is the reference standard handrub of the European Committee for Standardization.⁹

Tests were conducted with *Escherichia coli*, ATCC® 25922TM strain, a Biosafety Level 1 pathogen that is sensitive to all relevant antimicrobials, and can be safely handled on an open laboratory bench without special precautions.

2.1. Study design

Prospective. Volunteers were blinded to the identity of the products tested.

2.2. Human or animal subjects

None.

2.3. Data collection

Direct recording of experimental findings in the microbiology laboratory.

3. Experimental method:

Note: ABHRs were tested by the method specified in the following European Standard: EN 1500. Chemical disinfectants and antiseptics. Hygienic handrub. Test method and requirement (phase 2, step 2). 1997, with two exceptions:

1. EN 1500 requires every ABHR to be evaluated by 18 - 20 volunteers. We used only one volunteer, but that volunteer did the experiment in triplicate, making it the equivalent of three volunteers.
2. Instead of pour plates specified by EN 1500, we inoculated serial dilutions of bacterial suspensions in microtitre plates and used MPN tables based on Poisson's distribution rules to derive CFU count.¹⁰ This technique gave reproducible counts in our hands.

3.1. Experimental method

All ABHRs were tested separately in triplicate experiments and the same volunteers were used for all three experiments. This design was justified by the knowledge that hands were artificially contaminated for each experiment and that the artificial contamination resulted in highly reproducible bacterial counts.

Before each experiment, hands were first washed for one minute with soap (free from antibacterial agents) and tap water, and dried with paper towels. Afterwards, hands were disinfected with 60% iso-propanol for 20 seconds and allowed to dry for three minutes to allow all residual alcohol to evaporate before proceeding for the actual experiment.

E. coli, ATCC® 25922TM strain, was grown in two test tubes, each containing 5-ml of soybean casein digest broth (TSB), for 24 h at 36 ± 1°C. After 24 hours, the contents of each tube were inoculated into one Erlenmeyer flask each with 1-litre of TSB per flask; the flasks were then incubated at 36 ± 1°C for 24 hours to reach the stationary phase of growth. The contents of both flasks were then pooled in a 2-litre beaker to yield a suspension containing between 2 × 10⁸ and 2 × 10⁹ colony forming units (CFU) of *E. coli* / ml.

The right hand of the volunteer was then immersed in the *E. coli* culture up to the mid-metacarpal level for five seconds with the fingers spread apart. The hand was then withdrawn from the beaker and allowed to dry in air for three minutes to simulate the contamination of hands that occurs after patient care.

Fingertips were then rubbed in 10-ml Butterfield's phosphate buffer in a Petri dish for one minute to dislodge bacteria attached to the skin. This fluid with eluted bacteria was used to count the number of bacteria present on the hand before ABHR treatment. The fluid was referred to as the pre-fluid and the number of bacteria present in this fluid was referred to as the pre-value.

Hands were again washed with soft soap and tap water and dried with a paper towel. Subsequently the right hand was again immersed in the *E. coli* culture to artificially contaminate it and then withdrawn and dried. Afterwards, the hand was treated with 3-ml of an ABHR and rubbed for 20 seconds according to prescribed technique. After the ABHR dried, fingertips were again rubbed in 10-ml Butterfield's phosphate buffer for one minute to dislodge bacteria from the hand, and this fluid with eluted bacteria was used to count the number of bacteria present on the hand after ABHR treatment. This fluid was referred to as the post-fluid and the number of bacteria present in this fluid was referred to as the post-value. Pre-value and post-value for each handrub was tested in triplicate and the average value was taken for calculating bactericidal efficacy and comparing products.

For experiments involving CHG-containing handrub, the eluting buffer as well as the MacConkey broth contained a cocktail of CHG neutralizers as follows: 3% Tween-80, 3% saponin, 1% sodium dodecyl sulfate, 0.5% sodium thiosulfate, 0.3% lecithin, and 0.1% L-histidine. The suitability of these agents for neutralizing CHG has already been demonstrated.⁷

3.2. Counting bacteria

1. Pre-fluids and post-fluids were subjected to sequential 10-fold dilution in Butterfield's phosphate buffer in test tubes to achieve an ultimate dilution of one in hundred billion or 1:10.¹¹
2. 100- μ l of each dilution was then inoculated into each of 8 wells of one column in a 96-well microtitre plate, starting from neat in column 1 and ending with 1:10¹¹ in column 12.
3. 100- μ l of double-strength MacConkey broth with bromocresol purple was then added to each well. Plates were moved 10 times along a circular path on a horizontal surface to ensure complete mixture of the diluted inoculum with the double-strength broth. Plates were sealed with tape on the top to prevent evaporation, and incubated at $36 \pm 1^\circ\text{C}$ for 24 hours. A change of color in a well from purple to yellow

was considered indicative of growth, and was evidence that the particular well had been inoculated with one or more colony forming units (CFUs). Wells that remained purple were considered free from bacterial growth, and therefore not to have received even one CFU during inoculation.

4. The number of wells at each dilution that had evidence of growth was counted and tabulated. The mean probable number of CFU / ml was then calculated from an MPN table.¹¹
5. Average CFU / ml in pre-fluid was then divided by average CFU / ml in post-fluid to derive the fraction of bacteria killed by ABHR; the \log_{10} reduction of bacterial colony count after ABHR was then calculated from a log table.

4. Results

The average \log_{10} reduction in the colony count of *E. coli* after the use of different ABHRs under standardized conditions is shown below in Table 1. All values have been calculated from the mean of results obtained from experiments performed in triplicate.

Table 2 The prices (per 100 ml) of different ABHRs were as follows:

5. Discussion

This is the first study comparing the efficacy of ABHRs available in the Indian market. Our study had some limitations because of resource limitations and these are mentioned below.

EN 1500 requires every ABHR to be evaluated by 18 - 20 volunteers. We used only one volunteer, but that volunteer did the experiment in triplicate, making it the equivalent of three volunteers. We consider this to be enough because bacterial counts in the triplicate experiments were always within 5% of the mean.

EN 1500 uses the pour-plate method on serial dilutions of bacterial suspensions and derives direct CFU counts by counting. We inoculated serial dilutions of bacterial suspensions in microtitre plates and used MPN tables based on Poisson's distribution rules to derive CFU count.¹¹ This technique gave precise estimates on CFU count in our hands, and took much less time to set up and read than pour plates.

Therefore, our project should be considered a pilot experiment that should be followed up with more detailed studies.

To conclude, only three ABHRs showed a statistically significant difference from 60% iso-propanol, of which Product H and Product 3A were better while Product D proved to be actually worse. Product 3C did not prove to be superior to 60% iso-propanol when it was tested after neutralizing the residual activity of CHG. Product L

Table 1: Summary Results of Bacterial Counts in Artificially Contaminated Hands before and after using ABHR, with Mean log₁₀ Reduction and SD

Product	Average log ₁₀ MPN before ABHR	Average log ₁₀ MPN after ABHR	Mean log ₁₀ reduction in MPN	Standard Deviation (SD)
60% iso-propanol	7.235856725	3.556748618	3.679108108	0.131823
75% iso-propanol (WHO)	7.334119983	2.857275083	4.4768449	0.874794
Product L	5.335552628	2.14987755	3.185675078	0.117045
Product D	5.338885961	2.474289592	2.86459637	0.467831
Product H	7.092917867	2.289587319	4.803330548	0.235642
Product R	7.092917867	3.167329958	3.925587909	0.411103
Product 3A	7.152091638	0.679407546	6.472684091	0.681064
Product 3C	7.667820177	3.116749339	4.551070838	0.654325

ANOVA F=14.29. D.F. = 7, P = 0.0001

Table 2: Price of Commonly Available ABHR Products in India

Product	Price/100 ml (INR)
Product 3C	135
Product 3A	130
Product D	100
Product L	110
Product H	100
Product R	110
75% iso-propanol handrub (WHO)*	30
60% iso-propanol*	22

* Prices of 60% iso-propanol and 75% iso-propanol calculated from the wholesale prices of iso-propanol and glycerol (glycerol present only in the WHO formulation), and includes the price of plastic dispensers (assuming they are washed and reused five times before disposal) and 10% overhead for manpower and electricity.

and Product R did not perform any better than 60% iso-propanol either despite costing five-times and three-times more respectively.

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8. Conflict of Interest

The authors declare they have no conflict of interest.

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