Evaluation of clinical efficacy and safety of PureHands in hand hygiene

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ABSTRACT
Nosocomial infections lead to prolonged hospital stays, substantial morbidity and mortality. The hands of HCWs are the primary mode of transmission of multidrug-resistant pathogens and proper hand hygiene is the single most important, simplest and least expensive means of preventing nosocomial infections. This study was planned to evaluate the clinical efficacy and safety of “PureHands” in hand hygiene in HCWs.

The study was a double-blind, randomized and controlled clinical trial involving 16 HCWs who were without any signs of skin abrasions and infections. Informed written consent was obtained from all HCWs and they were divided into 2 groups. The first group used PureHands, while the second group used placebo for ensuring hand hygiene. On the first day of the study, 2 swabs were taken from both hands of all HCWs, which were inoculated and 2 samples per person were cultured in Mac Conkey’s and blood agar media. PureHands gel was squeezed out on the palms of the HCWs and they were asked to rub it thoroughly on their palms, back of the hands, fingernails and grooves briskly until dry. Subsequently, 2 swab samples were taken after 3, 10 and 30 minutes, which were cultured. All the HCWs were advised to use the PureHands at least for 5 times-a-day from the second day to the seventh day. On the 3rd, 5th and 7th, two samples from each person were cultured. Subsequently, all HCWs were advised not to use PureHands for next 2 days and on 10th day, two more samples were taken from each of the HCW’s hands. At each follow-up visit (day 3, 5, 7 and 10), the investigators recorded any information about adverse events and local examination of hands was done. All adverse events were recorded with information about severity, duration and action taken regarding the study drug. The predefined primary end points were reduction in the absolute microbiological count for following microorganisms: Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella species and Pseudomonas aeruginosa. The predefined secondary end points were absence of any adverse reactions and reduction in the total residual microbial count on 3rd, 5th and 7th day. The statistical analysis was conducted on an intention-to-treat basis.

There was no significant difference in the microbial load between drug and placebo groups at baseline. There was a significant reduction in the total microbial load with drug as compared to placebo, at 3, 10 and 30 minutes on day 1. There was a highly significant reduction in the total microbial load with drug as compared to placebo, at day 3, 5 and 7. There were no adverse reactions during the entire study period and overall compliance to the drug was excellent. Therefore, this study concludes that PureHands is clinically effective and safe for use as a hand sanitizer, to reduce the risk of nosocomial infections amongst HCWs.
INTRODUCTION
Infections that are acquired, while a patient is admitted in a hospital or when a patient is using healthcare services (diagnostic or preventive) are referred as “nosocomial infections” (a term derived from 'nosos' the Greek word for 'disease') or “iatrogenic infections”. Historically, the term ‘nosocomial infections’ was used to describe the infections acquired in the hospitals only, but later it was realized that, patients who have utilized health care services also have been found to develop certain nosocomial infections. Nosocomial infections represent a major problem in health care facilities, resulting in prolonged hospital stays and substantial morbidity and mortality. About one patient in 10 of those who come in contact with hospital and health care settings acquires a nosocomial infection. In the United States, the nosocomial infection rate is about 5 to 6 hospital-acquired infections for every 100 admissions and nosocomial infections have contributed to more than 88,000 deaths (one death every 6 minutes). No such data is available about India due to lack of healthcare data linkage.

Nosocomial infections affect nearly 10% of HCWs. In addition, multidrug-resistant pathogens are commonly involved in such infections and render effective treatment challenging. The hands of HCWs are the primary route of transmission of these multidrug-resistant pathogens. However, inconsistent isolation practices, misuse of antimicrobials, ability of these organisms to survive in dry-state for long periods, failure to conduct active surveillance cultures and poor adherence to hand hygiene practice all have a key role to play in increasing the incidence of nosocomial infections. Proper hand hygiene is the most important, simplest, and least expensive means of preventing nosocomial infections and the spread of antimicrobial resistance.

PureHands is an alcohol-based polyherbal hand sanitizer and it contains extracts of Coriandrum sativum, Citrus limon, Azadirachta indica, Vetiveria zizanioides, Coleus vettiveroides in ethyl alcohol (w/w 60%). This study was planned to evaluate the clinical efficacy and safety of PureHands in ensuring hand hygiene in case of HCWs.

Study Aim
The aim of the study was to evaluate the antimicrobial efficacy and safety (short- and long-term) of PureHands as a hand sanitizer for HCWs in hospital settings.

MATERIALS AND METHODS
Study design
The study was a double-blind, randomized and controlled clinical trial involving 16 HCWs during May 2004 at a 150-bedded teaching hospital at Pune. The study was conducted in accordance with the principles stated in the Declaration of Helsinki, with strict adherence to the GCP guidelines.

Inclusion criteria
A total of 16 HCWs (4 doctors, 4 nurses, 4 ward boys and 4 laboratory technicians), without any signs of abrasion/s, wound/s and infection/s on the skin of hand were included in the study.

Exclusion criteria
Healthcare workers with any visible signs of abrasions, wounds or infections on the skin of hand and those who were unwilling to give informed consent were excluded from the study.
Study procedures
Informed, written consent was obtained from all HCWs in the structured format. Each HCW’s demographic characteristics and a detailed medical history were recorded in structured a CRF. All enrolled HCWs were randomly divided into 2 groups with help of computerized random-number generator programme. The first group used PureHands, while the second group used placebo for ensuring hand hygiene. After stratification, double-blinding was done and decoding of the drug was done only after the end of the trial.

On the first day of the study, 2 swabs were taken (from both the hands: ventral and dorsal surface, including nails and fingers) with sterile cotton swab sticks from all the HCWs. These swabs were inoculated and 2 samples per person were cultured in Mac Conkey’s and blood agar media. Approximately 5 ml of PureHands gel was squeezed out on the palms of the HCWs and they were asked to rub thoroughly on their palms, back of the hands and fingernails briskly until dry. Subsequently, 2 swab samples were taken after 3, 10 minutes and 30 minutes and the swabs were cultured. The petri dishes were incubated at 37°C for 48 hours. Smear prepared from the culture or colony were stained by Gram’s stain and were examined microscopically.

All the HCWs were advised to use the PureHands at lease for 5 times-a-day from the 2nd day to the 7th day. On the 3rd, 5th and 7th day, two samples from each person were cultured for microbial growth. Subsequently, all the HCWs were advised not to use PureHands for the next 2 days and on 10th day, two more samples were taken from each HCW’s hands.

Follow-up and monitoring
At each follow-up visit (day 3, 5, 7 and 10), the investigators recorded any information about adverse events (if any) and local examination of the hands was done.

Adverse events
All adverse events reported or observed by HCWs were recorded in the CRF, with information about onset, severity (mild, moderate or severe), duration and action taken regarding the study drug.

Relation of adverse events to the study medication were described as ‘Unrelated’ (a reaction that does not follow a reasonable temporal sequence from the time of administration of the drug), ‘Possible’ (follows a known response pattern to the suspected drug, but could have been produced by the HCW’s clinical state or other modes of therapy administered to the HCW) and ‘Probable’ (follows a known response pattern to the suspected drug; that could not be reasonably explained by the known characteristics of the HCW’s clinical state). Treatment failure was judged by absence of any reduction in the absolute microbiological count, from 3rd day onwards.

Primary and secondary end points
The predefined primary end points were reduction in the absolute microbiological count for following microorganisms: Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella species and Pseudomonas aeruginosa. The predefined secondary end points were absence of any adverse reactions (burning sensation, skin rash, local irritation and erythema) and reduction in the total residual microbial count on 3rd, 5th and 7th day.

Analysis of data
All the analysis was conducted on an intention-to-treat basis. Statistical analysis was performed using “Unpaired ‘t’ Test” and “Repeated Measures ANOVA Test”, followed by
"Dunnett's Multiple Comparison Test". The minimum level of significance was fixed at 95% confidence limit and a 2-sided $p$ value of $<0.05$ was considered as significant.

**RESULTS**

There was no significant difference in the microbial load between drug and placebo groups at baseline (Mean ± SEM of PureHands and placebo group (CFU/cm$^2$): 271.1 ± 68.02 and 277.0 ± 107.7, difference between means: -5.875 ± 127.4, 99% confidence interval= -385.2 to 373.5, R squared=0.0001518, F=2.509, P=0.2480, NS, (F test to compare variances) and $t$=0.04611, df=14, $p=0.9639$, NS (Unpaired ‘$t$’ test) (Figure 1).

There was a significant reduction in the total microbial load between PureHands group as compared to the placebo group at 3 minutes on day 1 (Mean ± SEM of PureHands and placebo group (CFU/cm$^2$): 45.38 ± 10.09 and 204.8 ± 68.75, difference between means: -159.4 ± 69.48, 99% confidence interval= -366.2 to 47.48, $t$=2.294, df=14, $p=0.0378$, S (Unpaired ‘$t$’ test) and R squared=0.2731, F=46.43, $p<0.0001$, S, (F test to compare variances) (Figure 2).

There was a significant reduction in the total microbial load between PureHands group as compared to the placebo group at 10 minutes on day 1 (Mean ± SEM of PureHands and placebo group (CFU/cm$^2$): 20.63 ± 4.251 and 160.5 ± 49.83, difference between means: -139.9 ± 50.02, 95% confidence interval= -247.2 to –32.59, $t$=2.797, df=14, $p=0.0143$, S (Unpaired ‘$t$’ test) and R squared=0.3584, F=137.4, $p<0.0001$, S, (F test to compare variances) (Figure 3).

There was a significant reduction in the total microbial load between PureHands group as compared to the placebo group at 30 minutes on day 1 (Mean ± SEM of PureHands and placebo group (CFU/cm$^2$): 10.00 ± 4.559 and 90.75 ± 29.38, difference between means: -80.75 ± 29.73, 95% confidence interval= -144.5 to –16.98, $t$=2.716, df=14,
There was a highly significant reduction in the total microbial load in the PureHands group as compared to the placebo group, at day 3, 5 and 7 (F=6.217, R squared=0.4373, p<0.0001, S) (Repeated Measures ANOVA Test) (Table 1).

There was no significant difference in the microbial load between drug and placebo groups at day 10 (t=1.880, p=0.0810, NS) (Unpaired ‘t’ test).

Staphylococcus aureus was the commonest isolated microorganism from all HCWs and the other isolated microorganisms were coagulase negative Staphylococci, Bacillus subtilis and a few fungal colonies. There were nonsignificant isolates of gram negative bacilli (Klebsiella species, Escherichia coli). There was not a single isolate of Pseudomonas aeruginosa and Enterococcus faecalis.

There were no adverse reactions (either reported or observed) during the entire study period and overall compliance to the drug was excellent.

DISCUSSION

Modern understanding of nosocomial infection pre-dates the infancy of microbiology as a discipline and the entire concept of infection control is grounded in the work of Ignaz Semmelweis, who in the 1840's demonstrated the importance of hand hygiene in controlling the transmission of infection in hospitals. In 1985, the Center for Disease Control published a study report on the efficacy of nosocomial infection control, in which role of 4 key infection control components (hospital epidemiologist, clinical microbiologist, active surveillance mechanisms and ongoing control efforts) were emphasized and following implementation of these measures nosocomial infection rates were reduced by one third.

Normal human skin harbors bacteria usually between \(10^2\) and \(10^6\) CFU/cm\(^2\). Resident flora (Corynebacterium diphtheriae, Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus viridans), which colonize deeper layers of the skin are more resistant to mechanical removal and have lower pathogenic potential. Transient flora (Staphylococcus aureus, gram-negative bacilli, Candida species), which colonize the superficial layers of the skin for a short period are responsible for most of the healthcare-associated infections\(^3\).

During daily activity, HCWs progressively accumulate microorganisms on their hands from direct patient contact or from contact with contaminated environmental surfaces and devices\(^4\).
A number of risk factors have been linked with the development of nosocomial infections. These risk factors overlap, but may be considered broadly as underlying host defects (immunosuppression, old age) and mechanical predispositions (being bedridden, exposed to invasive medical devices like intravascular catheters). The most important contributing factor is that many HCWs fail to follow basic infection control procedures such as hand washing between patient contacts. In ICUs, emergency rooms and pre- and post-operative rooms, asepsis is often overlooked in the rush of crisis care. A 2nd contributing factor is the overuse of antimicrobials. Widespread use of cephalosporins is often cited as a cause for the emergence of *Enterococci* and MRSA. This led to the overuse of vancomycin and now medical institutions are faced with a resident flora of "super-bugs", resistant to the most potent antimicrobials. A 3rd contributing factor is the dust in the hospital environment dust and suspended particulate matter, which contain many pathogenic fungal spores, toxic molds that can cause severe nosocomial fungal infections and illness due to atypical pathogens (*Legionella pneumophilia*).

“Hand washing” refers to the process of application of a non-antimicrobial soap by rubbing the hands together for a minute, rinsing them with water and drying thoroughly with a disposable towel. The cleaning activity is attributed to detergent property, which result in mechanical removal of dirt and loosely adherent flora. The term "hand antisepsis" indicates hand hygiene with an antiseptic agent (an antimicrobial soap or an alcohol-based hand rub). In contrast to ‘hand washing’, the objective of ‘hand antisepsis’ is more effective and rapid reduction of skin flora by killing (not mechanically removing microorganisms) the microorganisms. In “hand antisepsis”, vigorous friction, rinsing with water and drying with a towel are unnecessary. Instead, the technique consists of rubbing alcohol onto both the hands until it completely evaporates (15 to 30 seconds). Most dispensers deliver 1.5 to 2.5 mL of alcohol per application; therefore, 2 applications are usually necessary to completely cover both the hands.

Multiple studies have shown those understaffing and increased workloads are risk factors for healthcare-associated epidemics. Switching to alcohol-based hand rubs decreases the time necessary for hand hygiene and in addition, HCWs can use the alcohol-based hand rub while walking to the next patient, saving additional time and human resources.

Alcohol-based hand rubs eliminate the risk of hand contamination or microbial dispersal into the environment as alcohol kills rather than remove the microorganisms. Contamination of alcohol-based solutions with vegetative bacterial forms has not been reported and alcohol dispensers can be reused as long as they are not visibly soiled. Moreover, alcohol-based hand rubs cause substantially less skin irritation and dryness than washing with soap. Hand washing removes lipids from the skin, whereas alcohol compounds only redistribute them. Allergies to alcohol are extremely rare. Despite extensive use, there is no evidence of microbial resistance (*in vitro or in vivo*), suggesting that the mechanism of action (protein denaturation) or the rapid killing effect might not allow the development of resistance. In addition, the rapid evaporation of alcohol prevents extended exposure of microorganisms to sub-inhibitory concentrations of alcohol, possibly reducing the risk of emergence of resistance.

The present study observed a significant reduction in the total microbial load in the PureHands group, as compared to the placebo group at 3, 10 and 30 minutes, on day 1. There was a highly significant reduction in the total microbial load in the PureHands group, as compared to the placebo group at day 3, 5 and 7. This indicates excellent antimicrobial action of PureHands and its remarkable residual antimicrobial activity. The non-significant
difference in the microbial load between drug and placebo groups at day 10 confirms the fact that daily usage of PureHands is associated with overall reduction in the microbial load, which is important to prevent risk of transmitting nosocomial infections by HCWs.

The potent antimicrobial properties of PureHands might be due to the synergistic action of its ingredients. The antimicrobial properties of these ingredients have been well studied by various researchers. The principle ingredients of *Vetiveria zizanioides* are valencene, 9-octadecenamide, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, 1,2-benzendicarboxylic acid, di-isoocctyl ester and terpenoids (monoterpenes, sequiterpenes and triterpene)\(^8\). *Citrus limon* contains sugars (glucose, fructose, and sucrose), polysaccharides, organic acids, myoinositol, carotenoids, vitamins, flavonoids, limonoids (limonin and nomilin), volatile oil, alpha-terpinene, alpha-pinene, coumarins, mucilage, pectins and bioflavonoids (eriocitrin and hesperidan)\(^9,10\). Quercetin 3-glucuronide, isoquercitrin and rutin are the main flavonoids from *Coriandrum sativum*\(^11\) and the other active chemicals are monoterpenoids, monoterpenoid glycosides, monoterpenoid glucoside sulfates and aromatic glycosides\(^12\). The principal constituents of *Azadirachta indica* are nimbin, nimbinin and nimbidin.

The ingredients of PureHands have potent antibacterial activity. Chopra et al., reported the inhibition of gram-negative and gram-positive microorganisms by *Azadirachta indica*\(^3\). Satyavati et al., documented the potent inhibition of *Vibrio cholerae, Klebsiella pneumoniae, Mycobacterium tuberculosis* and *Mycobacterium pyogenes* by *Azadirachta indica*. In 2 studies, a strong inhibition of *Streptococcus mutans* and *Streptococcus faecalis* was observed\(^14,15\). De Castillo et al., reported inhibition of *Vibrio cholerae O1* biotype *Eltor* serotype Inaba tox+ by *Citrus limon*\(^16\). Dabbah et al., and Parish et al., observed the potent inhibition of *Salmonella* species (*S. oranienburg, S. montevideo, S. typhimurium, S. heidelberg* and *S. senftenberg*), *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* species (including *Pseudomonas aeruginosa* )\(^17,18\). Kubo et al., reported that the bactericidal action of *Coriandrum sativum* is due to its ability to act as a nonionic surfactant\(^19\). Singh et al., reported the inhibition of common gram-positive and gram-negative pathogenic bacteria by *Coriandrum sativum*\(^20\). Ethyl alcohol inhibits *Staphylococcus aureus, S. aureus* (methicillin resistant), *Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella sonnei, Clostridium difficile, Enterococcus faecalis, E. faecalis* (Vancomycin resistant), *Enterococcus faecium, E. faecium* (Vancomycin resistant), *Escherichia coli*, E. coli (O157: H7), *Klebsiella ozaenae, Listeria monocytogenes, Proteus mirabilis* and *Serratia marcescens*\(^21\). The antimicrobial activity of alcohols is based on protein denaturation. They have excellent and rapid (within seconds) germicidal activity against vegetative bacteria. For hand rubs, ethyl alcohol, isopropanol, and/or n-propanol are used. Alcohol concentrations of 60% to 95% (v/v) kill 3.4 to 5.8 log\(^10\) CFU in 30 seconds, with higher concentrations having better antibacterial activity. However, concentrations of greater than 95% are less potent because water is essential for protein denaturation\(^6\).

The main ingredients of PureHands have strong antifungal action. Khan et al., and Jacobson et al., reported that *Azadirachta indica* inhibits *Candida albicans, Epidermophyton floccosum, Trichophyton ruberum, Trichophyton violaceum, Microsporum nanum, Trichosporon, Geotricum* and *Mentagrophytes*\(^22-24\). The potent antibacterial activity of *Azadirachta indica* is due to the inhibition of cell membrane synthesis in the bacteria\(^16\). Ezzat et al., observed a strong inhibition of *Candida albicans* by *Citrus limon*\(^25\). Chapel et al., observed clinical benefits of *Citrus limon* in Athlete's foot\(^26\), and Alderman et al., documented the anti-aspergillus effect of *Citrus limon*\(^27\). *Citrus limon* is also effective as a natural biocide to disinfect drinking water\(^28\). *Coriandrum sativum* inhibits *Saccharomyces cerevisiae*\(^29\). Ethyl
alcohol inhibits *Candida albicans*, *Epidermophyton*, *Histoplasma capsulatum*, *Microsporum*, *Trichophyton*, *Blastomyces dermatitidis*, *Coccidioides immitis* and *Cryptococcus neoformans*\(^{21}\).

The ingredients of PureHands have been studied for strong antiviral action also. *Azadirachta indica* inhibits *Vaccinia virus*\(^{23}\), *chikungunya virus*, *measles virus*\(^{23}\), and *Group-B Coxsackie viruses*\(^{30}\). *Citrus limon* inhibits *rabies virus*\(^{31}\). Ethyl alcohol has better virucidal activity than other alcohols. In general, alcohol-based hand rubs are approximately 100 times more effective against viruses than any form of hand washing\(^6\). Ethyl alcohol inhibits *adenovirus*, *coronavirus*, *coxackievirus – A and B*, *cytomegalovirus*, *Epstein-Barr Virus*, *Hepatitis (A, B, C, D, E) Virus*, *Herpes Simplex Virus (1 and 2)*, *Human Immunodeficiency (HIV) Virus*, *Human T-lymphotropic Virus*, *Influenza Virus*, *Measles Virus*, *Mumps Virus*, *Norwalk Virus*, *Papilloma Virus*, *Parainfluenza Virus*, *Polio virus*, *Respiratory Syncytial Virus*, *Rhinovirus*, *Rotavirus*, *Rubella Virus* and *Varicella-Zoster Virus*\(^{32}\).

The laboratory and clinical evidence, and ease of use support the use of alcohol-based hand rubs for hand hygiene amongst HCWs. The alcohol-based hand rub technique is microbiologically more effective, more accessible, less likely to cause skin problems and saves time and human resources. As a consequence, alcohol-based hand rubs are associated with substantially better adherence to hand hygiene than hand washing. The only drawback of alcohol-based hand rubs is the occasional dryness of skin. However, this study did not observe any such adverse reaction/s (either reported or observed) during the entire study period, which might be due to the presence of *Coleus vettiveroides* and *Vetiveria zizanioides*, which are potent emollients and act as moisturizing and soothing agents. Therefore, to summarize, the use of alcohol-based hand rubs should replace hand washing as the standard for hand hygiene amongst HCWs, in healthcare settings in all situations in which the hands are not visibly soiled.

**CONCLUSION**

Nosocomial infections represent a major problem in health care facilities, resulting in prolonged hospital stays and substantial morbidity and mortality. The hands of HCWs are the primary route of transmission of multidrug-resistant pathogens. Proper hand hygiene is the most important, simplest, and the least expensive means of preventing nosocomial infections and the spread of antimicrobial resistance. This study was planned to evaluate the clinical efficacy and safety of PureHands in hand hygiene in HCWs.

This study observed a significant difference in the microbial load between drug and placebo groups at 3, 10 and 30 minutes, on day 1, along with significant difference in the microbial load between drug and placebo groups, at day 3, 5 and 7. This study also observed no adverse reaction during the entire study period and an excellent compliance.

Therefore this study concludes that PureHands is clinically effective and safe for use as a hand sanitizer, to reduce the risk of nosocomial infections amongst HCWs.

**REFERENCES**


