Evaluation of the antimicrobial efficacy and safety of PureHands herbal hand sanitizer in hand hygiene and on inanimate objects

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ABSTRACT
Nosocomial infections are increasing alarmingly and have emerged as a critical issue in hospital care outcome. Opportunistic microorganisms primarily cause nosocomial infections; and multidrug-resistant pathogens that are commonly involved in nosocomial infections are difficult to treat. The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients. This study was aimed to evaluate the antimicrobial efficacy and safety of PureHands Herbal Hand Sanitizer in healthy volunteers and also on inanimate objects. The present study was an open, non-comparative prospective study. Two hundred healthy volunteers without any clinical signs of dermal abrasion, trauma and infection were included in the study. Sterile cotton swab sticks were used to take swabs from both hands and swabs were inoculated on the part of the petri dishes marked before application (before) in both aerobic and anaerobic media. Approximately 0.5 ml of PureHands Herbal Hand Sanitizer was squeezed out on the palms of the subjects and they were asked to rub the gel thoroughly on the palms, back of the hands, fingernails until the hands became dry and inoculation was done on the respective dishes, in the part marked (after). The same procedure was repeated for seven consecutive days on all subjects. For evaluating the efficacy and safety of PureHands Herbal Hand Sanitizer on inanimate objects preparation similar procedure was followed. All detected isolates Escherichia coli, Proteus mirabilis, Shigella sonnennei, Staphylococcus aureus and Staphylococcus epidermidis were eliminated, from the hands of all volunteers over the period of 7 days and also from surface of inanimate objects. PureHands Herbal Hand Sanitizer was found to be effective, safe and less likely to cause adverse skin reactions and saves time and human resources. It may be concluded that PureHands Herbal Hand Sanitizer has a significant bacteriostatic effect on the bacteria present on the hands surface and on the surface of inanimate objects.

INTRODUCTION
Nosocomial infections are those that originate or occur in a hospital or health care setting. The incidence of nosocomial infections is alarmingly increasing and has emerged as a critical issue in hospital care outcome; resulting in extended hospitalization, substantial morbidity and mortality, and excessive costs.

Nosocomial infections are the result of three factors occurring in tandem: high prevalence of pathogens, high prevalence of compromised hosts, efficient mechanisms of transmission from patient to patient. These three factors lead not just to a higher likelihood of transmission of
pathogens within hospitals, but also potentially to an evolution of enhanced disease causing potential among microorganisms present within hospitals.

Opportunistic microorganisms primarily cause nosocomial infections and *Enterococcus spp.*, *Escherichia coli*, *Pseudomonas spp.*, and *Staphylococcus aureus* are commonly involved. The sites of nosocomial infections include (in order from most to least common) urinary tract, surgical wounds, respiratory tract, skin (especially burns), blood (bacteremia), gastrointestinal tract, and central nervous system. These pathogens also tend to become incorporated into the normal flora of health care workers. In addition, multidrug-resistant pathogens (*Vancomycin resistant Enterococci*, *Vancomycin intermediately resistant Staphy. aureus*, or *Vancomycin resistant Staphy. aureus*) that are commonly involved in nosocomial infections are difficult to treat. In hospitalized patients and immunosuppressed people *Pseudomonas aeruginosa* is the most commonly detected microorganism. Opportunistic fungal infections have become very important esp. in HIV patients and the highest frequency of opportunistic fungal infections documented are candidiasis, aspergillosis and cryptococcosis.

The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients. Though proper hand hygiene is the single most important, simplest, and least expensive means of preventing health care-associated infections and the spread of antimicrobial resistance; but, unfortunately poor hand-hygiene practices are observed due to lack of scientific knowledge, unawareness of risks, misconceptions (eg, glove use obviates the need for hand hygiene), unavailability of hand-hygiene facilities, understaffing and patient overcrowding.

Normal human skin harbours bacteria (between 102 and 106 CFU/cm²) and during daily activity, health care workers progressively accumulate microorganisms on their hands from direct patient contact or contact with contaminated environmental surfaces and devices. Traditionally, microorganisms residing on the hands are divided into resident and transient flora. Resident flora (e.g. *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridans*) colonizing deeper skin layers are more resistant to mechanical removal has lower pathogenic potential. Transient flora (e.g. *Staphylococcus aureus*, *Gram-negative bacilli*, *Candida species*) colonizes the superficial skin layers for short periods, is usually acquired by contact with a patient or contaminated environment and these microorganisms are easily removed by mechanical means such as hand washing and are responsible for most health care-associated infections and the spread of antimicrobial resistance.

Currently, hand washing and alcohol hand-rub are two fundamentally different hand-hygiene procedures are in use. Hand washing refers to the application of a non-antimicrobial or antimicrobial soap; and mechanical friction is generated by rubbing the hands together for 1 minute, followed by rinsing with water, and then drying thoroughly with a disposable towel. The alcohol hand-rub procedure involves the use of alcohol rather than water. In contrast to hand washing, the objective of alcohol hand-rub is a more effective and rapid reduction of skin flora by killing all transient flora and most resident flora.
PureHands Herbal Hand Sanitizer contains extracts of Hrivera (*Coleus vettiveroides*), Dhanyaka (*Coriandrum sativum*), Nimbuka (*Citrus limon*), Ushira (*Vetiveria zizanioides*), and Nimba (*Azadirachta indica*) processed in Prasanna (*Ethyl Alcohol w/w 60 %). *Coleus vettiveroides* has anti-bacterial, deodorant and cooling properties, fruits of *Coriandrum sativum* has coriandrol which are antimicrobial, *Citrus limon* juice possesses bactericidal and astringent properties, while it protects the skin from oxidative damage, *Vetiveria zizanioides* oil is reported to be used as a astringent, soothing and antibacterial properties, and *Azadirachta indica*’s (*Melia azadirachta*) principal constituents (nimbin, nimbinin and nimbidin) possess antibacterial, fungicidal and antiviral properties. Present study was aimed to evaluate the antimicrobial efficacy and safety of PureHands Herbal Hand Sanitizer in healthy volunteers and also on inanimate objects.

**MATERIAL AND METHODS**

The present study was an open, non-comparative prospective study. Two hundred healthy volunteers without any clinical signs of dermal abrasion, trauma and infection were included in the study. Sterile cotton swab sticks were used to take swabs from both hands (Ventral and Dorsal), including nails and fingers. Sterile petri dishes were serially numbered and were divided into four quarters and 2 parts were marked as before application (Bf) and other 2 parts were marked as after application (Aft). Aerobic and anaerobic media (Nutrient agar and Mac conkey agar) was prepared on the petri dishes and were inoculated on the part of the petri dishes marked before application (Bf) in both aerobic and anaerobic media. Approximately 0.5 ml of PureHands Herbal Hand Sanitizer was squeezed out on the palms of the subjects and they were asked to rub the gel thoroughly on the palms, back of the hands, fingernails until the hands became dry. After 15 seconds of application the swabs were taken by the same method and inoculation was done on the respective dishes, in the part marked (Aft). The petri dishes were incubated in an incubator at 37°C for 24 to 48 hours. The smear prepared from the culture was stained by Gram’s stain and were examined for bacterial presence. The same procedure was repeated for seven consecutive days on all subjects.

For evaluating the efficacy and safety of PureHands Herbal Hand Sanitizer on inanimate objects preparation of the petri dishes was done in similar fashion to that of the in vivo trial. Swabs were taken from laboratory furniture and medical equipments, while bloodstains along with other contaminated sites were excluded from the study. These swabs were inoculated on the part of the petri dishes marked before application (Bf). A little amount of PureHands Herbal Hand Sanitizer was squeezed out and applied on the surface of the same objects, from where the swabs were taken, with the help of a sterile cotton swab and was rubbed till the gel evaporated making the surface dry. After that swabs were taken by the same method and inoculation was done on their respective dishes, in the part marked (Aft). The petri dishes were incubated in an incubator at 37°C for 24 to 48 hours and the smear prepared from the culture was stained by Gram’s stain and were examined for bacterial presence.

The predefined primary efficacy parameter was the microbiological count percent reduction for following microorganisms after 15 second hand rub: *Clostridium difficile, Enterococcus faecalis, Enterococcus faecalis* (VR), *Enterococcus faecium, Enterococcus faecium* (VR), *Escherichia coli, Escherichia coli* (O157, H7), *Klebsiella ozaenae, Listeria monocytogenes,*
Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus epidermidis and Streptococcus pyogenes. The above-mentioned organisms are commonly involved in nosocomial infections. The predefined secondary outcome parameters were concerned to adverse reactions to the drug, which included burning sensation, rash, irritation, allergic reactions, excessive dryness and dermal erythema.

RESULTS
All the detected isolates (Escherichia coli, Proteus mirabilis, Shigella sonnei, Staphylococcus aureus and Staphylococcus epidermidis) were eliminated, from the hands of all volunteers over the period of 7 days (Figure).

Most of the other bacteria isolated were aerobic commensals and a few cases yielded Klebsiella ozaenae. Bacterial growth was seen on all the petri dishes in the part marked before; but most of the culture plates showed no growth in the part marked as after. Similar results were observed in all the plates where culture was done consecutively for seven days. There was no anaerobic bacterial growth observed in any of the anaerobic culture plates.

The culture from inanimate objects yielded B.subtilis in all the cultures with before marking, and there was no bacterial growth was seen in any of the culture plates marked after.

DISCUSSION
The goal of hand hygiene is a sufficient reduction of microbial counts on the skin to prevent cross-transmission of pathogens among patients. It is easier to keep the hands clean than to make them clean. The critical density of microorganisms on the hands needed for the spread of pathogens remains unknown and it may depend on the type and duration of contact, the type of microorganism, the patient's resident flora and their colonization resistance. According to recently revised hand-hygiene guidelines, the use of an alcohol-based hand rub is the preferred method of hand hygiene.

The antimicrobial activity of alcohols is based on protein denaturation and they have excellent, rapid (within seconds) germicidal activity against vegetative bacteria, fungi, and many viruses. Alcohol rubs are highly effective against mycobacteria (the bacteria most resistant to the disinfection process) and multidrug-resistant pathogens. Alcohol rubs are approximately 100 times more effective against viruses than any form of hand washing. The
virucidal activity of alcohol against enveloped viruses (Coronavirus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis B, C and delta Virus, Herpes Simplex Virus 1 and 2, Human Immunodeficiency Virus, Influenza Virus, Polio virus, Varicella-Zoster Virus) and non-enveloped viruses (Adenovirus, Coxsackievirus – A and B, Hepatitis A and B Virus, Norwalk Virus) is good.

Alcohol hand rub offer numerous advantages over non-alcoholic hand disinfectants. Vigorous friction, rinsing with water, and drying with a towel are not needed; as, rubbing alcohol onto both hands until it completely evaporates, usually requires only 15 to 30 seconds.

Multiple studies have shown that understaffing and increased workload is risk factor for health care-associated epidemics. In a mathematical model with 3 opportunities for hand hygiene per health care worker per hour, 100% adherence would result in 1.3 hours of hand washing per shift (17% of total nursing time). Switching to alcohol hand disinfection would decrease the time necessary for hand hygiene to 0.3 hours (4% of total nursing time), and importantly, health care workers simply cannot afford to use almost one fifth of their time for hand washing.

Washed hands can become recontaminated from faucets or by splashes from traps or sinks and the pathogen like Pseudomonas aeruginosa is commonly found in tap water. In addition, plain soaps may become contaminated during use, and waterborne bacteria from the plumbing system may be present in the tap water. In contrast, alcohol hand rubs eliminate the risk of hand contamination or microbial dispersal into the environment because alcohol kills rather than removes 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. In fact, none of the hand-washing techniques, durations of washing, or types of soaps have demonstrated antimicrobial activity equal to or better than that of alcohol-based hand rubs. Multiple in vitro and in vivo experiments have indicated considerably better antimicrobial killing with alcohol hand disinfectants than with hand washing, and the use of alcohol-based hand rubs has been associated with a decrease in nosocomial infection rates.

Alcohol rubs are preferred over alcohol solutions as alcohol solutions lack persistent activity on resident skin flora. The resident flora regrows within hours after exposure to alcohol, presumably from bacteria residing in hair follicles. The addition of disinfectants (chlorhexidine, quaternary ammonium compounds, triclosan, or octenidine) may delay the regrowth of bacteria. This effect is usually desirable only in surgical hand antisepsis, in which long-term antimicrobial effectiveness under gloved hands may be beneficial and till date, no clinical studies have addressed this issue. Alcohol-impregnated wipes are not as effective as alcohol hand rubs and are not recommended for routine hand hygiene.

It is important to recognize that examination gloves do not provide complete protection against acquisition of microorganisms. Microorganisms from patients have been recovered from the hands of up to 30% of HCWs who wore gloves. Gloves should be used only when contact with blood, body fluids, or other potentially infectious materials, mucous membranes,
and nonintact skin are anticipated. Each pair of gloves should be used for the care of only 1 patient. They should be removed immediately after caring for the patient (before touching any surface) and should be changed between care of contaminated and clean body sites on the same patient. Failure to remove gloves after patient contact may result in the spread of nosocomial pathogens among patients or in contamination of surfaces. Gloves should not be washed or reused. Powderless gloves are preferred because alcohol may interact with residual powder and produce a gritty feeling on the hands\textsuperscript{19, 20}.

Despite extensive use, there is no evidence that such resistance has emerged in vitro or in vivo, suggesting that the mechanism of action (protein denaturation) or the rapid killing effect may not allow the development of resistance. In addition, the rapid evaporation of alcohol prevents extended exposure of microorganisms to subinhibitory concentrations of alcohol, possibly reducing the risk of emergence of resistance.

\textit{E. coli}, which is a facultative anaerobic, gram-negative bacteria and is an important opportunistic pathogen responsible for common nosocomial infection as cystitis in catheterised patients. \textit{Proteus mirabilis} is second only to \textit{E. coli} as a cause of acquired urinary tract infections and the enzyme urease, which catalyzes the splitting of urea into ammonia and carbon dioxide, causes the pH of urine to rise, allow unchecked growth of this bacteria. The higher pH is also toxic to renal cells and potentiates the formation of urinary stones. These stones can cause proteus infections to be chronic, as organisms can remain viable within the stones during therapy. Proteus can also cause wound infections, septicemia and pneumonias, in hospitalized patients. \textit{Shigella sonennei}, is a facultative anaerobic, gram-negative bacterium, is strictly a human pathogen and is highly communicable. Hospitalized children are considered to be at high risk, and acute gastroenteritis is due to the invasion of the colonic epithelial cells and multiplication of the organisms within these cells and the lamina propria with attendant cell death and tissue destruction leading to acute inflammation and ulceration of the mucosa. \textit{Staphylococcus aureus} is a facultative anaerobic, gram-positive bacterium and is highly prevalent in the hospital environment as a cause of nosocomial infection. This ubiquitous and hardy organism can produce, as attested to by the long (and probably not complete) list of diseases above, suppurative lesions of virtually any organ in the body. The typical lesion is an abscess, but other presentations are common and toxin-producing strains can cause a number of systemic diseases, notably toxic shock and scalded skin syndromes.

The organism is also a common cause of pneumonia and is the most common cause of food poisoning. Virtually 100\% of \textit{S. aureus} strains are resistant to penicillin G by virtue of a plasmid encoded beta lactamase. Multiply resistant \textit{S. aureus} (MSRA) is becoming more and more common with some strains even developing resistance to the antibiotic of last resort, vancomycin. \textit{Staphylococcus epidermidis} is a common member of the normal flora of skin and mucous membranes. Its large numbers and ubiquitous distribution make it one of the most commonly isolated organisms in the clinical laboratory. The hydrophobic nature of the organism's cell surface facilitates its adherence to synthetic devices. \textit{Klebsiella ozaenae} is facultative anaerobic, gram- bacteria responsible for severe conditions like wound infection, septicaemia, burns infection and cystitis. PureHands Herbal Hand Sanitizer totally eliminated \textit{Klebsiella ozaenae}, suggesting its role in preventing nosocomial infections.
PureHands Herbal Hand Sanitizer showed reliable efficacy in eliminating all common bacteria from the on the surface of the inanimate objects. The excellent antimicrobial activity of PureHands Herbal Hand Sanitizer is indicative of the additive role of *Coleus vettiveroides, Coriandrum sativum, Vetiveria zizanioides,* and *Azadirachta indica* in addition to alcohol. Thus, PureHands Herbal Hand Sanitizer seems to be a promising agent to prevent the nosocomial infections by the most commonly encountered microorganisms.

There was no adverse reaction in PureHands Herbal Hand Sanitizer and probably *Coleus vettiveroides, Citrus limon* and *Vetiveria zizanioides* may have contributed in the total freedom from adverse reactions as like skin drying or irritation; as hand washing removes lipids from the skin; which is responsible for skin drying or irritation, whereas alcohol compounds only redistribute them.

**CONCLUSION**

The hands of health care workers are the most common mode of transmission of pathogens to patients and proper hand hygiene can prevent health care-associated infections and the spread of antimicrobial resistance. Scientific evidence and ease of use support the use of alcohol-based hand rubs for hand hygiene during patient care.

PureHands Herbal Hand Sanitizer was found to be effective, safe and less likely to cause adverse skin reactions and saves time and human resources. It may be concluded that PureHands Herbal Hand Sanitizer has a significant bacteriostatic effect on the hands surface and on the surface of inanimate objects.

**REFERENCES**


CORRIGENDUM (The Antisepitic (2004), 101(3), 112)

In this article title, “Evaluation of the antimicrobial efficacy and safety of PureHands herbal hand sanitizer in hand hygiene and on inanimate objects” by Dr. Sunanda Mondal & Dr. Kolhapure S.A. published in “The Antisepitic”, Vol. 101, No. 2, February 2004 issue vide page numbers 55-58, in page 58, in the II column, Conclusion 2nd paragraph (last 5 lines), i.e. “Microbial per cent count reduction of Escherichia coli, Proteus mirabilis, Shigella sonnennet and Staphylococcus aureus after use of PureHands Herbal Hand Sanitizer” has been wrongly included and please delete the same.

The error is regretted.