Evaluation of efficacy and safety of PureHands in hand hygiene: A randomized, double-blind, placebo-controlled, phase III clinical trial

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INTRODUCTION
Infections acquired by the patient in hospital or while using diagnostic, curative or preventive healthcare services are referred as nosocomial infections or iatrogenic infections. The incidence of nosocomial infections is increasing alarmingly and they represent major problems in healthcare facilities resulting in prolonged hospital stays, and substantial morbidity and mortality.

Modern understanding of nosocomial infections pre-dates the infancy of microbiology as a discipline. The entire concept of infection control is grounded in the work of Ignaz Semmelweis, who in 1840 demonstrated the importance of hand hygiene in controlling infection transmission in hospitals. In the Vienna General Hospital, his investigations led him to conclude that medical students were carrying cadaveric material from the dissection classrooms on their hands and that it was this material that led to the deadly puerperal infections. After considerable struggle with the Viennese medical establishment, he insisted on a strict protocol of hand washing after dissection and before moving to the delivery ward. The effect was a dramatic reduction in the mortality rate.

Despite such dramatic results, infection control efforts remained neglected for almost a century. In 1976, the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) published accreditation standards for infection control, creating the impetus and need for hospitals to provide essential support for infection control programs. In 1985, the Centers for Disease Control published a study report on the Efficacy of Nosocomial Infection Control, in which the role of 4 key infection control components (hospital epidemiologist, clinical microbiologist for every 250 beds, active surveillance mechanisms and ongoing control efforts) was emphasized, and following implementation of these measures, nosocomial infection rates were reduced by one-third.

Insufficient handwashing by HCWs contributes more to the transmission of enteric pathogens than to the transmission of bloodborne or airborne pathogens. PureHands is an alcohol based polyherbal hand sanitizer and it contains the extracts of Coriandrum sativum, Citrus limon, Azadirachta indica, Vetiveria zizanioides, Coleus vettiveroides in 60% w/w ethyl alcohol. This study was planned to evaluate the efficacy and safety of PureHands in hand hygiene for HCWs.

Aim of the study
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.
MATERIAL AND METHODS

Study design
This study was a randomized, placebo-controlled, double-blind, phase III clinical trial, conducted at Kolkata Medical College, Kolkata, India, as per the Declaration of Helsinki, with strict adherence with the good clinical practice ethical guidelines, in July 2004. The study protocol, CRFs, regulatory clearance documents, product related information and informed consent form (in Bengali and English) were submitted to the Institutional Ethics Committee, and were approved by the same.

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

Exclusion criteria
Healthcare workers with any visible signs of abrasion/s, wound/s or infection/s to 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 and each HCW’s demographic characteristics and a detailed medical history was recorded in a structured CRF. All enrolled HCWs were randomly divided into 2 groups with help of computerized random number generator program. The first group used PureHands and the 2nd 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 study.

On the 1st day of the study, 2 swabs were taken (ventral and dorsal surface, including nails and fingers) with sterile cotton swab sticks from both hands of all 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 the palms, back of the hands and fingernails briskly until dry. Subsequent 2 swab samples were taken after 3, 10 minutes and 30 minutes, which were cultured. The petri dishes were incubated at 37ºC 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 least for 5 times a day from the 2nd day to the 7th day. On 3rd, 5th and 7th day, 2 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 the 10th day, 2 more samples were taken from each of the HCW’s hands.

Follow-up and monitoring
At each follow-up visit (3rd, 5th, 7th and 10th day), the investigators recorded any information about adverse events (either reported or observed) and thorough hand examination 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 efficacy end points were reduction in the absolute microbiological count of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella species* and *Bacillus subtilis*. The CFUs of above microorganisms were counted and a predefined score scale was used, which was based on the number of the CFUs (<10 CFUs=0, 11-100 CFUs=1, 101-500 CFUs =2 and >501 CFUs=3).

The predefined secondary safety 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 analyses were conducted on an intention-to-treat basis. Statistical analysis was performed using *Unpaired ‘t’ Test*, *F Test to Compare Variances*, *Repeated Measures ANOVA Test* and *Dunnett’s Multiple Comparison Test*. The minimum level of significance was fixed at 99% confidence limit and a 2-sided *p* value of <0.001 was considered as significant.

**RESULTS**

There was no significant difference in the mean score for microbial load between drug and placebo groups, at zero hour on first day (mean ± SEM, difference between mean, 99% confidence interval, lower 99% CI, upper 99% CI of PureHands and placebo were: 2.688 ± 0.1197 and 2.625 ± 0.1250, 0.0625 ± 0.1731, -0.4134 to 0.5384, 2.335 and 2.257, 3.040 and 2.993, R²=0.004329, t=0.3612, *p*=0.7205, NS (Unpaired ‘t’ Test) and F Test to Compare Variances: F=1.091, DFn=15, Dfd=15, *p*=0.8684, NS).

*S. epidermidis* and *S. aureus* were the commonest isolated microorganism and the other isolated microorganism were *P. aeruginosa*, *B. subtilis*, *K. species*, *E. coli* and a few fungal colonies. There was not a single isolate of *Enterococcus faecalis*.

The mean microbial load score for *S. epidermidis* decreased significantly from 3.00 to 0.00, 0.00 and 0.38 after 3, 10 and 30 minutes respectively in the PureHands group (F=64.53, R²=0.8114, *p*<0.0001, highly significant); while in the placebo group the respective values were 2.94, 2.88, 2.56 and 2.50 (F=1.894, R²=0.1121, *p*=0.0777, non-significant). When compared within the group the mean microbial load score in PureHands group were significantly (*p*<0.001) reduced on day 3, 5 and 7; while there was no significant difference (*p*>0.05) in the placebo group.

![Figure 1: Effect of PureHands and placebo on number of Staphylococcus epidermidis CFUs](image-url)
the 10th day the mean microbial load score was non-significant in both the groups. (Table 1 and Figure 1).

### Table 1: Effect of PureHands and placebo on number of *Staphylococcus epidermidis* CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PureHands</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
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<td>0 Min.</td>
</tr>
<tr>
<td>Mean</td>
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<td>2.94</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Lower 99% CI</td>
<td>3.00</td>
<td>2.75</td>
</tr>
<tr>
<td>Upper 99% CI</td>
<td>3.00</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Repeated Measures ANOVA Test

F=64.53, R²=0.8114, \( p<0.0001 \), Highly significant

### Table 2: Effect of PureHands on number of *Staphylococcus aureus* CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 Min.</strong></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Mean</td>
<td>2.63</td>
<td>2.56</td>
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<td>2.69</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.62</td>
<td>0.52</td>
<td>0.50</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.15</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Lower 99% CI</td>
<td>2.17</td>
<td>-0.11</td>
<td>-0.18</td>
<td>-0.18</td>
<td>-0.18</td>
</tr>
<tr>
<td>Upper 99% CI</td>
<td>3.08</td>
<td>0.48</td>
<td>0.68</td>
<td>0.68</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Repeated Measures ANOVA Test

F=52.21, R²=0.7564, \( p<0.0001 \), Highly significant

The mean microbial load score for *S. aureus* decreased significantly from 2.63 to 0.00, 0.00 and 0.19 after 3, 10 and 30 minutes respectively in the PureHands group (F=52.21, \( R^2=0.7564, \ p<0.0001 \), highly significant). When compared within the group the mean microbial load score in the PureHands group were significantly (\( p<0.001 \)) less on day 3, 5 and 7 and were in the order 0.25, 0.25 and 0.44 respectively; while there was no significant difference (\( p>0.05 \)) in the placebo group. On 10th day the mean microbial load score for *S. aureus* was non-significant in both the groups (Table 2 and Figure 2).
Table 3: Effect of PureHands on number of *Pseudomonas aeruginosa* CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th></th>
<th></th>
<th>Day 3</th>
<th></th>
<th>Day 5</th>
<th></th>
<th>Day 7</th>
<th></th>
<th>Day 10</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Min.</td>
<td>3 Min.</td>
<td>10 Min.</td>
<td>30 Min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.81</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Lower 99% CI</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.52</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Upper 99% CI</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.11</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Repeated Measures ANOVA Test

\[ F=882.6, R^2=0.9833, p<0.0001, \text{Highly significant} \]

The mean microbial load score for *P. aeruginosa* decreased significantly from 3.00 to 0.00, 0.00 and 0.81 after 3, 10 and 30 minutes respectively in the PureHands group (\( F=882.6, \ R^2=0.9833, \ p<0.0001, \text{highly significant} \)). When compared within the group the mean microbial load score in PureHands group were significantly (\( p<0.001 \)) less on day 3, 5 and 7 and were in the order 0.00, 0.00 and 0.00 respectively; while there was no significant difference (\( p>0.05 \)) in the placebo group. On the 10th day the mean microbial load score for *P. aeruginosa* was non-significant in both the groups (Table 3 and Figure 3).

Table 4: Effect of PureHands on number of *Escherichia coli* CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th></th>
<th></th>
<th>Day 3</th>
<th></th>
<th>Day 5</th>
<th></th>
<th>Day 7</th>
<th></th>
<th>Day 10</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Min.</td>
<td>3 Min.</td>
<td>10 Min.</td>
<td>30 Min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.20</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
<td>0.00</td>
<td>0.40</td>
</tr>
<tr>
<td>Std. Deviation</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.22</td>
<td>0.41</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.82</td>
<td>0.00</td>
<td>0.82</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.09</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
<td>0.00</td>
<td>0.18</td>
</tr>
<tr>
<td>Lower 99% CI</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.09</td>
<td>-0.06</td>
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<td>-0.13</td>
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<td>-0.13</td>
</tr>
<tr>
<td>Upper 99% CI</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.19</td>
<td>0.46</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.93</td>
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<td>0.93</td>
</tr>
</tbody>
</table>

Repeated Measures ANOVA Test

\[ F=226.7, R^2=0.9227, p<0.0001, \text{Highly significant} \]

The mean microbial load score for *E. coli* decreased significantly from 3.00 to 0.00, 0.00 and 0.05 after 3, 10 and 30 minutes respectively in the PureHands group (\( F=882.6, \ R^2=0.9833, \ p<0.0001, \text{highly significant} \)). When compared within the group the mean microbial load score in PureHands group were significantly (\( p<0.001 \)) less on day 3, 5 and 7 and were in the order 0.20, 0.00 and 0.00 respectively; while there was no
significant difference ($p>0.05$) in the placebo group. On the 10th day the mean microbial load score for *E. coli* was non-significant in both the groups (Table 4 and Figure 4).

### Table 5: Effect of PureHands on number of *Klebsiella* species CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 Min.</th>
<th>3 Min.</th>
<th>10 Min.</th>
<th>30 Min.</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.19</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Std. Deviation</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
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</tr>
<tr>
<td>Std. Error</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Lower 99% CI</td>
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<tr>
<td>Upper 99% CI</td>
<td>3.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.48</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Repeated Measures ANOVA Test</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean microbial load score for *K. species* decreased significantly from 3.00 to 0.00, 0.00 and 0.19 after 3, 10 and 30 minutes respectively in the PureHands group ($F=873.8$, $R^2=0.9831$, $p<0.0001$, highly significant). When compared within the group the mean microbial load score in the PureHands group were significantly ($p<0.001$) less on day 3, 5 and 7 and were in the order 0.00, 0.00 and 0.00 respectively; while there was no significant difference ($p>0.05$) in the placebo group. On the 10th day the mean microbial load score for *K. species* was non-significant in both the groups (Table 5 and Figure 5).

### Table 6: Effect of PureHands on number of *Bacillus subtilis* CFUs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 Min.</th>
<th>3 Min.</th>
<th>10 Min.</th>
<th>30 Min.</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.36</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
<td>0.09</td>
<td>0.09</td>
<td>0.00</td>
<td>0.82</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.81</td>
<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
<td>0.30</td>
<td>0.30</td>
<td>0.00</td>
<td>0.87</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.24</td>
<td>0.00</td>
<td>0.00</td>
<td>0.12</td>
<td>0.09</td>
<td>0.09</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Lower 99% CI</td>
<td>1.59</td>
<td>0.00</td>
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<td>-0.20</td>
<td>-0.20</td>
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</tr>
<tr>
<td>Upper 99% CI</td>
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<td>0.00</td>
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<td>0.38</td>
<td>0.00</td>
<td>1.65</td>
</tr>
<tr>
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<td></td>
</tr>
</tbody>
</table>

The mean microbial load score for *B. subtilis* decreased significantly from 3.00 to 0.00, 0.00 and 0.18 after 3, 10 and 30 minutes respectively in the PureHands group ($F=31.96$, $R^2=0.7617$, $p<0.0001$, highly significant). When compared within the group the mean microbial load score in the PureHands group were significantly ($p<0.001$) less on day 3, 5 and 7 and were in the order 0.00, 0.00 and 0.00 respectively; while there was no significant difference ($p>0.05$) in the placebo group. On the 10th day the mean microbial load score for *B. subtilis* was non-significant in both the groups (Table 6 and Figure 6).
difference ($p>0.05$) in the placebo group. On the 10th day the mean microbial load score for \textit{B. subtilis} was non-significant in both the groups (Table 6 and Figure 6).

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

**DISCUSSION**

Various epidemiological studies have documented that insufficient handwashing by HCWs contributes more to the transmission of various pathogens, responsible for nosocomial infections. In salmonellosis, institutional disease accounts for about 10% to 30% of all cases\(^1\), and hospitals, nursing homes, pediatric wards and nurseries are the commonly involved sites.\(^2\) In one study the highest attack rate of salmonellosis was seen in laundry workers, suggesting that transmission may occur through contaminated linen.\(^3\) Outbreaks of shigellosis have been reported in day care centers\(^4\) and at a teaching hospital salmonella accounted for 10% cases of nosocomial diarrhea.\(^5\)

The documented overall prevalence rate of HAV infection in HCWs is about 35-54% and in a report, the rates of antibodies to HAV were significantly higher in nurses than in office workers.\(^6\) Many outbreaks of HAV have been reported in pediatric (PICUs), neonatal intensive care units (NICUs)\(^7\) and orphanages.\(^8\) Eating on the hospital ward was found to be the most important risk factor for nosocomial infections, and nosocomial outbreaks have resulted from the consumption of contaminated food.\(^9\)-\(^11\) Eating hospital food and drinking hospital beverages expose HCWs to the same risks faced by patients and visitors, as shown by hospital food-related outbreaks of salmonellosis, hepatitis A, yersiniosis\(^12\), campylobacteriosis\(^13\), cyclospora infection\(^14\) and typhoid fever.\(^15\)

Several recent reports have examined the seroprevalence of antibodies to \textit{Helicobacter pylori} among HCWs.\(^16\) One study found that seroprevalence was higher among endoscopists (69%)\(^17\), while two other studies found that about 52% of endoscopists were seropositive for \textit{H. pylori}.\(^18,19\) However, dentists have been shown to exhibit no increased seroprevalence despite contact with saliva. These findings suggest that contact with contaminated equipment, rather than routine patient care or contact with saliva, is an important mode of transmission.\(^18\)

\textit{Clostridium difficile} has emerged as an important cause of hospital-acquired diarrhea and has been cultured from the hands of asymptomatic HCWs during outbreaks.\(^20\) Possible nosocomial transmission of fatal \textit{C. difficile} infection to otherwise healthy HCWs has recently been reported.\(^21\) Several nursing home outbreaks of infection with the Norwalk virus have resulted transmission to HCWs\(^22\) and in one outbreak, HCWs had an incidence rate of 92%.\(^23\) In an outbreak of \textit{E. coli} O157: H7-associated hemorrhagic colitis in a nursing home\(^24\), 13% HCWs developed symptoms and 27% had bloody diarrhea. Infections like Cholera also had been reported amongst HCWs.

Infection may spread to HCWs as a result of direct contact and outbreaks of scabies among HCWs have been reported from several hospitals.\(^25\) In another outbreak, secondary spread to the spouses of HCWs was documented.\(^26\) A large, sustained outbreak at an extended-care facility resulted in the infection of 26% of the HCWs, including half of all nurses.\(^27,28\) Cutaneous herpes is an occupational hazard for HCWs (esp. dentists, anesthesiologists, dialysis technicians, physiotherapists, physicians\(^29\) and nurses in intensive care units (ICU)).\(^30\) Molecular epidemiologic techniques have shown that dermatologists also face risk of cutaneous herpes associated with laser treatment of warts.\(^31,32\) \textit{Tinea corporis} may spread to HCWs further thwarting control efforts.\(^33\)
Laboratory personnel, pathologists, surgeons, dentists, anesthesiologists, laundry workers, veterinarians and animal handlers are at risk of an array of specific infections. In addition, the concerns of pregnant HCWs are considerable and unique because certain otherwise mild infections may affect fetal development. Laboratory-acquired infections have been extensively studied. Collins identified 2168 infections and 48 deaths from diseases ranging from brucellosis to rabies. *Neisseria meningitidis* has been shown to fatally infect laboratory workers. The potential danger of working in laboratories is dramatically illustrated by the fate of Ricketts, who died of laboratory-acquired rickettsiosis.

80% of nosocomial infections are caused by the microbial flora brought by the patients at the time of admission to the hospital. This stay-at-home flora appears to be opportunistic to the new environment and is able to take advantage of new routes of transmission that medical procedures offer. Other nosocomial infections (20%) develop following contamination with microbial organisms found within the hospital environment (via the hands, instruments of HCWs or contact with contaminated hospital materials). Person-to-person spread of infections in the healthcare setting can occur via direct contact, droplet, airborne, fecal-oral, and blood-borne routes.

A number of risk factors have been linked with the development of nosocomial infections and the most important factor is the prior treatment with broad-spectrum antibiotics, which has been shown to suppress symbiotic intestinal normal microbial flora. Presence of a persistent infection and an extended stay in hospital are other risk factor for acquisition of antibiotic-resistant pathogenic infections. Individuals may also have multiple risk factors and can accordingly be at high risk for nosocomial infections. These risk factors overlap, but may be considered broadly as underlying host defects (i.e. immunosuppression, old age) and mechanical predispositions (being bedridden, invasive medical devices like intravascular catheters).

The most important contributing factor for increase in occurrence of nosocomial infection rates 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 areas, asepsis is often overlooked in the rush of crisis care. During daily activity, HCWs progressively accumulate microorganisms on their hands from direct patient contact or contact with contaminated environmental surfaces and devices. Transient flora colonizes the superficial skin layers for short periods and is usually acquired by contact with a patient or contaminated environmental surfaces and devices. These microorganisms are easily removed by mechanical means such as hand washing. A second contributing factor is the overuse of antimicrobials and the widespread use of cephalosporins is often cited as a cause for the emergence of Enterococci and methicillin resistant *S. aureus* (MRSA), as nosocomial pathogens. This has resulted in the overuse of vancomycin (in response to concerns about MRSA and for treatment of vascular catheter associated infections by resistant coagulase-negative Staphylococci). Now, medical institutions are faced with a resident flora of superbugs, which are resistant to the most potent antimicrobials. A third contributing factor is the hospital environmental dust and suspended particulate matter, which contain many pathogenic fungal spores, toxic molds leading to severe nosocomial fungal infections and illness due to other pathogens, such as *Legionella pneumophilia*.

Despite the seemingly limitless number of infections that HCWs can acquire on the job, the interventions to prevent transmission are simple, well known and effective. Compliance with handwashing, vaccination and appropriate isolation of infected patients can control transmission dramatically. Handwashing is the oldest, simplest, and cheapest way to control the nosocomial spread of infectious organisms.
Hand washing refers to the application of a plain (non-antimicrobial) or antiseptic (antimicrobial) soap, mechanical friction generated by rubbing the hands together for 1 minute (covering all surfaces of the hands and fingers), rinsing with water, and drying thoroughly with a disposable towel (which is then used to turn off the faucet). The cleaning activity is attributed to the detergent properties, which result in mechanical removal of dirt (soil and organic substances) and loosely adherent flora (most transient flora and a small portion of the resident flora) from the hands.

The term hand antisepsis indicates hand hygiene with an antiseptic agent, either washing the hands with an antimicrobial soap or using an alcohol-based hand rub. In contrast to hand washing, the objective of this procedure is a more effective and rapid reduction of skin flora by killing, and not just mechanically removing microorganisms (all transient flora and most resident flora). Therefore, the alcohol hand-rub procedure should not be confused with hand washing, and vigorous friction, rinsing with water, and drying with a towel in case of alcohol hand-rub procedures are unnecessary. Instead, the technique consists of rubbing alcohol onto both hands until it completely evaporates, usually requiring 15 to 30 seconds. Most dispensers deliver 1.5 to 2.0 mL of alcohol per application; therefore, 2 applications are usually necessary to completely cover both hands.

On skin, alcohols (50% to 70% isopropyl alcohol) are effective against common microorganisms involved in nosocomial infections. 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. For hand rubs, ethanol, isopropanol, and/or n-propanol are used. In general, alcohol rubs are approximately 100 times more effective against viruses than any form of hand washing. Transmission of viruses is of concern in a broad range of healthcare institutions, including pediatric wards, bone marrow transplantation units and long-term care facilities. The virucidal activity of alcohol against enveloped viruses (such as influenza virus or human immunodeficiency virus) is good.

Alcohol hand-rub technique is better than hand washing due to various benefits. At least 1 to 2 minutes are required for hand washing compared with 15 to 30 seconds for the alcohol hand-rub technique. In the intensive care unit, where as many as 40 opportunities for hand hygiene per hour of care occur, time constraint becomes the most important limiting factor. Multiple studies have shown that understaffing and increased workload are risk factors for healthcare-associated epidemics. In a mathematical model with 3 opportunities for hand hygiene per HCW per hour, 100% adherence would result in 1.3 hours of hand washing per shift (or 17% of total nursing time). Switching to alcohol hand disinfection would decrease the time necessary for hand hygiene to 0.3 hours (or 4% of total nursing time). In addition, HCWs can use the alcohol rub while walking to the next patient, saving additional time and human resources.

Washed hands can become recontaminated from faucets or by splashes from traps or sinks (*P. 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. Moreover, alcohol 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 and allergies to alcohol are extremely rare.
The increasing use of alcohol for hand hygiene raises concern about the risk for emergence of resistant microorganisms, however, despite extensive use, there is no evidence that such resistance either *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. Scientific evidence and ease of use support the use of alcohol-based hand rubs for hand hygiene during patient care. The alcohol hand-rub technique is microbiologically more effective, more accessible, and less likely to cause skin problems and saves time and human resources. As a consequence, alcohol hand rubs are associated with substantially better adherence to hand hygiene than hand washing.

The other available chemical agents for hand hygiene have numerous limitations as compared to alcohol. Chlorhexidine is less effective against gram-negative bacteria, only fairly active against fungi and minimally active against *Mycobacterium tuberculosis*. Hexachlorophene has a slow onset of action, minimal gram-negative activity and the potential for absorption with resultant neurotoxicity has limited its use. *Para-chloro-meta-xylene* is less effective in reducing skin flora and is neutralized by nonionic surfactants. Triclosan is a poor fungicide and may be irritating. Currently iodophors are used for handwashing, surgical scrubs, and skin preparations, however allergic adverse events and reduction of antimicrobial activity in the presence of organic materials (e.g., blood or sputum) limit its usage.

This study observed a highly significant reduction in the total microbial load in PureHands group, as compared to the placebo group at 3, 10 and 30 minutes respectively, 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 remarkable residual antimicrobial activity. The non-significant difference in the microbial load between drug and placebo groups at 10th day confirms the fact that daily usage of PureHands is associated with overall reduction in 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 actions of its ingredients and 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-isoocyt ester and terpenoids (monoterpenes, sequiterpenes and triterpene).\(^{36}\) *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).\(^{37,38}\) Quercetin 3-glucuronide, isoquercitrin and rutin are the main flavonoids from *Coriandrum sativum*\(^{39}\) and other active chemicals are monoterpenoids, monoterpenoid glucosides, monoterpenoid glucoside sulfates and aromatic glycocisides.\(^{40}\) The principal constituents of *Azadirachta indica* are nimbin, nimbinin and nimbidin.

The ingredients of PureHands have potent antibacterial activity. Chopra et al. reported inhibition of Gram-negative and Gram-positive microorganisms by *Azadirachta indica*.\(^{41}\) The potent antibacterial activity of *Azadirachta indica* is due to the inhibition of cell membrane synthesis in the bacteria.\(^{42}\) Satyavati et al. documented 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. De Castillo et al. reported inhibition of *V. cholerae* O1 biotype Eltor serotype Inaba tox+ by *Citrus limon*. Dabbah et al. and Parish et al. observed potent inhibition of *Salmonella* species (*S. oranienburg, S. montevideo, S. typhimurium, S. heidelberg* and *S. senftenberg*), *E. coli, S. aureus* and *P. species* (including *P. aeruginosa*). Kubo et al. reported that the bactericidal action of *Coriandrum sativum* is due to its ability to act as nonionic surfactant. Singh et al. reported the inhibition of common Gram-positive and Gram-negative pathogenic bacteria by *Coriandrum sativum*. Ethyl alcohol inhibits *S. aureus, S. aureus* (Methicillin resistant), *S. pyogenes, P. aeruginosa, Salmonella typhimurium, Shigella sonnei, C. difficile, Enterococcus faecalis, E. faecalis* (Vancmycin resistant), *Enterococcus faecium, E. faecium* (Vancomycin resistant), *E. scherichia coli, E. coli* (O157; H7), *K. lebisia ozaenae, Listeria monocytogenes, Proteus mirabilis* and *Serratia marcescens*. The antimicrobial activity of alcohols is based on protein denatureation. 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<sub>10</sub> 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. The 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, Geotrichum* and *Mentagrophytes*. Ezzat et al. observed strong inhibition of *C. albicans* by *Citrus limon*. Chapel et al. observed the clinical benefits of *Citrus limon* in athlete's foot, and Alderman et al. documented anti-aspergillus effect of *Citrus limon*. *Citrus limon* is also effective as a natural biocide to disinfect drinking water. *Coriandrum sativum* inhibits *Saccharomyces cerevesiae*. Ethyl alcohol inhibits *C. albicans, Epidermophyton, Histoplasma capsulatum, Microsporum, Trichophyton, Blastomyces dermatitidis, Coccidioides immitis* and *Cryptococcus neoformans*. The ingredients of PureHands have been studied for strong antiviral action also. *Azadirachtra indica* inhibits *Vaccinia virus, chikungunya virus, measles virus*, and *Group-B Coxsackie viruses*. *Citrus limon* inhibits *rabies virus*. 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. Ethyl alcohol inhibits *adenovirus, coronavirus, coxsackievirus – 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-lymphotrophic 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*. The laboratory and clinical evidence, and ease of use support the use of alcohol-based hand rubs for hand hygiene by HCWs. The alcohol 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 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 observed any such adverse reaction/s (either reported or observed) during the entire study period, which might be due to the *Coleus vettiveroides* and *Vetiveria zizanoides*, which are potent emollients and act as moisturizing, soothing agents. Therefore, to summarize, the use of alcohol-based hand rubs should replace hand washing as the standard for hand hygiene for HCWs in healthcare settings in all situations in which the hands are not visibly soiled.
Healthcare workers are responsible for preventing and controlling nosocomial infections. Identifying the patients at risk, proper handwashing by HCWs, improving sterilization and disinfection can control and eliminate many of the common nosocomial infections. All HCWs play an essential roll in this effort including those that touch the skin of an individual patient, as well as those who sterilize, disinfect and store the materials. Together, HCWs can make a safer environment, one free from the diseases found within hospital doors!

CONCLUSION
Infections which are acquired, while a patient is admitted in a hospital or when patient is using healthcare services are referred as nosocomial infections or iatrogenic infections. The incidence of nosocomial infections or iatrogenic infections is increasing alarmingly and is a global health issue. Nosocomial infections represent major problems in healthcare facilities, resulting in prolonged hospital stays and substantial morbidity and mortality. Insufficient handwashing by HCW contributes more to the transmission of enteric pathogens than to the transmission of bloodborne or airborne pathogens. This study was planned to evaluate the clinical efficacy and safety of PureHands in hand hygiene for HCWs.

This study observed a highly significant reduction in the total microbial load in PureHands group, as compared to the placebo group at 3, 10 and 30 minutes respectively, on day 1. There was a highly significant reduction in the total microbial load in the PureHands group, as compared to the placebo group on the 3rd, 5th and 7th day. The non-significant difference in the microbial load between drug and placebo groups on day 10 confirms the fact that daily usage of PureHands is associated with overall reduction in microbial load, which is important to prevent risk of transmitting nosocomial infections by HCWs. This study did not observe any adverse reaction/s (either reported or observed) during the entire study period and overall compliance to PureHands was excellent. Therefore it may be concluded that PureHands is clinically effective and safe for use by HCWs for ensuring hand hygiene.

REFERENCES


