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The impact of alcohol-based and chlorhexidine-based disinfectants on hand microorganisms, skin tolerance and nosocomial infections among healthcare workers

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Abstract: **Background**: Hand hygiene is critical for preventing hospital-acquired infections (HAIs), yet the comparative effects of alcohol-based versus chlorhexidine-based disinfectants on microbial load, skin tolerance and infection rates among healthcare workers (HCWs) remain underexplored within the same clinical setting. **Objectives**: To compare the impact of alcohol-based and chlorhexidine-based disinfectants on hand microbiota reduction, skin tolerance and the incidence of HAIs among HCWs. **Methods**: This retrospective cohort study analyzed 136 healthcare workers at a single hospital. Participants were divided into two phases: an alcohol-based disinfectant phase from January 1, 2022, to June 30, 2022 and a chlorhexidine-based disinfectant phase from July 1, 2022, to December 31, 2022. Bacterial counts pre- and post-disinfection, skin condition assessments and hospital acquired infection (HAI) rates were analyzed. Results: Immediate bactericidal efficacy was superior in the alcohol group (1.60 ± 0.15 CFU/cm²) compared to the chlorhexidine group (2.80 ± 0.61 CFU/cm², $P < 0.001$). Chlorhexidine exhibited more sustained antimicrobial effects, with superior efficacy at 4 hours post-disinfection (7.20 ± 1.32 CFU/cm² vs. 9.53 ± 1.66 CFU/cm² for alcohol, $P < 0.001$). Skin tolerance was better for chlorhexidine. HAI incidence was slightly lower in the chlorhexidine group (3.80%) compared to the alcohol group (5.76%, $P = 0.427$). **Conclusion**: Alcohol provides immediate bactericidal action and chlorhexidine offers prolonged effects. It is recommended to use disinfectants according to the preferences of healthcare personnel.

Keywords: Alcohol-based disinfectants; Bacterial reduction; Chlorhexidine; Hand hygiene; Hospital-acquired infections; Skin tolerance

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INTRODUCTION

Effective hand hygiene practices are universally acknowledged as a vital element in preventing nosocomial infections (hospital-acquired infections, HAIs), which are a significant concern in healthcare settings worldwide (Bredin *et al*., 2022; Spruce, 2021; Van Wicklin, 2021). The World Health Organization and Centers for Disease Control and Prevention have consistently emphasized the importance of hand hygiene in reducing the transmission of infectious agents in healthcare environments (Kramer *et al*., 2022). At the heart of these hand hygiene practices is the utilization of antiseptic solutions, predominantly alcohol-based and chlorhexidine-based disinfectants. These agents are extensively employed to reduce the microbial load on the hands of healthcare professionals (Croke, 2022; Speth, 2023). However, while the efficacy of these disinfectants was well-documented (Akita *et al*., 2022; Voniatis *et al*., 2023). There remains a need for further investigation into their comparative impacts on hand microorganisms, skin tolerance and the consequent rates of HAIs.

Alcohol-based disinfectants are recognized for their broad-spectrum antimicrobial efficacy, rapid action and capacity to denature proteins, thereby disrupting microbial membranes almost instantaneously (Gold *et al*., 2026; Oh *et al*., 2023). These characteristics render alcohol-based solutions highly effective for immediate sanitization purposes. However, the inherent volatility of alcohol leads to a transient biocidal effect, which may require frequent reapplication to maintain efficacy. In contrast, chlorhexidine-based formulations demonstrate a slower onset of bactericidal activity but provide extended antimicrobial protection (Bertasi *et al*., 2022; Kucuker *et al*., 2023). This sustained action can be attributed to chlorhexidine's capacity to adhere to the keratin layer of the skin, thereby facilitating prolonged microbial reduction. The distinct temporal profiles exhibited by these disinfectants warrant a comprehensive evaluation to determine their efficacy across various time intervals and under diverse healthcare conditions.

In addition to their antimicrobial efficacy, the repeated use of these solutions raises concerns regarding skin tolerance among healthcare workers, who are required to sanitize their hands numerous times daily as part of infection

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control protocols. Alcohol-based disinfectants can lead to skin dryness and irritation over time due to their dehydrating properties. Conversely, chlorhexidine offers persistent antimicrobial benefits; it may also affect skin barrier function; however, it is generally regarded as less irritating than alcohol-based solutions. The balance between maintaining effective hand hygiene and ensuring dermal health is delicate, as skin irritation and damage can result in poor compliance with hygiene protocols, thereby undermining efforts to control the spread of infections (Lotfinejad *et al*., 2021; Abbas and Stevens, 2024).

The dynamics of microbial presence on the skin of healthcare workers, coupled with the potential development of resistant bacteria, further underscore the importance of selecting appropriate disinfectants. Healthcare-associated infections were often compounded by resistant strains of bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing organisms (Nazli *et al*., 2024). Both types of disinfectants have exhibited varying degrees of efficacy against resistant pathogens, underscoring the importance of assessing their performance on both common pathogens and drug-resistant strains in clinical environments.

While numerous studies have independently examined alcohol-based and chlorhexidine-based disinfectants, comparative analyses conducted within the same institutional environment and time frame remain limited. Furthermore, existing literature often emphasizes either microbial efficacy or skin health, rather than providing a comprehensive evaluation that encompasses both parameters alongside infection rates in healthcare settings.

This study aims to fill these gaps by conducting a retrospective cohort analysis of healthcare personnel at a single hospital, with a standardized transition from alcohol-based to chlorhexidine-based hand disinfectants over the course of a year.

MATERIALS AND METHODS

Case selection

This study utilizes a retrospective cohort design, involving 136 healthcare personnel from our institution as participants. On July 1, 2022, our hospital implemented a uniform transition of disinfectants from alcohol-based solutions to chlorhexidine-based alternatives. Consequently, the study was divided into two distinct phases: the first phase spanned from January 1, 2022, to June 30, 2022, during which healthcare workers utilized alcohol-based disinfectants (designated as the alcohol group); the second phase extended from July 1, 2022, to December 31, 2022, when chlorhexidine-based disinfectants were universally adopted (designated as the chlorhexidine group). The primary objective of this research was to assess the impact of different disinfectants on hand microbiota composition, skin tolerance levels and hospital infection rates by comparing data collected during these two periods. The flowchart of study participants is displayed in Fig. S1.

The study was approved by the Ethics Committee of Shanxi Bethune Hospital (SX-2022-010). Due to the retrospective nature of the study, informed consent was not required, as the data utilized was anonymized and posed no risk to patient care. The waiver for informed consent was granted by both the Institutional Review Board and Ethics Committee, in accordance with regulatory and ethical standards for conducting retrospective research.

Inclusion and exclusion criteria for healthcare personnel

Inclusion criteria: Medical staff within the hospital, including doctors, nurses and medical technicians. Individuals who have been employed at the hospital for at least six months prior to the study. Personnel who worked continuously at the hospital from January 1, 2022, to December 31, 2022. Those who have received training in proper hand hygiene techniques and are familiar with correct procedures. Individuals aged 18 years or older.

Exclusion criteria: Personnel with severe chronic diseases or immune system disorders. Medical staff with a history of allergies to alcohol or chlorhexidine. Personnel with severe skin conditions, such as severe eczema or dermatitis. Pregnant or breastfeeding female healthcare workers. Personnel who used other types of disinfectant products during the study period.

Treatment approach

In the alcohol disinfection group, healthcare personnel performed hand sanitation using an alcohol-based disinfectant. Adhering to the six-step handwashing technique, they thoroughly cleaned their hands up to the lower third of their forearms and subsequently dried them with a clean towel. They then dispensed 6 mL of the disinfectant onto their palms, ensuring even distribution by rubbing their hands together effectively (Iversen *et al*., 2024). This was followed by sequentially rubbing their palms, backs of their hands, between their fingers, backs of their fingers, thumbs, fingertips and the lower third of their forearms, allowing the disinfectant to air dry.

In the chlorhexidine disinfection group, personnel disinfected their hands with a chlorhexidine-based solution, following the same six-step handwashing technique up to the lower third of the forearm and then drying with a towel. They placed 2 mL of the disinfectant in the left palm and used the fingertips of the right hand to mix and then applied it to the right forearm, rubbing until dry. The process was repeated with another 2 mL of disinfectant applied in the right palm for the left forearm, using the left hand to perform the procedure (Huang *et al*., 2023).

Natural colony counts of pathogenic and resistant bacteria

The isolated bacterial strains were identified using standard biochemical reactions and the automated identification system VITEK 2. This process aimed to determine common pathogenic bacteria present on the hands of healthcare personnel, such as *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus sciuri*, *Micrococcus*, *Klebsiella pneumoniae*, *Actinomyces israelii*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *A*cinetobacter baumannii, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Fungi*, *Aeromonas hydrophila* and other. Additionally, the VITEK 2 system was employed to conduct susceptibility testing on the isolated strains to evaluate their sensitivity to commonly used antibiotics (Wormald *et al*., 2022).

A comparative statistical analysis of the natural colony count on the hands of healthcare personnel from both groups was conducted (Papanikolopoulou *et al*., 2022). Before and after disinfection, participants were asked to close their fingers and a sterile swab soaked in the appropriate neutralizing wash solution was used to swab the flexor surfaces of their fingers from base to tip twice. The portion of the swab that touched the operator was then snipped off and placed into a test tube containing 10 mL of the corresponding neutralizing wash solution. Sterile saline was used as a diluent, from which 1.0 mL of the wash solution was taken to achieve an initial dilution ratio of 1:10. A series of sterile test tubes, each containing 9 mL of diluent, was prepared. From the initial wash solution, 1.0 mL was added to the first test tube to achieve a dilution of 1:100. This process was repeated, adding 1.0 mL from the 1:100 dilution to the next tube, resulting in a 1:1000 dilution and so on. From each dilution level, 1.0 mL was dispensed onto nutrient agar in a sterilized petri dish and evenly spread using a spreader. The plates were incubated at a constant 37°C for 7 days. Post incubation, plates with colony counts between 30 and 300 were selected for counting. The actual colony number for each dilution was calculated by multiplying by the respective dilution factors to determine the total bacterial count in the original sample. The disinfection efficacy was then calculated using the formula: Disinfection rate = (Total bacteria count before disinfection - Total bacteria count after disinfection) / Total bacteria count before disinfection × 100% (Russotto *et al*., 2023). To evaluate the prolonged antimicrobial effects of the disinfectants in public areas, the sampling and cultivation process was repeated at intervals of 1, 2 and 4 hours post-disinfection (Papanikolopoulou *et al*., 2022).

Skin assessment

The condition of the hands was evaluated using three different scoring methods. Two scores were determined by a consistent observer throughout the study, while the third was a self-assessment score.

(i) Larson's Skin Assessment Rating Scale (Iversen *et al*., 2024) was an ordinal scale with a maximum of 21 points, with seven points allocated to each factor evaluated: appearance, integrity and moisture. A lower 'Larson Score' indicates poorer skin condition.

(ii) A five-level dermatological assessment scale was employed by a dermatologist to evaluate skin condition. This scale includes level 0, no visible skin changes; level 1, slightly dry skin; level 2, dry skin with redness; level 3, very dry skin with redness but no wrinkles; and level 4, significant skin damage with fissures and wrinkles. The resulting score was termed the "Sauermann Score (Fourie *et al*., 2023).

(iii) The Larson scale was also used for self-assessment by the study participants, who evaluated the same factors along with a fourth factor: 'skin sensation.' This version of the scale has a maximum score of 28 points (Thom *et al*., 2023).

The assessment of skin condition was conducted by measuring transepidermal water loss (TEWL) on the back of the hand, as TEWL was a validated indicator of skin damage (Prakoeswa *et al*., 2022). Measurements were taken using a Tewameter TM-210 Evaporimeter (Courage & Khazaka GmbH, Cologne, Germany) (Hempstead *et al*., 2023). Before taking measurements, each subject was asked to relax for 10 minutes under controlled environmental conditions set at 22°C. The actual TEWL values were adjusted to a standard skin temperature of 30°C using the following formula: $\log \text{TEWL}(30) = \log \text{TEWL}(T) + 0.035(30 - T)$, where TEWL (T) represents the TEWL at a given skin temperature (T) and TEWL (30) was the corrected TEWL for a standardized reference temperature of 30°C.

Hospital infection status

The infection records from the hospital's infection control department covering the period from January 1, 2022, to December 31, 2022, were analyzed. The monitoring indicators for hospital infections were compared in accordance with the "Quality Control Indicators for Hospital Infection Management (2015 Edition)" (National Health Office Medical Letter [2015] No. 252). Indicators included the hospital infection rate, the pathogen submission rate before antibiotic therapy among inpatients and the detection rate of multidrug-resistant bacteria. The hospital infection rate was calculated as follows: (Number of patients with hospital infections / Total number of patients) × 100% (Rakshit *et al*., 2023).

In addition, comparisons related to healthcare personnel's knowledge and implementation of infection control measures were based on the "Disinfection Technical Specifications in Healthcare Settings" (WS/T 367-2012), "Hospital Sanitation Standards for Disinfection"

(GB15982-2012) and the "Regulations on the Management of Medical Waste." The aspects evaluated included environmental cleaning and disinfection, the handling of disinfected and sterile items, as well as medical waste management. The rate of awareness regarding infection control knowledge was calculated using the following formula: (Number of personnel aware / Total number of healthcare personnel) × 100%. The rate of implementation of infection control measures (including environmental cleaning and disinfection, handling of disinfected and sterile items and medical waste management) was determined by: (Number of qualified personnel / Total number of healthcare personnel) × 100% (Senges *et al*., 2024).

Statistical analysis

The data were analyzed utilizing SPSS statistical software version 29.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as [n (%)]. The chi-square test was applied using the basic formula when the sample size was ≥40 and the theoretical frequency (T) was ≥5, with the test statistic expressed as χ^2. When the sample size was ≥40 but $1 \leq T < 5$, the chi-square test was adjusted using a correction formula. For cases where the sample size was < 40 or T < 1, Fisher's exact probability method was used for statistical analysis (Stead *et al*., 2024).

Continuous variables were initially tested for normal distribution using the Shapiro-Wilk method. If the data followed a normal distribution, they were expressed as (X ± s). For non-normally distributed data, the Wilcoxon rank-sum test was employed and results were presented as [median (25th percentile, 75th percentile)]. A p-value of less than 0.05 was considered statistically significant. Correlation analysis was conducted using Pearson's correlation for continuous variables and Spearman's correlation for categorical variables (Farooq *et al*., 2024).

RESULTS

Baseline characteristics of participants

In this study, evaluating the impact of alcohol-based and chlorhexidine-based disinfectants, baseline characteristics of 136 medical personnel were assessed (Table 1). The cohort comprised 76 males (55.88%) and 60 females (44.12%) with a mean age of 33.21 years (SD = 3.61) and a professional experience averaging 9.27 years (SD = 1.54). Educationally, 105 participants (77.21%) held a bachelor's degree or higher, while 31 (22.79%) had an associate degree or below. Professional activities were dominated by nurses, accounting for 96 of the participants (70.59%), followed by nursing assistants (15.44%) and doctors (13.97%). Distribution across professional fields was most notable in medical wards (38.97%) and surgical wards (34.56%), with smaller representations in intensive care (15.44%), gynecology/obstetrics (5.88%) and pediatrics (5.15%). The level of risk of contamination predominantly featured medium-risk procedures, affecting 65 individuals (47.79%), followed by low-risk (36.03%) and high-risk procedures (16.18%). These characteristics ground the context for analyzing how disinfectant types impact hand microorganisms, skin tolerance and nosocomial infection rates.

Hand microbes

The study compared bactericidal efficacy between alcohol-based and chlorhexidine-based disinfectants by analyzing bacterial counts on the hands of 136 healthcare workers per group. Initial bacterial counts before disinfection were similar between the alcohol group (24.36 ± 3.57 CFU/cm²) and the chlorhexidine group (24.85 ± 3.14 CFU/cm²), showing no significant difference ($t = 1.204$, $P = 0.230$) (Fig. 1A). However, immediately after disinfection, the alcohol group demonstrated significantly lower bacterial counts (1.60 ± 0.15 CFU/cm²) compared to the chlorhexidine group (2.80 ± 0.61 CFU/cm²) ($t = 22.243$, $P < 0.001$) (Fig. 1B). One hour post-disinfection, bacterial counts remained significantly lower in the alcohol group (4.98 ± 1.16 CFU/cm²) versus the chlorhexidine group (5.34 ± 1.19 CFU/cm²) ($t = 2.535$, $P = 0.012$) (Fig. 1C). At two hours, the trend persisted with the alcohol group showing higher bacterial counts (8.26 ± 1.62 CFU/cm²) than the chlorhexidine group (7.73 ± 1.24 CFU/cm²) ($t = 3.049$, $P = 0.003$) (Fig. 1D). Notably, after four hours, the chlorhexidine group exhibited significantly lower bacterial counts (7.20 ± 1.32 CFU/cm²) compared to the alcohol group (9.53 ± 1.66 CFU/cm²) ($t = 12.827$, $P < 0.001$) (Fig. 1E). These results illustrate different efficacy profiles, with alcohol showing superior immediate bactericidal action, while chlorhexidine offered more sustained bactericidal effects over time.

The bacterial reduction rates for post-disinfection were compared between the alcohol- and chlorhexidine-based disinfectant groups among 136 healthcare workers in each cohort. Immediately after disinfection, the alcohol group exhibited a significantly higher bacterial reduction rate of 93.46% (SD = 8.92) compared to 89.96% (SD = 8.11) in the chlorhexidine group ($t = 3.393$, $P < 0.001$) (Fig. 2A). One hour after disinfection, the alcohol-based disinfectant continued to show a superior reduction rate at 83.74% (SD = 9.21) versus 81.31% (SD = 5.87) observed in the chlorhexidine group ($t = 2.604$, $P = 0.01$) (Fig. 2B). Interestingly, at the two-hour mark, the chlorhexidine group demonstrated a slightly higher bacterial reduction rate of 66.66% (SD = 4.38) compared to 65.34% (SD = 4.12) in the alcohol group, with the difference being statistically significant ($t = 2.558$, $P = 0.011$) (Fig. 2C). At four hours post-disinfection, the trend intensified, with the chlorhexidine group's reduction rate further surpassing that of the alcohol group, achieving 65.54% (SD = 2.65) versus 63.51% (SD = 1.34), respectively ($t = 7.994$, $P < 0.001$) (Fig. 2D). These findings suggest that while alcohol-based disinfectants provide a more immediate reduction in bacterial load, chlorhexidine-based disinfectants may offer prolonged efficacy over time.

The classification of bacteria present on the hands of healthcare personnel before and after disinfection with alcohol-based and chlorhexidine-based disinfectants was assessed (Table 2). Initially, there was no statistically significant difference in the counts of gram-positive (11.92 ± 2.00 CFU/cm² for alcohol and 12.16 ± 1.66 CFU/cm² for chlorhexidine; $t = 1.049$, $P = 0.295$) and gram-negative bacteria (12.44 ± 2.09 CFU/cm² for alcohol and 12.69 ± 1.94 CFU/cm² for chlorhexidine; $t = 1.037$, $P = 0.301$) between the two groups. Immediately after disinfection, the alcohol group exhibited significantly lower counts of both gram-positive (0.79 ± 0.14 CFU/cm²) and gram-negative bacteria (0.81 ± 0.11 CFU/cm²) compared to the chlorhexidine group (0.86 ± 0.26 CFU/cm² for gram-positive; $t = 2.822$, $P = 0.005$ and 0.92 ± 0.35 CFU/cm² for gram-negative; $t = 3.449$, $P < 0.001$). At one hour post-disinfection, this trend continued with gram-positive bacteria counts at 1.67 ± 0.24 CFU/cm² for alcohol and 1.76 ± 0.33 CFU/cm² for chlorhexidine ($t = 2.375$, $P = 0.018$) and gram-negative bacteria at 2.23 ± 0.21 CFU/cm² versus 2.34 ± 0.47 CFU/cm², respectively ($t = 2.424$, $P = 0.016$). Two hours after disinfection, the chlorhexidine group demonstrated lower gram-positive (3.62 ± 0.81 CFU/cm²) and gram-negative bacteria counts (4.32 ± 0.67 CFU/cm²) compared to the alcohol group (3.83 ± 0.45 CFU/cm² for gram-positive; $t = 2.598$, $P = 0.010$ and 4.56 ± 0.51 CFU/cm² for gram-negative; $t = 3.282$, $P = 0.001$). After four hours, the chlorhexidine group maintained a superior bactericidal effect with gram-positive (4.31 ± 0.53 CFU/cm²) and gram-negative bacterial counts (4.98 ± 0.72 CFU/cm²) remaining lower compared to the alcohol group (4.59 ± 0.56 CFU/cm² for gram-positive; $t = 4.231$, $P < 0.001$ and 5.22 ± 0.75 CFU/cm² for gram-negative; $t = 13.852$, $P < 0.001$). These findings underscore the immediate efficacy of alcohol-based disinfectants and the prolonged bactericidal effect of chlorhexidine-based disinfectants.

Staphylococcus epidermidis was detected in 80.15% of the alcohol group and 76.47% of the chlorhexidine group ($\chi^2 = 0.541$, $P = 0.462$) (Table 3). *Escherichia coli* was present in 68.38% of the alcohol group versus 69.12% in the chlorhexidine group ($\chi^2 = 0.017$, $P = 0.896$). Similarly, *Staphylococcus sciuri* was identified in 55.15% and 53.68% of the respective groups ($\chi^2 = 0.059$, $P = 0.808$). Additional microorganisms such as *Micrococcus*, *Klebsiella pneumoniae*, *Actinomyces israelii*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were detected at comparable rates between groups, with *P values* ranging from 0.620 to 0.889. Less prevalent organisms, including *Streptococcus pyogenes* (14.71% vs 13.97%, $\chi^2 = 0.030$, $P = 0.863$), *Fungi* (5.15% vs 5.88%, $\chi^2 = 0.071$, $P = 0.791$) and *Aeromonas hydrophila* (2.21% vs 3.68%, $\chi^2 = 0.129$, $P = 0.720$), also showed no significant variation. Lastly, other microorganisms were present in 16.91% of the alcohol group and 19.12% of the chlorhexidine group ($\chi^2 = 0.224$, $P = 0.636$). This baseline parity in microorganism detection supports the assessment of disinfectant efficacy without initial bias.

The positive detection rates of key pathogens among healthcare personnel were assessed before and after the application of alcohol-based and chlorhexidine-based disinfectants (Table 4). Before disinfection, there were no significant differences between the two groups for the top five pathogens, including *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus sciuri*, *Micrococcus* and *Klebsiella pneumoniae*. Immediately post-disinfection, a significant reduction in *Staphylococcus epidermidis* was observed in the alcohol group (5.88%) compared to the chlorhexidine group (13.97%) ($\chi^2 = 4.975$, $P = 0.026$), whereas no significant differences emerged for other pathogens. At 1 hour post-disinfection, detection rates remained similar across both groups for all pathogens examined. At 2 hours post-disinfection, again, no significant differences were noted in the prevalence of any pathogens between groups. However, at 4 hours post-disinfection, *Staphylococcus epidermidis* was significantly less prevalent in the chlorhexidine group (33.09%) than in the alcohol group (47.06%) ($\chi^2 = 5.527$, $P = 0.019$), with other pathogens showing no statistically significant differences. These results suggest that although both disinfectants were effective in reducing pathogen presence immediately, chlorhexidine may offer more sustained efficacy against certain pathogens over time.

The rates of detection of resistant bacteria among healthcare personnel were compared between the alcohol-based and chlorhexidine-based disinfectant groups, both before and after disinfection (Table 5). Prior to disinfection, there were no significant differences in detection rates for any of the resistant bacteria, including methicillin-resistant *Staphylococcus epidermidis (MRSE)*, extended-spectrum *beta-lactamase-producing Escherichia coli (ESBLs-Ec)*, methicillin-resistant *Staphylococcus sciuri* (*MRSS*), drug-resistant *Micrococcus* (*DRM*) and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (*ESBLs-Kp*), with *P values* ranging from 0.680 to 0.839. Immediately following disinfection, both groups showed reductions in the presence of these pathogens, yet no statistically significant differences were evident between them. For instance, *MRSE* detection reduced to 2.94% with alcohol and 1.47% with chlorhexidine ($\chi^2 = 0.170$, $P = 0.680$). At subsequent time points—1, 2 and 4 hours post-disinfection—persistent reductions were observed that did not differ significantly between groups. For example, *MRSE* was detected in 8.82% of the alcohol group and 6.62% of the chlorhexidine group at 4 hours post-disinfection ($\chi^2 = 0.464$, $P = 0.496$). Both disinfectants effectively reduced bacterial resistance on hands across all measured intervals without significant intergroup differences, highlighting their comparable efficacy in this context.

Skin assessment

The Observer's Larson Score was higher in the chlorhexidine group (18.06 ± 2.36) compared to the alcohol group (17.13 ± 2.36), indicating a statistically significant difference ($t = 3.251$, $P = 0.001$) (Fig. 3A). The Subject's Larson assessment reflected a different trend, being significantly higher in the alcohol group (22.45 ± 3.11) than in the chlorhexidine group (19.36 ± 3.57), with a t value of 7.617 ($P < 0.001$) (Fig. 3B). There was no significant difference in the Sauermann Score between the groups (alcohol: 0.93 ± 0.14; chlorhexidine: 0.90 ± 0.13; $t = 1.813$, $P = 0.071$) (Fig. 3C). Instrumental assessment of TEWL also showed no significant variation between the alcohol group (23.41 ± 2.64 $g\ m^{-2}\ h^{-1}$) and the chlorhexidine group (22.92 ± 3.41 $g\ m^{-2}\ h^{-1}$) ($t = 1.335$, $P = 0.183$) (Fig. 3D). These results suggest differing perceptions of skin condition between observers and subjects, with no significant instrumental differences in skin barrier function as measured by TEWL.

Comparison of hospital infection

Knowledge of hospital infections was reported by 80.15% of participants in the alcohol group compared to 84.56% in the chlorhexidine group ($\chi^2 = 0.911$, $P = 0.340$) (Table 6). Regarding environmental cleaning and disinfection, compliance was similar between the groups, with 85.29% in the alcohol group and 83.09% in the chlorhexidine group ($\chi^2 = 0.249$, $P = 0.618$). Management of disinfected items showed a slight difference, with 75.00% in the alcohol group and 78.68% in the chlorhexidine group ($\chi^2 = 0.516$, $P = 0.472$). For the management of sterile items, high compliance was observed in both groups, 95.59% for the alcohol group and 93.38% for the chlorhexidine group ($\chi^2 = 0.635$, $P = 0.426$). Handling of medical waste was also similarly reported between groups, with 92.65% compliance in the alcohol group and 94.85% in the chlorhexidine group ($\chi^2 = 0.565$, $P = 0.452$). These findings suggest comparable levels of knowledge and implementation of infection control measures among healthcare personnel, regardless of the disinfectant used.

The overall incidence of HAIs was 5.76% in the alcohol group and 3.80% in the chlorhexidine group ($t = 0.631$, $P = 0.427$) (Table 7). Respiratory tract infections were reported in 2.16% of the alcohol group and 1.27% of the chlorhexidine group, while urinary tract infections occurred at 0.72% and 0.63%, respectively. Incidents of bloodstream infections were absent in the alcohol group but occurred in 0.63% of the chlorhexidine group. Gastrointestinal infections and skin and soft tissue infections were similarly rare in both groups and other infections occurred in 1.44% of the alcohol group and 0.63% of the chlorhexidine group. Analyzing departmental infection rates, internal medicine reported infections in 3.60% of the alcohol group and 1.90% of the chlorhexidine group ($t = 0.295$, $P = 0.587$), while surgery reported 0.72% and 1.90%, respectively ($t = 0.141$, $P = 0.707$). Other departments saw infections in 1.44% of the alcohol group, with none reported in the chlorhexidine group ($t = 0.643$, $P = 0.423$). These results indicate comparable infection rates across both disinfectant groups and departments, suggesting no significant advantage of one disinfectant over the other in reducing HAIs.

DISCUSSION

In this study, we evaluated the effects of alcohol-based and chlorhexidine-based disinfectants on hand microorganisms, skin tolerance and HAIs among healthcare workers. The immediate reduction in bacterial load achieved by alcohol-based disinfectants was widely supported by the literature, attributed to their ability to rapidly denature proteins, effectively disrupting microbial cell membranes (Alajlan *et al*., 2022; Oh *et al*., 2025). This innate mechanism confers quick bactericidal action, making alcohol-based solutions particularly effective for immediate hand sanitization needs. Our findings align with previous research demonstrating the superior immediate bactericidal efficacy of alcohol-based disinfectants (Lechuga *et al*., 2025). However, the rapid evaporation rate of alcohol also suggests a relatively short duration of action, which was evident in the temporal reduction in efficacy observed within the first few hours post-disinfection.

Conversely, chlorhexidine-based disinfectants, while slower to act initially, demonstrated more sustained bactericidal effects over time. The prolonged antimicrobial activity of chlorhexidine may be attributed to its ability to adsorb onto the skin's stratum corneum and remain active for extended periods (Chen *et al*., 2024; Duyu *et al*., 2022; Thomas *et al*., 2024). This property was due to its cationic nature, which enables it to bind strongly to negatively charged sites on the skin and cell surfaces, providing a residual effect that extends its antimicrobial action beyond the immediate application (Widmer *et al*., 2024). The data from our study were consistent with existing knowledge that chlorhexidine's persistent activity makes it effective for situations where ongoing antimicrobial action was desirable (Wade *et al*., 2021).

Exploring the mechanism behind the reduction in both gram-positive and gram-negative bacteria, we note that both disinfectants exhibited efficacy, but the temporal dynamics varied. Alcohol-based disinfectants effectively reduced initial bacterial loads across both bacterial groups due to their broad-spectrum action. However, chlorhexidine's effect became more apparent, particularly in gram-positive bacteria over time, suggesting a robust durability that was advantageous in sustained infection control. Both the alcohol group and chlorhexidine were effective in reducing bacterial resistance on the hands at all measured intervals and there were no significant differences in the elimination and inhibition of resistant bacteria between the two disinfectants.

Table 1: Baseline characteristics of medical personnel.

Parameters	Medical personnel (n = 136)
Gender (*Male/Female*)	76 (55.88%)/60 (44.12%)
Age (years)	33.21 ± 3.61
Body mass index (BMI)	21.32 ± 2.27
Professional experience (years)	9.27 ± 1.54
Educational level [n (%)]	
-Associate degree or below	31 (22.79%)
-Bachelor's degree or above	105 (77.21%)
Professional activity [n (%)]	
-Nurses	96 (70.59%)
-Doctors	19 (13.97%)
-Nursing assistants	21 (15.44%)
Professional field [n (%)]	
-Medical ward	53 (38.97%)
-Surgical ward	47 (34.56%)
-Gynaecology/obstetrics	8 (5.88%)
-Paediatrics	7 (5.15%)
-Intensive care	21 (15.44%)
Level of risk of contamination [n (%)]	
-Low risk procedure [a]	49 (36.03%)
-Medium risk	65 (47.79%)
-High risk	22 (16.18%)

Note: [a] Level of risk of contamination was ranked according to the scale proposed by Fulkerson (Herdt and Ikner, 2023).

Table 2: Comparison of bacterial classification before and after disinfection for healthcare personnel.

Time	Parameters (CFU/cm^2)	Alcohol group (n = 136) [Mean(%)± SD]	Chlorhexidine group (n = 136) [Mean(%) ± SD]	t	p
Before disinfection	Gram-positive bacteria	11.92 ± 2.00	12.16 ± 1.66	1.049	0.295
	Gram-negative bacteria	12.44 ± 2.09	12.69 ± 1.94	1.037	0.301
Immediate post-disinfection	Gram-positive bacteria	0.79 ± 0.14	0.86 ± 0.26	2.822	0.005
	Gram-negative bacteria	0.81 ± 0.11	0.92 ± 0.35	3.449	< 0.001
1 Hour post-disinfection	Gram-positive bacteria	1.67 ± 0.24	1.76 ± 0.33	2.375	0.018
	Gram-negative bacteria	2.23 ± 0.21	2.34 ± 0.47	2.424	0.016
2 Hours post-disinfection	Gram-positive bacteria	3.83 ± 0.45	3.62 ± 0.81	2.598	0.010
	Gram-negative bacteria	4.56 ± 0.51	4.32 ± 0.67	3.282	0.001
4 Hours post-disinfection	Gram-positive bacteria	4.59 ± 0.56	4.31 ± 0.53	4.231	< 0.001
	Gram-negative bacteria	5.22 ± 0.75	4.98 ± 0.72	13.852	< 0.001

Table 3: Comparison of positive detection rates of hand microorganisms in healthcare personnel before disinfection.

Parameters	Alcohol group (n = 136)	Chlorhexidine group (n = 136)	χ^2	p
Staphylococcus epidermidis [n (%)]	109 (80.15)	104 (76.47)	0.541	0.462
Escherichia coli [n (%)]	93 (68.38)	94 (69.12)	0.017	0.896
Staphylococcus sciuri [n (%)]	75 (55.15)	73 (53.68)	0.059	0.808
Micrococcus [n (%)]	52 (38.24)	56 (41.18)	0.246	0.62
Klebsiella pneumoniae [n (%)]	40 (29.41)	38 (27.94)	0.072	0.789
Actinomyces israelii [n (%)]	37 (27.21)	39 (28.68)	0.073	0.787
Staphylococcus haemolyticus [n (%)]	35 (25.74)	34 (25.00)	0.019	0.889
Staphylococcus aureus [n (%)]	32 (23.53)	33 (24.26)	0.02	0.887
Acinetobacter baumannii [n (%)]	28 (20.59)	30 (22.06)	0.088	0.767
Pseudomonas aeruginosa [n (%)]	21 (15.44)	19 (13.97)	0.117	0.732
Streptococcus pyogenes [n (%)]	20 (14.71)	19 (13.97)	0.03	0.863
Fungi [n (%)]	7 (5.15)	8 (5.88)	0.071	0.791
Aeromonas hydrophila [n (%)]	3 (2.21)	5 (3.68)	0.129	0.72
Other [n (%)]	23 (16.91)	26 (19.12)	0.224	0.636

Table 4: Comparison of positive detection rates of pathogens among healthcare personnel before and after disinfection (Top 5 pathogens).

Time	Parameters	Alcohol group (n = 136)	Chlorhexidine group (n = 136)	χ^2	p
Before disinfection [n (%)]	*Staphylococcus epidermidis*	109 (80.15)	104 (76.47)	0.541	0.462
	Escherichia coli	93 (68.38)	94 (69.12)	0.017	0.896
	Staphylococcus sciuri	75 (55.15)	73 (53.68)	0.059	0.808
	Micrococcus	52 (38.24)	56 (41.18)	0.246	0.620
	Klebsiella pneumoniae	40 (29.41)	38 (27.94)	0.072	0.789
Immediate post-disinfection [n (%)]	*Staphylococcus epidermidis*	8 (5.88)	19 (13.97)	4.975	0.026
	Escherichia coli	4 (2.94)	2 (1.47)	0.170	0.680
	Staphylococcus sciuri	7 (5.15)	9 (6.62)	0.266	0.606
	Micrococcus	11 (8.09)	10 (7.35)	0.052	0.820
	Klebsiella pneumoniae	0 (0.00)	2 (1.47)	0.504	0.478
1 Hour post-disinfection [n (%)]	*Staphylococcus epidermidis*	11 (8.09)	8 (5.88)	0.509	0.475
	Escherichia coli	6 (4.41)	5 (3.68)	0.095	0.758
	Staphylococcus sciuri	8 (5.88)	7 (5.15)	0.071	0.791
	Micrococcus	13 (9.56)	11 (8.09)	0.183	0.669
	Klebsiella pneumoniae	1 (0.74)	3 (2.21)	0.254	0.614
2 Hours post-disinfection [n (%)]	*Staphylococcus epidermidis*	26 (19.12)	24 (17.65)	0.098	0.754
	Escherichia coli	16 (11.76)	13 (9.56)	0.347	0.556
	Staphylococcus sciuri	8 (5.88)	10 (7.35)	0.238	0.626
	Micrococcus	15 (11.03)	17 (12.50)	0.142	0.707
	Klebsiella pneumoniae	5 (3.68)	3 (2.21)	0.129	0.720
4 Hours post-disinfection [n (%)]	*Staphylococcus epidermidis*	64 (47.06)	45 (33.09)	5.527	0.019
	Escherichia coli	29 (21.32)	27 (19.85)	0.090	0.764
	Staphylococcus sciuri	14 (10.29)	18 (13.24)	0.567	0.452
	Micrococcus	26 (19.12)	25 (18.38)	0.024	0.877
	Klebsiella pneumoniae	11 (8.09)	8 (5.88)	0.509	0.475

Table 5: Comparison of positive detection rates of resistant bacteria in healthcare personnel.

Time	Parameters	Alcohol group (n = 136)	Chlorhexidine group (n = 136)	χ^2	p
Before disinfection [n (%)]	*MRSE*	21 (15.44)	19 (13.97)	0.117	0.732
	ESBLs-Ec	13 (9.56)	14 (10.29)	0.041	0.839
	MRSS	11 (8.09)	13 (9.56)	0.183	0.669
	DRM	10 (7.35)	9 (6.62)	0.057	0.812
	ESBLs-Kp	2 (1.47)	4 (2.94)	0.170	0.680
Immediate post-disinfection [n (%)]	*MRSE*	4 (2.94)	2 (1.47)	0.170	0.680
	ESBLs-Ec	2 (1.47)	0 (0.00)	0.504	0.478
	MRSS	3 (2.21)	1 (0.74)	0.254	0.614
	DRM	0 (0.00)	2 (1.47)	0.504	0.478
	ESBLs-Kp	2 (1.47)	0 (0.00)	0.504	0.478
1 Hour post-disinfection [n (%)]	*MRSE*	6 (4.41)	5 (3.68)	0.095	0.758
	ESBLs-Ec	5 (3.68)	3 (2.21)	0.129	0.720
	MRSS	3 (2.21)	1 (0.74)	0.254	0.614
	DRM	2 (1.47)	0 (0.00)	0.504	0.478
	ESBLs-Kp	2 (1.47)	0 (0.00)	0.504	0.478
2 Hours post-disinfection [n (%)]	*MRSE*	8 (5.88)	7 (5.15)	0.071	0.791
	ESBLs-Ec	6 (4.41)	4 (2.94)	0.415	0.519
	MRSS	5 (3.68)	3 (2.21)	0.129	0.72
	DRM	4 (2.94)	2 (1.47)	0.170	0.680
	ESBLs-Kp	4 (2.94)	2 (1.47)	0.170	0.680
4 Hours post-disinfection [n (%)]	*MRSE*	12 (8.82)	9 (6.62)	0.464	0.496
	ESBLs-Ec	8 (5.88)	7 (5.15)	0.071	0.791
	MRSS	7 (5.15)	4 (2.94)	0.853	0.356
	DRM	5 (3.68)	3 (2.21)	0.129	0.720
	ESBLs-Kp	7 (5.15)	6 (4.41)	0.081	0.776

Note: *MRSE*, methicillin-resistant *Staphylococcus epidermidis*; *ESBLs-Ec*, extended-spectrum *beta-lactamase-producing Escherichia coli*; *MRSS*, methicillin-resistant *Staphylococcus sciuri*; *DRM*, drug-resistant *Micrococcus*; *ESBLs-Kp*, extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*.

Table 6: Comparison of knowledge and implementation of hospital infection control measures among healthcare personnel.

Parameters	Alcohol group (n = 136)	Chlorhexidine group (n = 136)	χ^2	p
Knowledge of hospital infections [n (%)]	109 (80.15)	115 (84.56)	0.911	0.340
Environmental cleaning and disinfection [n (%)]	116 (85.29)	113 (83.09)	0.249	0.618
management of disinfected items [n (%)]	102 (75.00)	107 (78.68)	0.516	0.472
Management of sterile items [n (%)]	130 (95.59)	127 (93.38)	0.635	0.426
Handling of medical waste [n (%)]	126 (92.65)	129 (94.85)	0.565	0.452

Table 7: Comparison of the incidence of HAI in patients cared for by different sterilized medical staff.

Parameters	Alcohol Group (n = 139)	Chlorhexidine Group (n = 158)	t	p
Overall incidence of hospital-acquired infections [n (%)]	8 (5.76)	6 (3.80)	0.631	0.427
- Respiratory tract Infections	3 (2.16)	2 (1.27)		
-Urinary tract infections	1 (0.72)	1 (0.63)		
-Bloodstream infections	0 (0.00)	1 (0.63)		
-Gastrointestinal infections	1 (0.72)	1 (0.63)		
-Skin and soft tissue infections	1 (0.72)	0 (0.00)		
- Other infections	2 (1.44)	1 (0.63)		
Departmental infection rates [n (%)]				
-Internal medicine	5 (3.60)	3 (1.90)	0.295	0.587
- Surgery	1 (0.72)	3 (1.90)	0.141	0.707
- Other	2 (1.44)	0 (0.00)	0.643	0.423

Skin tolerance was another crucial aspect, as repeated use of hand disinfectants can lead to adverse skin conditions, impacting compliance with hand hygiene protocols. Our study found differences in skin tolerance, with chlorhexidine showing better observer-rated skin condition, whereas alcohol-based disinfectants were preferred by healthcare personnel for self-assessed skin sensation. This discrepancy may be explained by alcohol's capacity to dehydrate the skin, thus affecting the sensation and potentially contributing to a sense of dryness or discomfort (Lopes *et al*., 2022). Chlorhexidine, while effective, may also interact with skin lipids but appears to foster better overall skin condition due to its potentially less aggressive action on lipids compared to alcohols.

Infection rates within the hospital setting were not significantly different between the two groups, suggesting that both disinfectants were comparably effective in controlling the incidence of HAIs. This observation supports the notion that both forms of hand hygiene can be integral components of infection control measures. Despite minor differences in efficacy and skin tolerance, both disinfectants played a significant role in reducing the burden of HAIs, which underscores the need for their continued use and improvement. Maintaining a strategic balance between immediate and sustained disinfectant efficacy can optimize infection control protocols.

Our findings highlight not only the need for effective disinfectants but also the importance of compliance with hand hygiene practices as part of a holistic infection control strategy. The data emphasize that while the type of disinfectant used was crucial for microbial control, adherence to proper hand hygiene protocols was equally, if not more, essential in preventing HAI transmission (Boyce, 2023; Glowicz *et al*., 2023; Stadler and Tschudin-Sutter, 2020). The study underscores the importance of decision-makers in healthcare settings remaining informed about the individual properties and advantages of each disinfectant type, allowing facilities to tailor usage based on specific requirements, such as immediate germicidal action or prolonged antimicrobial effect.

Another focal point in our discussion was the interpretation and perception of skin conditions associated with the regular use of these disinfectants. The subjective preference for alcohol-based solutions among healthcare workers may be influenced more by immediate tactile sensations than by long-term skincare outcomes. This underscores the necessity for healthcare facilities to provide comprehensive education on the benefits and drawbacks of various disinfectants, addressing misconceptions that may impact personal choices and compliance with hygiene practices.

This study provides valuable insights into the efficacy and skin tolerance of alcohol-based and chlorhexidine-based disinfectants; however, it is important to acknowledge several limitations. The retrospective design of the study constrains our ability to draw causal inferences, while reliance on self-reported assessments of skin condition introduces potential biases regarding perceived irritation or dryness that may not accurately reflect objective measures of skin health. Furthermore, the controlled hospital environment may not adequately represent the variability in hand hygiene practices across different healthcare settings. The specific populations and hospital contexts examined could limit the generalizability of these findings to other healthcare environments. Additionally, the time frame of this study may not account for long-term effects on skin health and microbial resistance. Future research should address these considerations by employing prospective designs and exploring a wider range of healthcare settings to enhance both the generalizability and depth of understanding regarding the impacts of disinfectants.

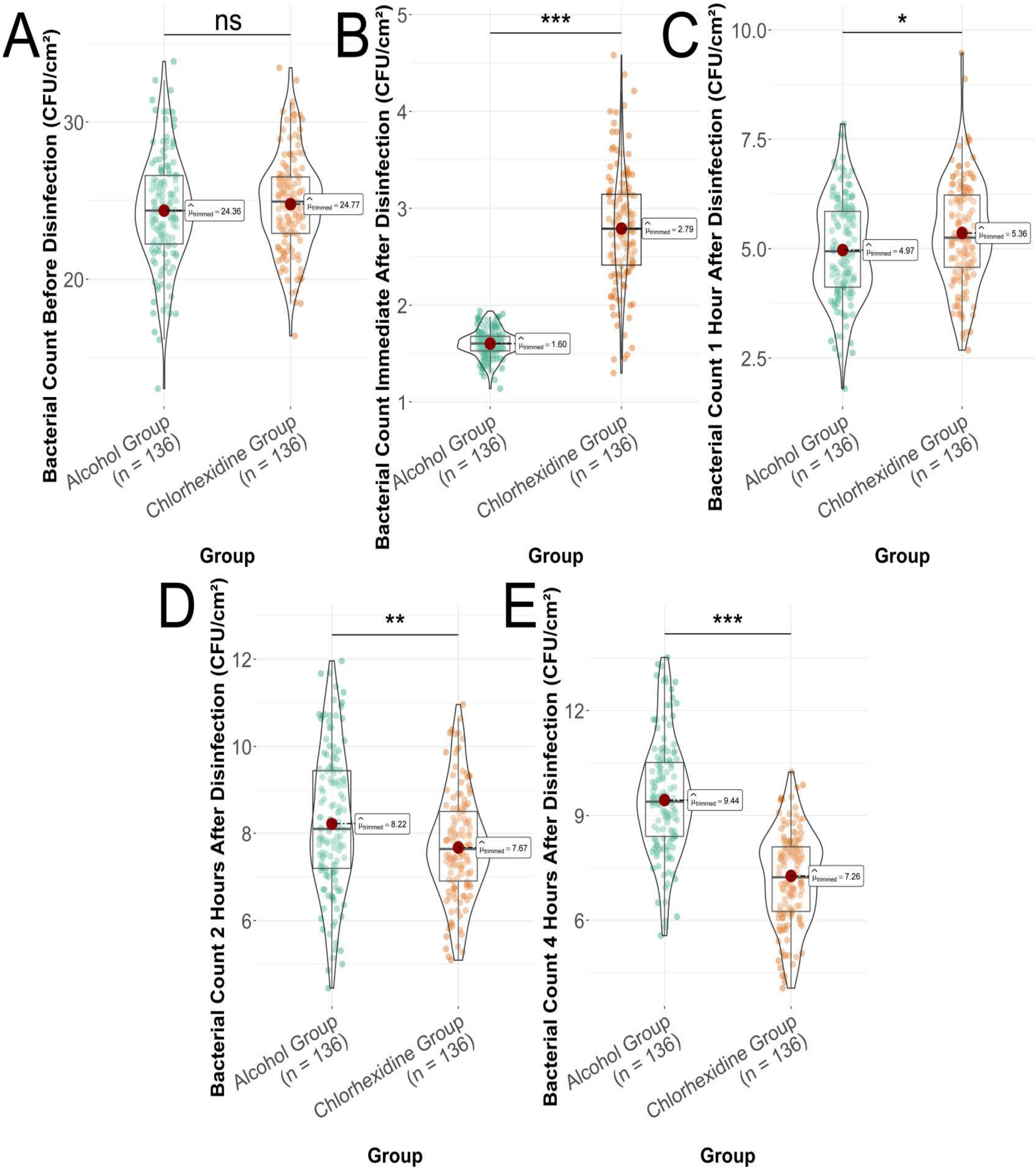

Fig. 1: Comparison of bactericidal efficacy between alcohol and chlorhexidine groups (Bacterial count in CFU/cm²).

(A) Bacterial count before disinfection; (B) Bacterial count immediately after disinfection; (C) Bacterial count 1 hour after disinfection; (D) Bacterial count 2 hours after disinfection; (E) Bacterial count 4 hours after disinfection *: $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: no significant difference.

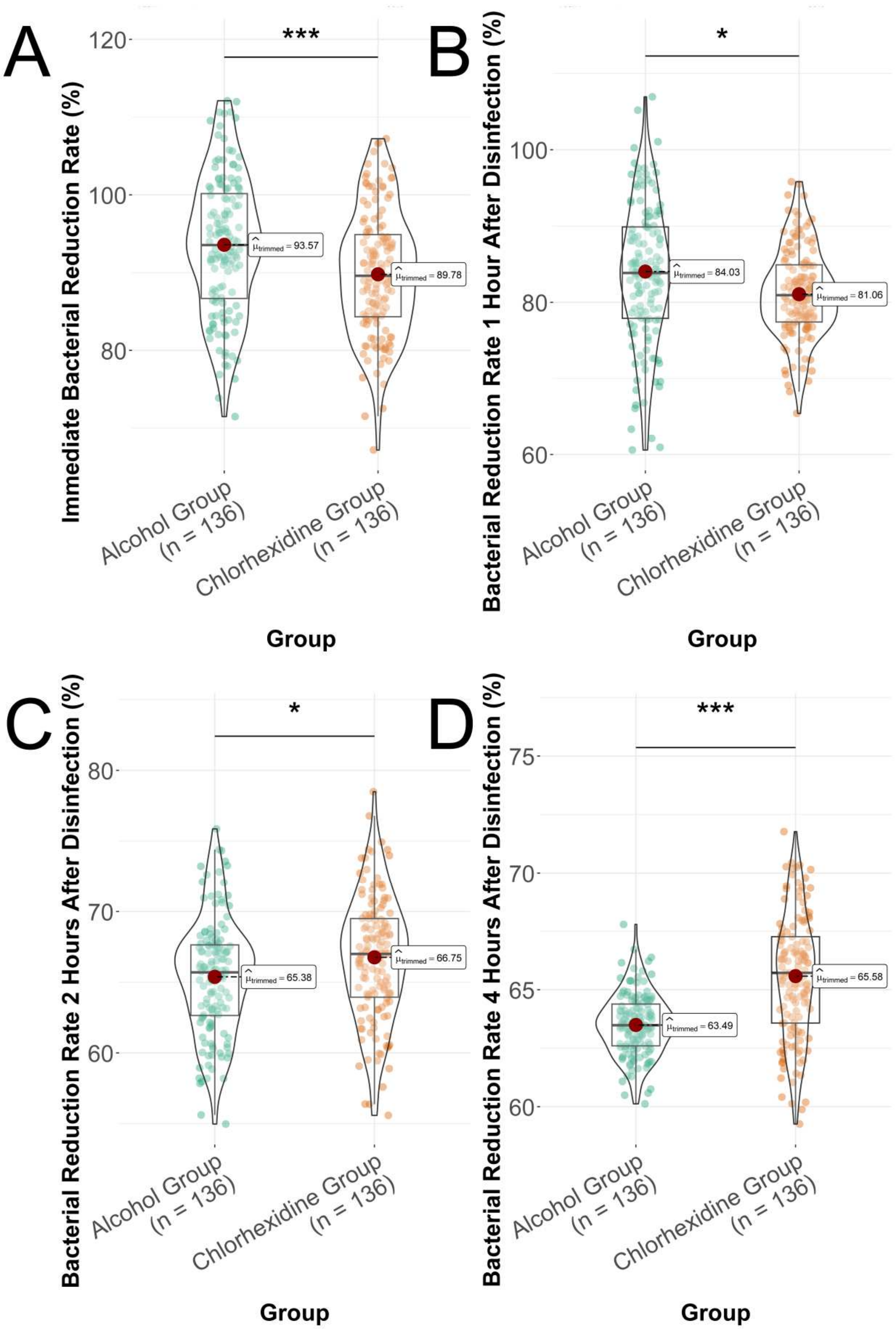

Fig. 2: Comparison of bacterial reduction rates between alcohol and chlorhexidine groups.

(A) Immediately bacterial reduction rate; (B) Bacterial reduction rate 1 hour after disinfection; (C) Bacterial reduction rate 2 hours after disinfection; (D) Bacterial reduction rate 4 hours after disinfection *: $P < 0.05$, *** $P < 0.001$.

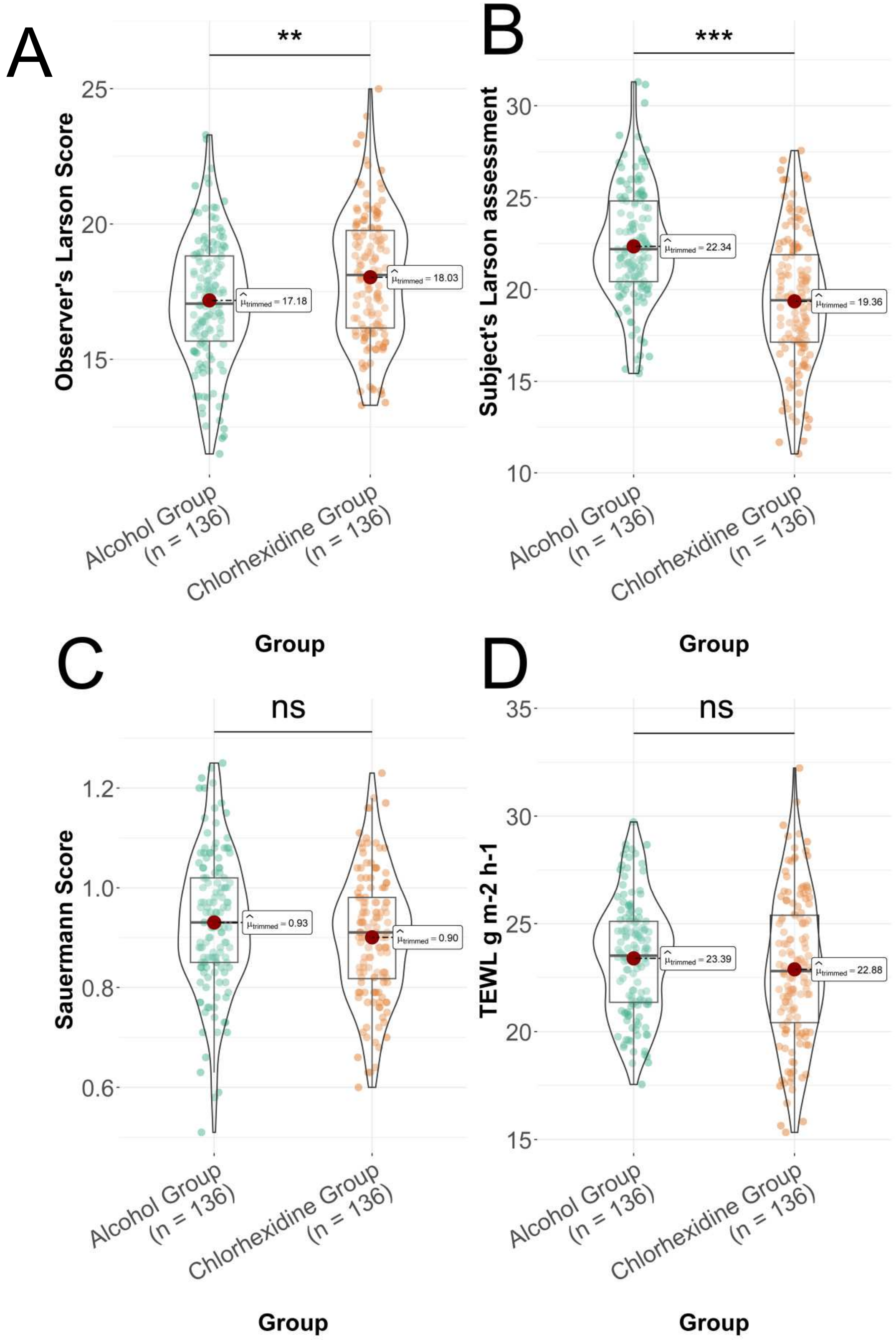

Fig. 3: Comparison of hand skin conditions in healthcare personnel.
(A) Observer's Larson Score; (B) Subject's Larson assessment; (C) Sauermann Score; (D) TEWL ** $P < 0.01$, *** $P < 0.001$, ns: no significant difference.
Note: TEWL: transepidermal water loss.

CONCLUSION

In conclusion, both alcohol-based and chlorhexidine-based disinfectants play pivotal roles in infection prevention through their specific mechanisms of action and skin interaction properties. Understanding their distinct characteristics offers an opportunity to enhance compliance strategies and optimization of disinfectant use, taking into consideration not just immediate microbial efficacy but also long-term skin health and healthcare worker satisfaction. Continued research and innovation in this field hold great potential to advance the effectiveness of infection control measures in hospitals globally.

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None.

Authors' contributions

YX and HYC: Involved in the conception and design and analysis and interpretation of the data; YX, HYC and WL: Drafting of the paper, revising it critically for intellectual content; WL: Final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The study was approved by the Ethics Committee of Shanxi Bethune Hospital (SX-2022-010). This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest

The authors declare no conflict of interest.

Supplementary data

https://www.pjps.pk/uploads/2026/06/SUP1781939131.pdf

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