Staphylococcus aureus and *Escherichia coli* levels on the hands of theatre staff in three hospitals in Johannesburg, South Africa, before and after handwashing

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Background. Hand hygiene is a fundamental component of infection control. Hand contamination with *Staphylococcus aureus* and *Escherichia coli* may contribute to infections.

Objectives. To assess the effectiveness of different handwashing methods in reducing the levels of bacterial flora, especially *S. aureus* and *E. coli*, on the hands of theatre staff.

Methods. A cross-sectional study was conducted among 70 staff in surgical theatres of three randomly chosen hospitals in Johannesburg, South Africa. Samples were taken before and after handwashing using the modified glove juice method and the fingernail press technique. Standard microbiological techniques were used to identify bacteria. Descriptive statistics and non-parametric analysis were used to compare the differences between hospitals and to determine the effects of handwashing on microbial flora and skin irritation.

Results. *S. aureus* organisms were isolated in the prewash samples of 29 (41%) and in the postwash samples of 20 (29%) workers. Of the 29 with positive prewash cultures, 19 (65.5%) showed decreased postwash counts, while 10 (34.5%) showed no change or increased counts. Four workers with a negative prewash count had a positive postwash count. No statistical differences were found between postwash counts categorised by the type of cleansing formula used and the washing technique. *E. coli* organisms were identified in the prewash count of the fingertip press of one worker.

Conclusions. Almost half of the theatre staff carried *S. aureus* isolates on their hands prior to handwashing and approximately one-third after handwashing. Closer monitoring of handwashing techniques should be introduced.

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Hospital theatres are considered to be one of the most complex and hazardous environments that pose a high risk of infection to medical staff (and patients) owing to long exposures to biological agents.[1] Hospital-acquired infections (HAIs) are a serious health issue and an economic burden worldwide, as they present a risk to healthcare workers (HCWs), patients and the community. The main sources of HAIs are contaminated air, contact surfaces and hands of medical staff. HCWs can also acquire these pathogens during direct contact with patients or contaminated environmental surfaces.[2,3] In spite of hand hygiene protocols and policies in healthcare facilities, poor handwashing compliance continues to exist among medical professionals.[4]

Skin flora contains resident bacteria that inhabit the deeper skin layers and are difficult to eradicate (e.g. *Micrococcus* spp.), as well as transient flora that colonise the superficial layer of the skin and are responsible for nosocomial infections, although they are easily removed by handwashing.[5] Numerous reports reflect the necessity for handwashing using various techniques to prevent the spread of pathogenic organisms to patients.[6] *Staphylococcus aureus* organisms cultured from healthy hands of medical professionals (10%) and from damaged hands (16.7%) were also noted.[3] Studies reported high levels of *S.aureus* on the hands of female and male HCWs, ranging from 5% to 20%.[7-9]

S. aureus is a dangerous pathogen that can cause serious and life-threatening diseases, such as severe septicaemia, pneumonia, meningitis, septic arthritis, folliculitis, impetigo, osteomyelitis and toxic shock syndrome.[4,10] Furthermore, methicillin-resistant *S. aureus* has become common in hospitals and communities.[11] From January to July 2012, there were 1 148 South African (SA) cases of confirmed *S. aureus* bacteraemia.[12] Of these, 619 (54%) were reported in Gauteng, and 289 (44%) were resistant to oxacillin/methicillin, with compliance of only 40% in intensive care units.

The objective of this study was to assess the efficacy of different handwashing techniques in reducing the levels of bacterial flora, especially *S. aureus* and *Escherichia coli*, on the hands of theatre workers.

Methods

A cross-sectional study was conducted from October to December 2013 and in July 2014 among 70 surgical theatres staff of three hospitals in Johannesburg, SA. These hospitals were selected randomly by their proximity to the testing laboratory and the willingness of management to participate in the study. To test for bacteria on the skin at the beginning of the work shift, the dominant hand of each participant was inserted into and massaged for 1 minute in a sterile polyethylene bag containing 75 mL of tryptic soy broth (TSB) with neutralisers (0.1% polysorbate 80, 0.03% lecithin and sodium thiosulphate). The procedure was performed before handwashing and repeated immediately after washing and before drying of the hands. The samples were transported on the same day in a cooler

bag with ice to the National Institute for Occupational Health, Johannesburg, for microbiological analysis. Standard microbiological techniques were employed for bacterial quantification and identification of *S. aureus* and *E. coli*. After vigorous mixing, TSB was diluted (1:10 and 1:100) and 0.1 mL was plated onto a tryptic soy agar (TSA) medium, 5% blood agar with gentamicin and mannitol salt agar (Diagnostic Media Products, SA) for isolation of *S. aureus*, using the aseptic spreading method. The inoculated culture plates were incubated aerobically at 37°C. Total bacterial count enumeration was performed after 72 hours and reported as colony-forming units (CFU) per mL.

The 5 fingertips of the less dominant hand of participants were pressed with equal pressure onto half plates containing 5% blood agar and MacConkey agar for 5 seconds to isolate Gram-negative bacteria (no bacterial counts were done). Isolated bacterial colonies from the agar plates were identified by conventional techniques (morphology, haemolysis, lactose fermentation, Gram-staining and microscopy) using a study guide on diagnostic bacteriology.[13] Descriptive statistics and non-parametric analysis using Stata 11 (StataCorp., USA) were employed to compare the differences between handwashing techniques and between hospitals. The level of significance was $p<0.05$.

Results

Seventy individuals (40 females, 30 males) took part in the study, of whom 32 were nurses, 31 medical doctors and 7 other participants. A total of 280 samples (140 hands and 140 fingerprints) for both pre- and postwashing were collected. Of the 70 participants, 8 had an increase, 58 had a decrease and 4 had no change in total bacterial counts after handwashing. There were no statistically significant differences between the prewash and postwash arithmetic mean counts of *S. aureus* (Table 1) or by hospital ($p\leq0.10$).

S. aureus organisms were isolated in the prewash samples of 29/70 (41%) workers and in 20/70 (29%) postwash samples. Of the 29 who had positive cultures of *S. aureus* before washing, 19 (65.5%) showed a decrease in the postwash count and 10 (34.5%) an increase or no change in bacterial load of the postwash count. The proportion of workers with *S. aureus* in the prewash samples differed between hospitals, even though the difference was not statistically significant (exact test, $p=0.08$). *S. aureus* was identified in 12/30 (40%) workers in hospital A, 5/20 (25%) in hospital B and 12/20 (60%) in hospital C. Four workers with a negative prewash count had a positive postwash count.

No statistical differences were found between postwash counts categorised by the type of cleansing formula used and between different techniques (scrub with a brush, scrub without a brush and ordinary handwashing). Chlorhexidine gluconate (Hibiscrub) washing solution was used by 54/69 (78%) participants, povidone iodine (Betadine) by 13 (19%) and 4% chlorhexidine gluconate (MediScrub) by 2 (3%). One person (2%) of the total number of participants ($N=70$) did not report the use of any soap; *S. aureus* was not isolated from this worker. No difference was seen in workers with and without *S. aureus* for postwash samples based on the type of handwashing agent used (exact test, $p=0.153$).

Table 1. Mean *Staphylococcus aureus* CFUs before and after handwashing

	Prewash ($n=29$)	Postwash ($n=20$)
Arithmetic mean, CFU/mL	1.9×10^3	0.5×10^3
Mean $\log_{10}$, CFU/mL*	3.28	2.70

CFU = colony-forming units.
*Zero count values are excluded from the mean $\log_{10}$ calculations.

E. coli organisms were isolated from the prewashed fingertip sample of 1 HCW. *Staphylococcus* spp. were isolated from fingertips, but could not be identified further for *S. aureus* using half-plates (MacConkey/blood agar).

Discussion

This is the first SA report to quantify the microbiological burden on the hands of theatre workers and to identify *S. aureus* and *E. coli*. The overall minimal bacterial counts for prewashing ranged from 0 to 4.3×10^5 CFU/mL, whereas counts after handwashing ranged from 0 to 1.4×10^5 CFU/mL. The mean $\log_{10}$ CFU/mL of *S. aureus* was 3.28 and 2.70 pre- and postwashing, respectively.

In contrast to our findings of 74% total bacterial reduction, in another study a decrease of 95 - 99% in bacterial load was demonstrated after performing hand hygiene.[14] The percentage of HCWs with *S. aureus* contamination on their hands is higher in our study than levels previously described.[3,7,15] However, Singh and Singh[14] isolated *S. aureus* in 70% of nurses, 60% of students and 40% of attendants. Persistent skin colonisation with *S. aureus* was reported,[8] and was associated with certain body sites and the environment.

The different hospitals showed no statistical differences in the arithmetic mean counts between prewashing and postwashing counts. This may be attributed to the small sample sizes. The handwashing protocols approved by theatre management did not differ between hospitals.

In 4 cases across the hospitals, the postwash count of *S. aureus* was elevated, although the prewash count was zero. Of the HCWs with *S. aureus* isolates on their hands, 10 had increased counts after handwashing. The elevation in bacterial counts could be owing to the hypothesis that transient microbes are easily removed from the hands by washes of <30 seconds, whereas resident bacteria embedded in deeper layers of the skin are not easily removed, regardless of the length of time spent handwashing.[16] The elevated postwash counts may have been due to cross-contamination with contaminated surfaces and/or equipment in theatres and between departments, as proven by researchers.[2-4]

In 3 HCWs no bacteria were isolated from their hands for both pre- and postwashing, which could be due to handwashing prior to sampling, even though they had stated that they had not washed their hands – possibly for fear of being excluded. However, in 2 of the 3 workers, *Staphylococcus* spp. were isolated from their fingerprints before handwashing. This suggests that although their palms or hands were not colonised by bacteria, their fingernails were contaminated.

Transient organisms such as *S. aureus* and Gram-negative bacteria are acquired by HCWs during contact with patients and contaminated surfaces and could lead to HAIs.[17] Our findings may be indicative of contamination of the soap dispenser lid, the hand lever or the soap residual at the tip of the container, which may have been contaminated by air particles. Studies showed that taps and door knobs are rarely cleaned and therefore contain the highest microbial load.[4] The presence of *S. aureus* on the hands of a large proportion of theatre staff, even after handwashing, should be reason for concern, as gloves are not puncture proof and there may be bacterial transmission to surgical sites from exposed skin. This is also supported by a study investigating handwashing practices of medical students, which showed an increase in bacterial load on the hands of those who washed with soap after toilet use; this increase was attributed to touching the toilet door knobs.[4]

Workers carrying *S. aureus* organisms on their hands may harbour these on other body sites, notably in the nose. Persistence of the organisms found on cultures after handwashing may imply that they are not easily removed from the hands and may be resident.[4] Hand

cleansing should be performed as per the '5 moments of hand hygiene' recommended by the World Health Organization (WHO), equipment should be decontaminated after use, and environmental sites should be regularly and effectively cleaned.[18]

Staphylococcus spp. were isolated from both prewash and postwash fingertip samples of the less dominant hand. Although not confirmed, it is possible that *S. aureus* was among the S*taphylococcus* spp. found on the fingertips. De Alwis *et al.*[4] found high bacterial loads on the dominant hand after toilet use. Two previous studies demonstrated the presence of *S. aureus* using the fingertip method.[19,20]

The current study showed that a higher percentage (23%) of HCWs who scrubbed their hands with a brush had skin irritation, even though the percentage was not statistically significant. While it is generally accepted that nosocomial infections in patients may be acquired from HCWs, and the importance of hand hygiene is stressed throughout the literature, the former is not always the case. For instance, although a previous study demonstrated postsurgical wound infection with *S. aureus* in 24 of 214 patients, genetic typing showed that HCWs were not the source of infection.[21] *E. coli* were isolated from the fingerprints of only 1 HCW (1%); this finding is similar to that of Singh and Singh,[14] who also reported 1% in a study done in India.

A major drawback of the current study is the small sample size. Furthermore, methicillin-resistant *S. aureus* was not identified and should be investigated in a larger population. The study warrants further research into the determinants of poor handwashing outcomes, such as duration of washing, amount of antimicrobial agent used and lather formation, sources of cross-contamination (e.g. taps and detergent containers) and behaviour practices.[21]

Conclusion

S. aureus contamination remains a challenge in healthcare facilities. It was found on the hands of almost half of theatre staff before handwashing for theatre and approximately one-third after handwashing. This may be regarded as medically significant. The type of handwashing technique was not shown to be a key determinant in reducing *S. aureus* counts; however, it may play a role in skin irritation. Handwashing has been reported as the most effective and inexpensive way to prevent transmission, and more advocacy is needed to ensure that HCWs adhere to strict handwashing and hand-care protocols in view of their fundamental role in infection control. In conclusion, our findings may be used to inform other hospitals that handwashing protocols should be reassessed at regular intervals and further analysed for adequacy. This may help to prevent potential *S. aureus* outbreaks in healthcare settings. Continuing education on behavioural changes to improve hygiene habits of all staff should be encouraged.

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Conflicts of interest. None.

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