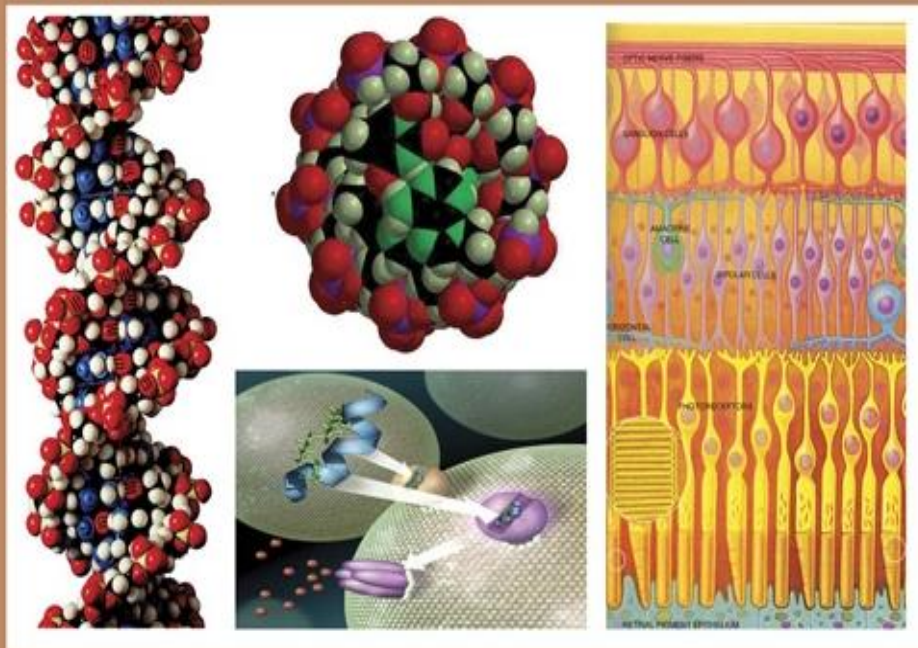




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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULAR BIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 13 No. 1 (2021)



Unused Non-sterile Latex Disposable Gloves Contamination with Healthcare-associated Bacteria and Contamination Reduction by Reminder Signs

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ARTICLE INFO

Article History

Received:30/10/2020

Accepted:3/1/2021

Keywords:

Hand hygiene;
healthcare-associated
infections;
infection control

ABSTRACT

Background: Non-sterile latex disposable gloves (NSLDGs) can prevent infections, but may also cause them if improperly used.

Aim: To study NSLDGs bacterial contamination and the effect of hand hygiene reminder notices.

Methods: A controlled interventional study was conducted through a quantitative bacterial sampling of NSLDGs ($n = 160$ gloves) obtained from boxes immediately after they were opened (baseline contamination) and 48 hours afterwards (per-intervention), and after hand hygiene reminder notices were placed (post-intervention). Bacteria were isolated and identified by 16S rRNA gene sequencing and antibiotic sensitivity testing.

Findings: Pre-intervention contamination was found to be 90% and 65% for skin commensals and pathogens, respectively. Post-intervention contamination by skin commensals and pathogens declined to 70% and 15%, respectively, representing a significant reduction in the prevalence of pathogens ($p = 0.0006244$). The average number of colony-forming units per glove pair was also reduced, from 57.05 to 4.95 ($p = 1.5 \times 10^{-5}$) and from 16.1 to 0.65 ($p = 0.003374$), for skin commensals and pathogenic bacteria, respectively.

Discussion: NSLDGs are potential pathogen transmission vehicles. Hand hygiene reminder notices placed on glove boxes can lead to reductions in bacterial contamination levels.

INTRODUCTION

Safety of healthcare workers and patients, including the response to the currently ongoing COVID-19 pandemic (Kraus *et al.*, 2020), requires hand hygiene and disinfection, which remains the main recommended practice aimed at reducing healthcare-associated infections (Haque *et al.*, 2020; Kingston *et al.*, 2016; Nishiwaki and Ichikawa 2014). Non-sterile latex disposable gloves (NSLDGs) are commonly used to protect healthcare workers' hands against the spread of pathogens (Picheansanthian and Chotibang 2015). Nonetheless, healthcare-associated infections remain a serious issue worldwide. Their high incidence is usually attributed to observed low compliance with hand hygiene and disinfection (Allegranzi *et al.*, 2013; Joshi *et al.*, 2013; Kingston *et al.*, 2016). For example, it has been shown that most healthcare-associated pathogens—such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and Enterobacteriaceae—are spread via healthcare workers' hands (Brooks *et al.*, 2013; Luebbert and Chinnes 2015). Thus, by improving hand hygiene practices, their proliferation can be controlled (Barnes *et al.*, 2014; Pelat *et al.*, 2016).

Moreover, even gloves can transmit bacteria, such as healthcare-associated multi-drug resistant *Acinetobacter baumannii* (Greene *et al.*, 2015; Morgan *et al.*, 2010; Ye *et al.*, 2015).

Findings of several studies indicate that NSLDGs can be contaminated with pathogens in intensive care units (Rock *et al.*, 2013) and wards within large hospitals (Hughes *et al.*, 2013). During a 9 day study, it was found that up to 81.6% of non-sterile disposable gloves used in a hospital orthopedic ward were contaminated with environmental bacteria, but this contamination level tended to fall down in terms of the number of positive samples (from 90% to 70-80%) and colony-forming units (cfu) per glove (from $\geq 10^3$ cfu/glove to > 40 but $< 10^2$ cfu/glove) within 9 days. On the other hand, skin commensals' contamination increased significantly (from 10% at baseline contamination at < 40 cfu/glove to 40-60% and a contamination load $\geq 10^2$ but $< 10^3$). In addition, pathogenic bacteria increased to clinically significant levels (Hughes *et al.*, 2013). However, no studies aiming to determine the presence of pathogens on NSLDGs in any primary healthcare centre, labour room or resuscitation room, or under any healthcare settings in Saudi Arabia, have been conducted thus far. Reproducibility of contamination levels over several days has not been previously studied either. In addition, the outcomes from placing hand hygiene reminder notices on the contamination levels of NSLDGs remain to be determined. Finally, it is still unknown why levels of environmental bacteria tend to decrease over time.

These gaps in the extant knowledge by analyzing environmental, skin, and pathogenic bacteria on unused NSLDGs were addressed. The data for the analyses were obtained before and after placing hand hygiene reminder notices directly on the glove boxes.

MATERIALS AND METHODS

Ethical Approval:

Approval to carry out the research was obtained from the Institutional Review Board, Faculty of Applied Medical Sciences, Albaha University. The research involved collecting and sampling gloves and placing hand hygiene reminder signage on glove boxes; hence, healthcare personnel was under no circumstances research subjects. As such, the study permitted healthcare personnel to be blinded from the research proposition to allow for bias elimination and to prevent any potential unconscious changes in the behaviors of healthcare personnel.

Study Design:

The study was (before-and-after) an epidemiological interventional study carried out in two primary healthcare centres, as well as in the labour and resuscitation rooms in one central hospital in Saudi Arabia. Exclusion criteria were: 1) a recorded outbreak of a healthcare-associated infection during the last 6 months prior to the study, and 2) irregular surveillance of healthcare-associated pathogens. Inclusion criteria were: 1) presence of an infection control unit and 2) the display of written infection control guidelines for hand hygiene on at least one noticeboard. In all locations, hand hygiene was facilitated by placing alcohol-based disinfectant dispensers by every clinic door and beside patients' examination beds. Hand hygiene basins, containing liquid soap and paper towels, were available in all examined locations and were ≤ 6 meters away from any tested glove box. Moreover, disinfection of surfaces with antiseptic solutions was performed daily. The healthcare personnel were not informed of the purpose of the research being conducted in their respective institutions and continued to perform their duties without any interference from the researcher.

On the first morning of the investigation, new unopened boxes of powdered NSLDGs (UNIMED[®], United Medical Industries, Riyadh, Saudi Arabia) were placed in the main labour room and the resuscitation room of a central hospital, with

a further two boxes placed in the main general practitioner clinic at both health centres. Once the boxes were opened, the first pair of NSLDGs was retrieved aseptically in order to measure baseline contamination. After 48 hours of standard usage by the personnel, another pair were aseptically removed from each box to serve as the samples for the study. Once all of the samples had been collected, the boxes were replaced with new boxes and the process was repeated four times. Thus, at each study location, five boxes were examined, resulting in 20 gloves (10 pairs). The same investigator retrieved NSLDGs on days 0 (baseline samples), 2, 4, 6 and 8 (baseline and pre-intervention samples), and 10 (pre-intervention samples only) during the pre-intervention stage of the study. The collection was performed aseptically by wearing a sterile surgical glove (UNIMED[®], United Medical Industries, Riyadh, Saudi Arabia) and immediately placing the NSLDGs into sterile specimen collection containers (urine collection container, SaudiPlast, Jeddah, KSA), which were placed on ice and immediately transported to the microbiology laboratory for processing. Once this pre-intervention stage of the study was complete, the intervention stage commenced. In this phase, the process described above was repeated; however, reminder notices stating “Wash your hands before retrieving gloves to prevent glove contamination” were attached perpendicular to each glove box, making them clearly visible to the users, and post-intervention samples were collected rather than pre-interventional samples. As above, this stage included the same four locations, and from each location, five glove boxes were examined as a collection of 20 gloves (another 10 pairs) over another 10-day period by the same investigator using the same method from each location.

Bacterial Elution:

Each pair of NSLDGs were mixed with 50 ml of trypticase soy broth with 0.1% Tween 80 (Sigma-aldrich Chemie GmbH, Steinheim, Germany) for 1 min at 250 rpm

at 37 °C in a shaker incubator (GFL type 1003, Gesellschaft für Labortechnik, Burgwedel, Germany). Next, 25 ml of the resulting solution was serially diluted in sterile trypticase soy broth. Each dilution was then filtered through a 47-mm-diameter, 0.45 µm-pore-size cellulose acetate MF grid filter membrane held in a steam-sterilized stainless steel filtration unit (Millipore Corp., Bedford, MA, USA), to which 20 ml of pre-warmed sterile trypticase soy broth had previously been added. Finally, an additional 5 ml of trypticase soy broth was added in order to force any residual sample to pass through the filter and ensure efficient deposition of bacteria on the membrane. Colony-forming units per glove (cfu/glove) was determined by counting the number of colonies appearing after culturing the filters on sheep blood agar (Becton Dickinson and Company, Riyadh, Saudi Arabia). Only plates with 30 – 200 colonies were used.

Determination of Bacterial Detection Limits:

The bacterial detection limit of the sampling method described above was determined using NSLDGs seeded with 100 µl trypticase soy broth cultures containing 1×10^6 cfu/ml of *Escherichia coli* DH10B, *Lactobacillus rhamnosus* GR-1, *Bacillus subtilis* ATCC 21332, *Staphylococcus aureus* SA11, or a mixture thereof (Tagg Laboratory Culture Collection, University of Otago, New Zealand). The seeding was performed after the NSLDGs were disinfected with 70% ethanol and left to dry inside a sterile Petri dish. Then, the described inoculum was spread on the palmar side of the glove and left to dry at room temperature inside the dish. Uninoculated gloves were also subjected to the disinfection and drying stages described and were used as negative controls. After being kept at room temperature for 0, 1, 2, 3, 4, 5, 6, 7, and 8 days, the inoculated and negative control samples were subjected to the bacterial elution step described above. Moreover, in a separate experiment, mixtures of *Escherichia coli* DH10B, *Lactobacillus rhamnosus* GR-1, *Bacillus*

subtilis ATCC 21332 and *Staphylococcus aureus* SA11 were used to determine if the co-presence of different species altered the recovery rate.

Bacterial Culture:

Membranes from the bacterial elution steps were transferred onto the surface of sheep blood agar plates (Becton Dickinson and Company, Riyadh, Saudi Arabia) and cultured for the recovery of bacteria. Then, the isolates were subcultured on rich, selective, and differential media used for the identification of *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and Enterobacteriaceae and incubated aerobically at 37 °C for 48 hours. The media used were sheep blood agar, Mannitol salt agar and MacConkey agar (Becton Dickinson and Company, Riyadh, Saudi Arabia). For *Clostridium difficile*, elution samples were inoculated into a cooked meat medium (Becton Dickinson and Company, Riyadh, Saudi Arabia) anaerobically at 37 °C for 48 hours. After incubations of the different media, several representative colonies were subcultured and subjected to microscopic and biochemical identification.

Molecular Identification Of Bacteria:

16S rRNA gene sequencing was carried out to identify the species of the isolates with ambiguous profiles using the previously described primers (Wilson *et al.*, 1990). Multiple isolates for each colony type were subjected to 16S rRNA gene amplification, after which, the generated amplicons were sequenced and BLASTN analyzed using the algorithm freely available online (<http://www.ncbi.nlm.nih.gov>). The extraction of DNA was performed via Qiagen DNeasy tissue kit (QIAGEN, Hilden, Germany). The ABI Prism kit, the ABI 3100 DNA sequencer (BigDye terminator sequencing kit, AmpliTaq DNA polymerase FS, GeneAmp PCR system 9700; ABI) was used to sequence the PCR amplicons by following the manufacturer's directions. *Staphylococcus aureus* SA11 served as a positive control.

Antibiotic Susceptibility Testing:

Susceptibility to methicillin (oxacillin), levofloxacin, metronidazole, vancomycin and clindamycin was tested on Mueller-Hinton with 5% sheep blood agar (Oxoid Ltd., Basingstoke, United Kingdom), using the E-tests and a 10⁸ cfu/ml exponential-phase inocula (AB Biodisk, Solna, Sweden). The breakpoints used corresponded to those described by the Clinical and Laboratory Standards Institute (Institute 2014).

Statistical Analysis:

The Statistical Package for Social Sciences version 20.0 (SPSS[®] Inc., Chicago, Illinois, USA) was used for statistical analysis. The number of contaminated gloves was expressed by proportions (%), and the contamination load (bacterial colony forming units (cfu)) was reported as mean ± standard deviation. A one-tailed z-test for two population proportions was used to determine if the differences in the percentages of contaminated gloves were significant. To determine whether the detected variations in mean bacterial cfu values were significant, a Student's *t*-test was used. Results were declared significant at *p*-value <0.05.

RESULTS

Determination of Bacterial Detection Limits:

The NSLDGs seeded with bacteria and sampled on day 0 showed that the bacterial elution and culturing method had an accuracy rate of 92% in determining bacterial counts and that the recovery rate of each of the different species was not affected by mixing them. The cfu/glove was found to decline at an average rate of 21.5% per day for *Escherichia coli*, 17.75% for *Lactobacillus rhamnosus* GR-1, 6.75% for *Bacillus subtilis* ATCC 21332 and 6.4% for *Staphylococcus aureus* SA11.

Baseline and Pre-Intervention Contamination:

Average bacterial cfu/pair are shown in Table 1 for each location and bacterial group. Bacteria were absent from 10% of the baseline samples (*n* = 20) and only environment bacteria were recovered from

the remaining baseline NSLDG samples (90%), except for one pair that showed two skin commensal bacteria. Bacterial counts from the baseline group during this stage of

the study ranged from 0 to 950 cfu/pair. The average environmental bacterial count recovered from NSLDGs before reminder notices were installed was 502.75 cfu/pair.

Table 1: CFU per glove per day for the different locations before reminder notice installation

Sampling day	Day									
	0 [©]	2 [©]	2*	4 [©]	4*	6 [©]	6*	8 [©]	8*	10*
Environmental bacteria										
Resuscitation Room	0	690	30	950	30	250	30	680	0	30
Labor Ward	770	830	0	570	29	290	44	820	36	28
Health Center 1	490	440	45	640	37	580	0	640	41	55
Health Center 2	690	35	43	0	0	280	27	410	33	45
Skin bacteria										
Rustication Room	0	0	49	0	70	0	66	0	7	57
Labor Room	0	0	76	0	0	0	82	0	58	68
Health Center 1	0	0	2	0	91	0	91	0	78	0
Health Center 2	0	0	87	0	59	2	39	0	82	79
Pathogens										
Rustication Room	0	0	68	0	35	0	7	0	32	0
Labor Room	0	0	0	0	3	0	3	0	3	17
Health Center 1	0	0	0	0	0	0	0	0	0	0
Health Center 2	0	0	71	0	60	0	6	0	1	16

Legend: [©], negative control results; *, sample results.

Skin isolates were recovered from 90% of the pre-intervention NSLDG samples. Skin isolates identified were mainly coagulase-negative *Staphylococcus*, *Corynebacterium* and *Dermabacter* species. Pathogens (*Staphylococcus aureus*, *Clostridium difficile*, *Enterococcus faecalis*, *Pseudomonas* species and Enterobacteriaceae) were recovered from 13 pairs (65%) from the pre-intervention NSLDGs ($n = 20$). Ten of these pre-intervention pairs (50%) yielded only a single opportunistic pathogen or pathogenic species, while mixed cultures were obtained from three pairs (15%). Pathogen counts pertaining to the pre-intervention NSLDGs sampled before the reminder notices were introduced ranged from 0 to 68 cfu/pair. On the other hand, the remaining seven pre-intervention sample pairs (35%) yielded no pathogenic species. All anaerobic cultures were found negative, i.e., no *C. difficile* isolates were obtained from any of the

gloves analyzed.

Post-intervention Contamination:

Bacterial counts from the baseline samples during this stage ranged from 23 to 818 cfu/pair, similar to the results obtained before the reminder notice signs were installed (0 to 950 cfu/pair). Likewise, skin isolates identified were mainly coagulase negative *Staphylococcus*, *Corynebacterium* and *Dermabacter* species, similar to the pre-intervention results. Moreover, average environmental cfu/pair of post-intervention baseline NSLDGs was 501.05 cfu/pair, in line with the previous findings (502.75 cfu/pair). On the other hand, the skin isolates were recovered from 14 of post-intervention pairs (70%), compared to 18 (90%) found pre-installation; however, this reduction was not statistically significant ($p = 0.057$). In contrast, following the introduction of notices, the average counts of skin bacteria in post-intervention pairs was 4.95 cfu/pair, a significant reduction from 57.05 cfu/pair

recovered from pre-intervention pairs ($p = 1.5 \times 10^{-5}$). Moreover, in the post-intervention NSLDGs, pathogenic bacteria (*Staphylococcus aureus*, *Clostridium difficile*, *Enterococcus faecalis*, *Pseudomonas* species and Enterobacteriaceae) were recovered from 3 pairs (15%), compared to 13 (65%) collected pre-intervention, which is also a statistically

significant result ($p = 0.0006244$). The average pathogen cfu/pair detected in pre-intervention gloves (16.1 cfu/pair) was also significantly higher ($p = 0.003374$) than after the reminder notice placement (0.65 cfu/pair). Figure 1 and Figure 2 depict the cfu/pair of gloves at baseline, pre-intervention and post-intervention stages for skin and pathogenic bacteria, respectively.

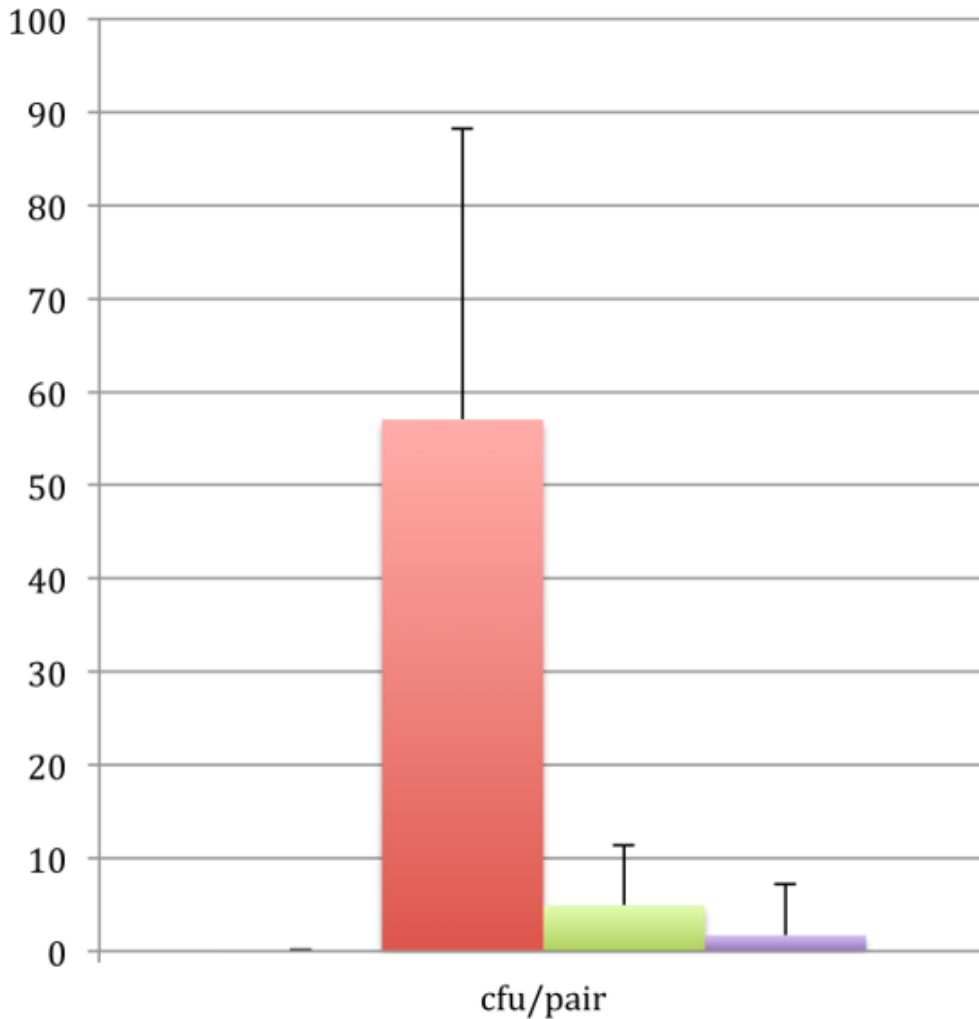


Fig. 1: Mean skin bacteria colony-forming units per pair of gloves.

Legend: Red, pre-intervention contamination of gloves; Green, post-intervention contamination of gloves; Purple, baseline contamination of gloves obtained from unused boxes.

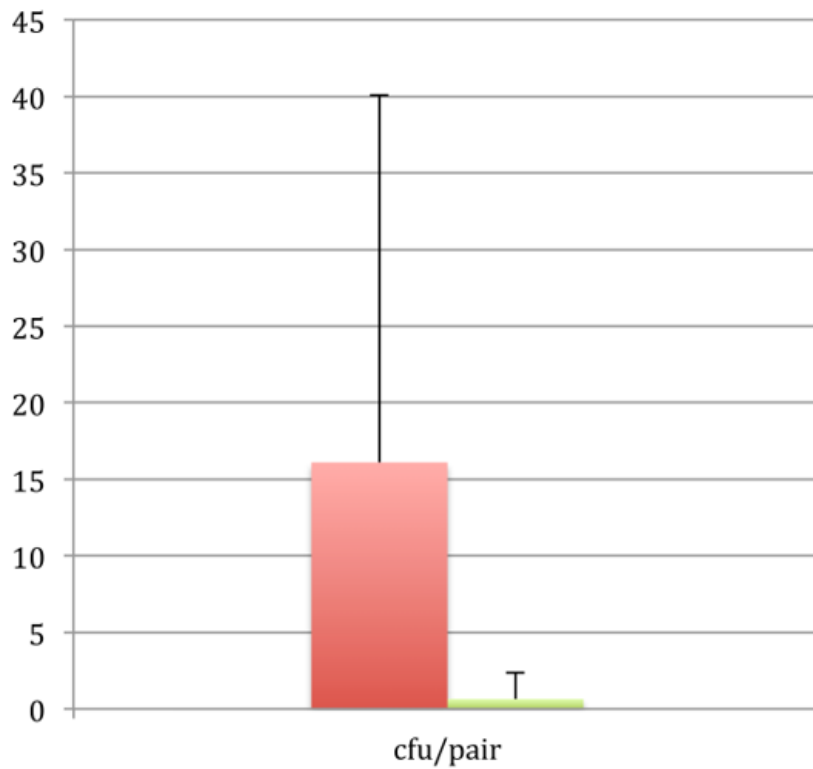


Fig. 2: Mean pathogenic bacteria colony-forming units per pair of gloves. Legend: Red, pre-intervention contamination of gloves; Green, post-intervention contamination of gloves. Baseline pathogenic bacteria colony-forming units per pair were zero.

Identification of Bacteria Through 16S rRNA Gene Sequence:

The identified environmental genera were *Aerococcus*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Curtobacterium*, *Deinococcus*, *Lactobacillus*, *Microbacterium*, *Paenibacillus*, *Pseudoclavibacter* and *Streptomyces*. *Bacillus* species were the most common isolate, identified in 75% of pairs, while *Lactobacillus* and *Deinococcus* were the least common, as each was identified in only 2.5% of pairs. Skin commensals belonged to one of four genera (*Corynebacterium*,

Dermabacter, *Micrococcus* and coagulase-negative *Staphylococcus*). The opportunistic and pathogenic genera were *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Pseudomonas* and coagulase-positive *Staphylococcus*.

Antibiotic Susceptibility Testing:

The identified opportunistic pathogens and pathogens' susceptibility to methicillin, chloramphenicol, ciprofloxacin, levofloxacin, metronidazole, vancomycin and clindamycin results are presented in Table 2. Of the tested bacteria, 24.5% were found to be resistant to ≥ 1 antibiotic

Table 2: CFU per glove per day for the different locations after reminder notice installation

Sampling day	Day									
	0 ^o	2 ^o	2*	4 ^o	4*	6 ^o	6*	8 ^o	8*	10*
Environmental bacteria										
Resuscitation Room	32	732	27	818	36	353	22	712	21	36
Labor Ward	632	791	33	763	31	196	0	757	36	53
Health Center 1	502	390	45	578	37	641	0	703	41	43
Health Center 2	729	181	43	78	23	23	14	410	33	33
Skin bacteria										
Rustication Room	0	0	0	0	3	0	4	0	0	4
Labor Room	0	0	0	0	8	0	0	0	12	14
Health Center 1	0	0	22	0	0	0	12	0	0	10
Health Center 2	0	0	11	0	9	2	14	0	18	10
Pathogens										
Rustication Room	0	0	0	0	0	0	0	0	0	0
Labor Room	0	0	0	0	3	0	7	0	0	0
Health Center 1	0	0	0	0	0	0	0	0	0	0
Health Center 2	0	0	0	0	0	0	0	0	3	0

DISCUSSION

In this study, bacterial contaminations of NSLDGs before and after hand hygiene reminder notices were placed were measured. Findings indicate that NSLDGs can easily and rapidly become contaminated with different skin and pathogenic bacteria, including species known to cause healthcare-associated infections. Moreover, even the gloves obtained from newly opened boxes were found to be contaminated with environmental bacteria. The results obtained are in line with the previously observed trend, whereby skin and pathogenic bacterial levels increase, while environmental bacteria decrease over time (Hughes et al., 2013). In this study, the death rates of *Escherichia coli* DH10B, *Lactobacillus rhamnosus* GR-1, *Bacillus subtilis* ATCC 21332 and *Staphylococcus aureus* SA11 on gloves over a 48-hour period were measured at 21.5%, 17.75%, 6.75% and 6.4%, respectively. We thus postulate that this is one potential factor that contributes to the reduction of environmental bacterial counts over time. The presence of skin commensals and

pathogenic bacteria, on the other hand, may remain high due to continuous contamination from the hands of healthcare personnel regularly retrieving gloves from the boxes.

A repetitive sampling of different glove boxes at four different locations during a 10-day period indicated that the baseline contamination of gloves obtained from freshly opened boxes is significantly different from that of gloves from boxes that have been in regular use for 48 hours. In previous studies, bacterial contamination on gloves was reported for samples collected from an intensive care unit and an orthopedic surgical ward (Hughes *et al.*, 2013; Ye *et al.*, 2015). Nonetheless, the bacterial counts obtained in this study for gloves obtained from boxes that have been in regular use for 48 hours were higher than those reported previously (Hughes *et al.*, 2013).

Pathogenic and skin commensal species, which are commonly implicated in healthcare-associated infections, were seldom detected in baseline samples. However, environmental saprophytes, such as *Bacillus* species, which are common

manufacturing contaminants (Berthelot *et al.*, 2006; Seiler *et al.*, 2012; Seiler *et al.*, 2013), were more prevalent. It should be noted that *Bacillus* species are occasional opportunistic pathogens and their presence is thus not without health implications, since they can cause significant diseases (Celandroni *et al.*, 2019; Jeurissen *et al.*, 2010; Sakihama and Tokuda 2016). Therefore, healthcare personnel may want to attempt to reduce the use of NSLDGs on compromised skin or mucosae of immunocompromised patients.

The detected pathogenic species such as *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Pseudomonas* and coagulase-positive *Staphylococcus*—are a common cause of human infection (Brooks *et al.*, 2013; Luebbert and Chinnes 2015). Alarming, 24.5% of glove-isolated bacteria were found to be resistant to at least one medically used antibiotic (Table 2). The hands of healthcare personnel can be contaminated when exposed to surroundings (FitzGerald *et al.*, 2013; Landelle *et al.*, 2014; Monistrol *et al.*, 2013). Similarly, bacteria are potentially transmitted to the glove box edges and unused gloves when fresh gloves are retrieved by contaminated hands. Thus, any subsequently retrieved gloves may also get contaminated. In our study, the high numbers of skin commensals (average 57.05 cfu/pair) and a contamination rate of 90% of NSLDGs collected from boxes that had been in use for 48 hours prior to the installation of reminder notices supports the hypothesis that contamination is transmitted by the hands of healthcare providers during the retrieval process. In addition, following the placement of reminder notices, the percentage of contaminated NSLDGs and average cfu/pair decreased to 70% and 4.95 cfu/pair, respectively. The reduction in cfu/pair was statistically significant ($p = 1.5 \times 10^{-5}$) while the reduction in positive pair frequency was approaching significance ($p = 0.057$). These findings indicate that contamination with skin commensals can

still take place even if reminder notices potentially increased hand hygiene frequency prior to glove retrieval, although at a significantly lower bacterial load since these are commensal organisms rather than transient colonizers. The reduction in pathogens due to the intervention was more prominent. Before the reminder notices were placed, 13 (65%) NSLDGs were found contaminated with pathogens, compared to only 3 (15%) after placement, representing a statistically significant reduction ($p = 0.0006244$). The average pathogen cfu/pair detected in NSLDGs per-intervention (16.1 cfu/pair) was also significantly higher ($p = 0.003374$) than post-intervention (0.65 cfu/pair).

Not only the detected pathogens or opportunistic pathogens but also the detected skin commensals, such as *S. epidermidis*, *S. saprophyticus*, *S. hominis*, *S. capitis* and *S. haemolyticus*, can cause many diseases, including device-related, urinary and skin infections (Talebi *et al.*, 2016; Widerstrom *et al.*, 2012). These findings justify the need for the introduction of additional controls aimed at preventing glove contamination and the transmission of healthcare-associated pathogens. The reminder notices described here are cost-effective and easy to implement a method with the potential to reduce the contamination levels. Additionally, the presence of spore-forming and other environmental bacteria, such as the *Bacillus* species identified from baseline gloves, could be controlled with manufacturing regulations, as previously recommended (Berthelot *et al.*, 2006; Celandroni *et al.*, 2019). This is important, given that the level of contamination detected reached infectious doses for some bacterial pathogens, which can be as low as 10 infective particles (viable cells) for some strains of *Escherichia coli* (Leggett *et al.*, 2012).

The study did not aim to calculate an association between contamination levels and healthcare-associated infections, rather, the contamination levels were determined

and intervention was used to attempt to reduce them. One implication of finding different bacteria at infectious doses that can be reduced with reminder notices include the need for future studies to determine the health implications of such strategies on patients and also determine cost-effectiveness. Moreover, the study did not determine if the intervention actually caused protracted sustainment of the reduction in contamination levels, due to more routine compliance to hand hygiene requirements beyond the 10-day period of the interventional stage of this study. Longitudinal behavioral studies could in the future address the effect of different reminder methods; including the one used herein, on compliance levels, and subsequently the rate of healthcare-associated infections. Directly measuring the behavioral changes related to hand hygiene practices before and after placement of the signage during an extended period of time, in addition to the rate of healthcare-associated infections, to see if the new behavior can become a habit with real health-related outcomes is warranted.

Conclusions

One implication of finding different bacteria at infectious doses that can be reduced with reminder notices is that there is a need for future infection control strategies to ensure patients' safety. In conclusion, hand hygiene reminder notices were found to improve glove safety, as their placement resulted in a reduction in their carriage rate and a load of skin and pathogenic bacteria. Thus, printing hand hygiene reminder notices on glove boxes could be an affordable strategy to significantly reduce glove contamination with bacteria.

Conflict of Interest

There are no conflicts of interest to declare.

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