Bacterial contamination of unused, disposable non-sterile gloves on a hospital orthopaedic ward

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RESEARCH

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Abstract

Background

Non-sterile disposable gloves are used on large hospital wards, however their potential role as a vehicle for pathogen transmission has not been explored in this setting.

Aims

This study investigates glove use on a hospital orthopaedic ward to examine whether pathogen contamination occurs prior to contact with patients.

Method

Glove samples were aseptically removed from boxes on a hospital orthopaedic ward on opening and days 3, 6 and 9 thereafter. Following elution of bacteria and viable counts, glove isolates were identified by standard techniques and 16s rDNA sequencing. Methicillin resistance of staphylococci was determined by disc diffusion, Epsilon tests and PCR. Gloves were inoculated to determine two isolate survival rates.

Results

Total bacterial counts ranged from 0 to 9.6 x 10^3 cfu/glove. Environmental bacteria, particularly *Bacillus* species, were present on 31/38 (81.6%) of samples. Half (19/38) the samples were contaminated with skin commensals; coagulase negative staphylococci were predominant. *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas* sp. or methicillin susceptible *Staphylococcus aureus* were recovered from 5/38 (13.2%) of samples. Significantly more skin commensals and pathogens were recovered from samples from days 3, 6, 9 than box-opening samples. *Staphylococcus epidermidis* and *Klebsiella pneumoniae* inoculated onto gloves remained viable for several days but counts decreased.

Conclusion

Health care workers introduced skin commensals and pathogenic bacteria into glove boxes indicating that unused, non-sterile gloves are potential pathogen transmission vehicles in hospitals. Findings highlight adherence to hand-washing guidelines, common glove retrieval practice, and glove-box design as targets for decreasing bacteria transmission via gloves on hospital wards.

Key Words

Bacteria, nosocomial pathogens, nosocomial infection, disposable gloves, contamination, methicillin resistance, hospital.

What this study adds:

1. In a hospital ward setting, unused non-sterile disposable gloves (NSDG) may become contaminated with skin commensals and pathogens during the act of glove retrieval.
2. Contaminated NSDG therefore have the potential to act as transmission vehicles for bacteria as demonstrated by these results.
3. Glove box design and glove withdrawal technique could be further examined to decrease the potential for pathogen transfer to unused gloves.

Background

Nosocomial (hospital acquired) infections (NI) are an ongoing problem in health care facilities worldwide with an estimated 5-10% of hospitalised patients acquiring a NI

during admission. In New Zealand, NI contribute approximately $136 million to health care budgets annually.[1] Implementation of proper hand hygiene practices amongst healthcare workers (HCW) is accepted as the single most important measure in controlling NI.[2,3] Compliance rates to hand hygiene standards are generally low,[4] indicating that common NI pathogens on HCW hands (such as Gram negative bacilli, staphylococci, enterococci and clostridia)[4,5] are at risk of being transmitted to patients and potentially causing NI.

An important supplement to hand hygiene practices is the correct use of gloves which can reduce the transmission of pathogens and thus help prevent NI.[3,5] However, when gloves are misused they can significantly increase the horizontal spread of pathogens.[6] Girou et al. have described gloves acting as a 'second skin' when worn for prolonged periods of time without changing, enabling the spread of NI pathogens not only to the patient, but to the surrounding environment as well.[6]

Previous investigations in intensive care and dental settings have identified pathogens on non-sterile disposable gloves (NSDG) before use, however whether pathogens exist on NSDG in the context of a large hospital ward remains unexplored.[7,8] The aim of this study was to investigate the potential of unused NSDG to act as vehicles for microbial transmission in a hospital ward setting. We hypothesised HCW hands could transmit NI pathogens to surrounding unused NSDG in in-use boxes. Such information is relevant to infection control agencies and healthcare outcomes in settings where NSDG are utilised.

Method

Study design

This study was a prospective audit of NSDG use on a single orthopaedic ward. Ten boxes of powder-free latex NSDG (USL Medical, Auckland, New Zealand; 100 gloves/box) were placed in randomly selected rooms (identified using a random number generator) on a 32-bed general orthopaedic ward at Dunedin Public Hospital (Dunedin, New Zealand). The ward carries both elective and non-elective orthopaedic cases of both surgical and non-surgical origin. It is staffed by six full-time nurses, one full-time nurse aide, four house surgeons, seven registrars and 11 consultants. Staff were not informed of the research purpose and continued to use the gloves for routine purposes during the study period. Existing handwashing procedures were congruent with the WHO guidelines.[3] This means washing hands before and after individual patient contact, regardless of whether gloves were used or not.

Unused glove samples (three gloves from each box on each occasion) were aseptically removed by one investigator (KH) from the boxes on opening (day 0) and on days 3, 6 and 9 thereafter. Collection occurred over a five-week period to ensure samples were not biased by staff roster or use over a short time period. Sterile forceps were used to withdraw glove samples from boxes; these were placed in sterile Whirlpool bags (Simport, Belloell, QC, Quebec, Canada) before being transported to the laboratory on ice.

Bacteria were eluted from the glove samples and plated onto different culture media for preliminary identification and viable counts. Enrichment culture to recover small numbers of bacteria was also carried out. Following presumptive identification, unidentifiable colonies and non-*Bacillus* species were identified using 16S rDNA amplification and sequencing. Staphylococci were investigated for methicillin resistance. In a separate experiment, two of the isolated bacteria were inoculated onto glove samples and their viability monitored over a two-week period.

J-CT (Professor and Head of Orthopaedic Surgery) and J. Stoddart (Infection Control, Dunedin Public Hospital) gave permission for the study; ethical approval was not required.

Bacterial culture of glove samples

Once the sterile Whirlpool bags containing gloves were transported to the laboratory, diluent (40 mL PBS with 1% tryptone [Bacto™, Becton Dickinson & Co., Sparks MD, USA]) was added to each bag and the contents mixed using a stomacher for 30 seconds. Triplicate 333L and 10L samples were cultured on Columbia sheep blood, Mannitol Salt and MacConkey agar plates (Fort Richard Laboratories Ltd., Auckland, New Zealand) incubated aerobically at 35±2°C and examined for growth after 24 and 48 h. The limit of detection for the plating method was 40 cfu/glove; counts below 8×10^2 cfu/glove (< 20 cfu/plate) were regarded as approximate. Enrichment cultures (10mL) were prepared in Brain Heart Infusion broth (Bacto™, Becton Dickinson & Co., Sparks MD, USA) and subcultured as for primary cultures. For *Clostridium perfringens* spores, sample diluent (1 mL) was heat-treated and cultured anaerobically in cooked meat medium (Fort Richard Laboratories Ltd., Auckland, New Zealand).

Multiple representatives of each morphological type were subcultured for identification tests. Isolates were presumptively identified by standard microbiological tests. Unidentifiable isolates and non-*Bacillus* species were stored in Brain Heart Infusion broth (Bacto™, Becton Dickinson &

Co., Sparks MD, USA) with 20% glycerol (Sigma-Aldrich, Castle Hill, NSW, Australia) at -80°C for further study.

Polymerase chain reactions and sequencing

Full identification of unidentifiable isolates and non-*Bacillus* species was achieved by 16s rDNA amplification and sequencing of multiple isolates of each colony type.[9] For potential MRSA isolates, the SCC*mec* and *orf*X regions were amplified.[10] MRSA NZCC 3529 (New Zealand Culture Collection, ESR, Porirua, New Zealand) was used as a positive control for both reactions. Sequencing was performed by the Genetic Analysis Services (Department of Anatomy, University of Otago, Dunedin, New Zealand) using an ABI 3730xl DNA analyser with the BigDye® Terminator Version 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems®, Life Technologies Ltd., Mulgrave, Victoria, Australia). Sequences were subjected to BLAST alignment searches within the GenBank DNA and/or the Ribosomal Database project (RDP) databases. To assist with data analysis, bacteria were assigned to one of three categories on completion of the identification tests: (i) bacteria primarily of environmental origin; (ii) skin commensals; (iii) pathogens associated with outbreaks of NI.

Methicillin susceptibility testing

The oxacillin disc diffusion test was performed on staphylococcal isolates as previously described.[11] Methicillin resistance was confirmed using Oxacillin M.I.C.E E-test strips (Oxoid, Basingstoke, Hampshire, UK) according to the manufacturer's instructions. CSLI breakpoints were applied for both tests.[12]

Glove Inoculation trial

NSDG aseptically removed from newly opened boxes were inoculated with either $1x10^5$ cfu/glove of *Staphylococcus epidermidis* (isolate N-13-1) or $1x10^5$ cfu/glove of *Klebsiella pneumoniae* (isolate ML-18-12). On days 0, 2, 4, 7 and 14 of the room temperature incubation period, samples were processed and viable counts performed on Columbia sheep blood agar plates (Fort Richard Laboratories Ltd.). Uninoculated gloves were the negative control.

Statistical analysis

P values were determined by Fisher's exact probability test, with significance at $P < 0.05$.

Results

Bacterial culture

Apart from boxes 2 and 6, which were empty by day 9, the remaining boxes still contained gloves at the end of the sampling period, estimated to be no more than 25% of the original content. In total, 38 samples comprising 114 gloves were examined. Culturable bacteria were absent from only two of the 38 glove samples (boxes 1 and 7 on day 3).

Average viable counts for the three groups of bacteria (environmental, skin commensals and pathogens) are shown in Table 1. Environmental bacteria were cultured from 31/38 (81.6%) glove samples and counts ranged from <40 cfu/glove to 9.6×10^3 cfu/glove. Skin commensals were cultured from half the samples (19/38) with counts ranging from <40 cfu/glove to 8.4×10^2 cfu/glove. Pathogens associated with outbreaks of NI were present in low numbers, approximately ≤80 cfu/glove, in 5/38 (13.2%) samples. Skin commensals and pathogens were absent from the samples taken from freshly opened boxes, apart from a single enrichment culture. Compared with day 0 samples, day 3, 6 and 9 samples were significantly more often contaminated with skin commensals/pathogens ($P = 0.03$, $P = 0.003$, $P = 0.03$ respectively). There was a trend towards increasing numbers of skin commensals and decreasing numbers of environmental bacteria over time (Table 1). Samples taken on day 0 during the glove inoculation trial indicated that the method used for recovering bacteria from the gloves had an average efficiency rate of 89%. Anaerobic cultures for *Clostridium* were all negative.

Identification of bacteria

Match scores for sequences within GenBank and/or RDP databases and were between 98.9% and 100%. Ten environmental genera were identified (*Aerococcus, Arthrobacter, Bacillus, Brevibacterium, Curtobacterium, Microbacterium Micrococcus, Paenibacillus, Pseudoclavibacter* and *Streptomyces*). *Bacillus* was predominant being recovered from 29/38 (76.3%) glove samples. Skin commensals identified were CoNS, *Dermabacter* and *Corynebacterium* species. CoNS, especially *S. epidermidis*, were prevalent accounting for 69/86 (80.2%) isolates identified by PCR. Species identification of CoNS is shown in Table 2. *Enterococcus faecalis, Klebsiella pneumoniae,* an unidentified *Pseudomonas* sp. and *Staphylococcus aureus* comprised the pathogen outbreak group. Eighteen glove samples yielded a single bacterial species on culture while mixed cultures were obtained from the remainder. The highest diversity of genera (7) was seen in a day 9 sample.

Methicillin susceptibility

Methicillin resistance was detected in 38/71 (53.5%) CoNS isolates (Table 2). The two *S. aureus* isolated were phenotypically methicillin sensitive and failed to amplify in the SCC*mec*/*orf*X PCR.

Glove inoculation trial

When inoculated onto glove samples, the viability of *S. epidermidis* decreased to approximately 50% after 24 h followed by a steady decline with <1% of the original inoculum recoverable on day 14. *K. pneumoniae* showed a similar decreasing trend in viability over the incubation period. *S. epidermidis* and *K. pneumoniae* were absent from the uninoculated control gloves.

Discussion

We investigated bacterial contamination of unused NSDG sampled from in-use boxes on a hospital orthopaedic ward over time and identified many different bacteria, including those that are known to cause NI.[13,14,15] Glove bacterial counts were much higher than those reported by Rossoff et al., who examined NSDG in an ICU setting.[7] Skin commensals and pathogens were rarely isolated from samples collected when the glove boxes were newly opened but environmental bacteria, mainly *Bacillus* spp., were common. *Bacillus* spp. are known manufacturing contaminants, however they are also occasional opportunist pathogens, and as such their presence is noteworthy.[13]

The increased frequency and number of skin commensals, especially CoNS, found on gloves from in-use boxes strongly suggests contamination from the hands of HCW occurred during glove retrieval. *S. epidermidis* was the most common skin contaminant and its association with a number of different NI, including device-related infections, is well known.[14] Methicillin resistance was common among CoNS, as noted elsewhere.[15] Of the other CoNS isolated, *S. caprae, S. capitis, S. haemolyticus* and *S. hominis* are clinically significant.[15]

While CoNS infections are mostly endogenous (infections occurring from bacteria colonising the person), nosocomial infections caused by *E. faecalis, K. pneumoniae*, *Pseudomonas* and *S. aureus* may be endogenous or exogenous (from an external source). Thus, they constitute a group of nosocomial pathogens with known transmissibility.[16–22] In the present study these pathogens were detected in some glove samples, but as counts were low it is unknown whether the observed levels constituted a direct threat to patient health.

It remains unclear whether pathogens levels detected in the present study represent a direct threat to human health, however previous publications have indicated the infectious dose for some pathogens is equal to or below the level of glove contamination seen in our study.[23,24] Kaiser *et al.* recorded a level of <10 cfu for *S. aureus* in an animal model of surgical wound infection, suggesting that even very low levels of glove contamination could be significant if the bacterial contaminant is a virulent strain.[23] Most of our values (Table 1) are above this level, suggesting that our findings are of clinical significance. Other factors such as the virulence of the strain, the route of infection, and whether the bacteria were scattered over the surface of the glove or clumped in an area that was likely to come into contact with a vulnerable part of the patient need also to be taken into account and may be important in determining an overall likelihood of transmission. These factors have not yet been examined in a clinical setting.

The presence of the identified bacteria indicates there is potential for unused NSDG to act as transmission vehicles for these organisms. *E. faecalis*, *K. pneumoniae* and *Pseudomonas* are not usually indigenous skin commensals, so the adequacy of hand hygiene practiced by HCW was likely an important factor in the observed glove contamination. These findings highlight the importance of hand-washing prior to using NSDG in patient contact, in accordance with the WHO and other guidelines.[3,4]

Bacterial survival on NSDG was tested in a glove inoculation trial using two of the glove isolates. While these non-sporing contaminants were not long-lived on the gloves, they would probably remain sufficiently viable in the short term for cross-transmission to occur and therefore indicate the potential for NI to arise from the use of any open glove boxes that were frequently accessed.

Limitations

The study was limited to one hospital ward over a defined period of time, therefore we are unable to determine whether such rates of contamination are similar on other wards or in other hospitals. Underestimation of numbers of slow-growing bacteria was a limitation of the culture technique used because the maximum incubation time was 48 hours. Direct plating of eluted bacteria meant that low bacterial counts were approximate and diluent filtration is recommended for future studies so that greater accuracy can be achieved.

Conclusion

This study found many different bacteria existing in open boxes of NSDG on a large hospital ward, with results suggesting that these bacteria were likely introduced from the hands of HWC. These bacterial levels were higher than amounts previously demonstrated as causes of wound infection.[23,24] Improvements to glove withdrawal technique, box design, or good hand-hygiene compliance have the potential to reduce contamination of unused NSDG with human-associated bacteria. Further research is required to

discover whether the type of contamination described here is a regular occurrence and whether there is a correlation with the type of ward and/or HCW hand hygiene. Such modifications could decrease the risk of pathogen cross-transmission in settings that utilise NSDG and potentially affect the overall incidence of NI on hospital wards. Results reinforce the necessity of appropriate hand hygiene on hospital wards to decrease to possibility of pathogen transmission to patients and lower the risk of subsequent NI.

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PEER REVIEW

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CONFLICTS OF INTEREST

One of the authors (JC) is a Senior Editor for the AMJ, another (HB) is an expert reviewer for the AMJ.

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ETHICS COMMITTEE APPROVAL

Ethics not required.

Table 1: Average viable counts of bacteria present on unused non-sterile disposable gloves sampled from in-use boxes over time

Sampling day	**0**	**3**	**6**	**9**
No. samples tested*	**n = 10**	**n = 10**	**n = 10**	**n = 8**
Environmental bacteria:				
Viable count average	+++	±	±	+
No. positive samples	9	7	7	8
Skin commensals:				
Viable count average	±	+	++	++
No. positive samples	1	6	8	4
Pathogens:				
Viable count average	Nil	+	Nil	+
No. positive samples		3		2

*Each sample comprised three gloves

± = <40 cfu/glove; + = ≥40 < 10^2 cfu/glove; ++ = ≥10^2 <10^3 cfu/glove; +++ = ≥10^3 cfu/glove

Table 2: Species identity of staphylococcal glove isolates and methicillin susceptibility determined by disc diffusion and E-test

Staphylococcus (n=71)	**Number of isolates**	**Methicillin susceptible**	**Methicillin resistant ([a]MIC range mg/L)**
S. epidermidis	27	5	22 (0.5–192)
S. haemolyticus	12	5	7 (4–>260)
S. pasteuri	12	10	2 (0.5)
S. warneri	7	5	2 (6–12)
S. capitis	7	5	2 (0.75–3)
S. aureus	2	2	0
S. caprae	2	0	2 (2–3)
S. pettenkoferi	1	0	1 (>260)
S. hominis	1	1	0

[a] MIC determined by oxacillin E-test