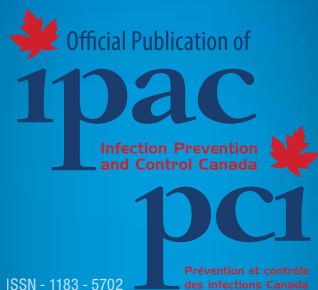


# CJIC

The Canadian Journal of Infection Control  
Revue canadienne de prévention des infections



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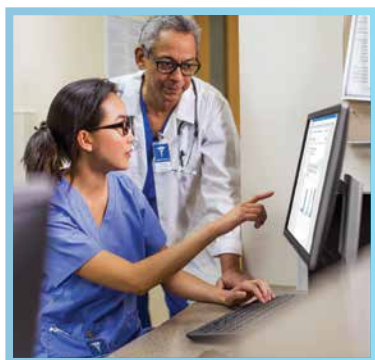
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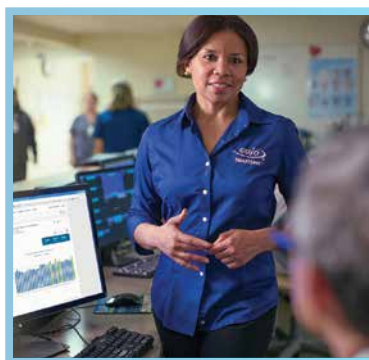
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# Comparative study on the efficacy of disinfectants against bacterial contamination caused by biofilm

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## ABSTRACT

The disinfection of surfaces containing biofilms is complex, since bacteria within biofilms use mechanisms of protection that lead to an increased bacterial resistance against commonly used disinfectants. To eliminate biofilm contamination, microorganisms within the biofilm must be destroyed and the biofilm structure itself must be removed from the surface. The study presented here use a standard method (ASTM standard test method E2871-12) for measuring efficacy of six disinfectants (Hydrogen peroxide, peracetic acid, chlorine dioxide, sodium hypochlorite, quaternary ammonium compound and enzyme) on removing biofilm and kill the bacteria within it. Results show that some disinfectant is good to kill bacteria (e.g., sodium hypochlorite) where other are better at removing the biofilm structure from the surface (e.g., enzyme). However, two disinfectants, peracetic acid and chlorine dioxide, are able to have a dual action on killing the bacteria and removing the biofilm from the surface. We also conclude that diffusion control the relative efficacy of each disinfectant.

## KEY WORDS:

Biofilm, disinfection, method, Sodium hypochlorite, Peracetic acid, quaternary ammonium compound, hydrogen peroxide, enzyme

## INTRODUCTION

Most discussions on infection control or environmental contamination tend to centre on planktonic bacteria (free bacteria in the liquid environment) [Ofek I, 1994 #1;Ofek I, 1994 #1;Buckingham-Meyer, 2007 #11][1]. However, it is known that bacteria will naturally stick to a surface and create a micro community called a biofilm [2]. This natural way of life for bacteria is in large part to blame for recurrent contamination on specific surfaces and a clear indicator of how we should disinfect surfaces [3]. Recent advances in microbiology shed new light on how bacterial communities are organized, raising serious concerns around disinfection protocols [4].

When bacteria encounter a surface, they can attach themselves to it. At this point, they change their metabolism and start to produce new metabolites, this decision is made in reaction to their surrounding environment [2]. When there are plenty of resources, bacteria will grow and multiply until resource become scarce. Then, bacteria will start to create a "cocoon" in which they can be protected and form a viable community [5]. This "cocoon", which is called an exopolysaccharide matrix (EPS), is the main difference between planktonic bacteria and a biofilm. Though this differentiation may seem meaningless, it has a huge impact on the bacteria and on the disinfection of the surface containing this biofilm. Four protection mechanisms are now generally accepted [3]. Firstly, the matrix creates a physical barrier that prevents dangerous agents from reaching bacteria due to limited diffusion. Secondly, some of the cells in a biofilm produce stress

responses at the expense of other cells, which are sacrificed. Thirdly, the cells lower their metabolic activity, which may make them less susceptible to antimicrobials. Finally, persistent cells accumulate in biofilms because they revert less readily and are physically retained by the biofilm matrix. These four mechanisms of protection lead to an increased bacterial resistance against commonly used disinfectants.

To obtain approval for a disinfection product's stated efficacy, one must show efficacy results based on different methods. However, these methods test product efficacy on newly grown bacteria that can be in a broth (e.g., EN1276, AOAC 960.09) or dried on a carrier (e.g., AOAC 955.14). These methods do not take into account the fact that, on environmental surfaces, bacteria tend to be hidden in a biofilm. Other microorganisms (i.e., viruses) can also hide in a biofilm, leading to increased protection [6, 7]. Various strategies have been suggested for attacking biofilms, but from the author's point of view, to eliminate biofilm contamination, the microorganism within the biofilm must be destroyed and the biofilm structure itself must be removed from the surface. If a disinfectant only kills the bacteria but leaves the biofilm structure in place, it may create a nest to which other bacteria can attach themselves in order to create a new biofilm more quickly. On the other hand, if a disinfectant removes the biofilm without killing the bacteria, it will release the planktonic bacteria, which will contaminate the surrounding environment.

Multiple studies have been conducted on biofilms over the years [3, 4, 8-14]. However, because of variability of methods

and the resulting high disparity of outcomes, conducting a meaningful comparison across the studies is challenging [4]. The objective of this paper is to present the efficacy of common disinfectants in killing and removing bacterial biofilm on surfaces. The results compare the effect of hydrogen peroxide, sodium hypochlorite, chlorine dioxide, quaternary ammonium compound (quat), enzymes and peracetic acid (PAA) on a pseudomonas biofilm. This will shed light on the efficacy of each method and demonstrate how the molecular characteristics of various disinfectants can explain these outcomes.

## MATERIALS AND METHODS

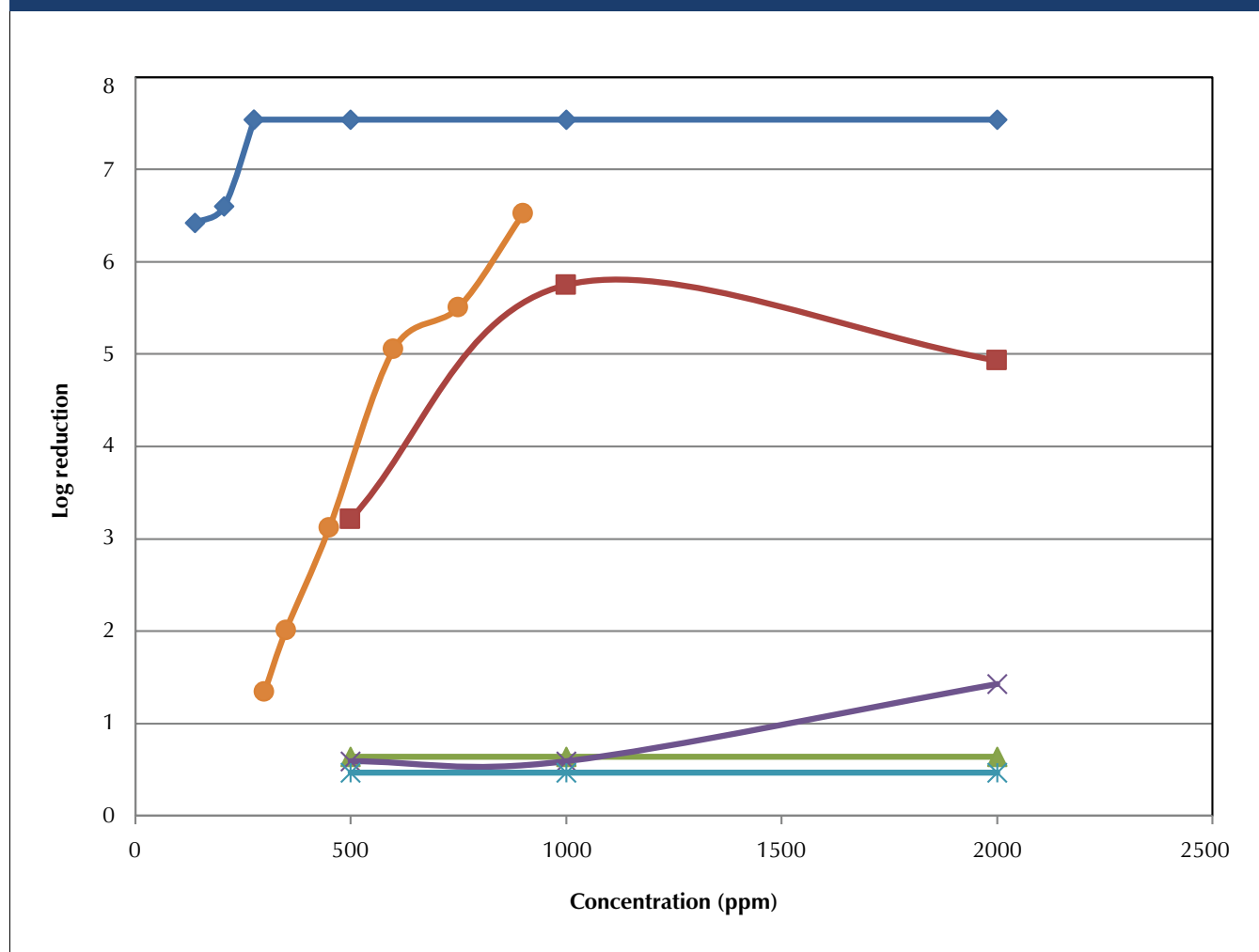
### Materials

The hydrogen peroxide solution was obtained by diluting 50% hydrogen peroxide from Arkema inc. (Colombes, France) to the required test concentration. The sodium hypochlorite solution was obtained by diluting 12% sodium hypochlorite from UBA Inc. to the required test concentration. The chlorine dioxide solution was prepared with the product

Activator™ and the product Ecosan™ from Sani Marc Inc. (Victoriaville, Quebec, Canada). The quat solution was obtained by diluting an 80% first generation quat from Stepan Co. (Northfield, Illinois, États-Unis) to the required test concentration. A concentrated enzymatic solution was made using a mix of three types of enzymes: a protease, a lipase and an amylase from Novozymes Inc. (Gledsaxe, Denmark): Everlase 16 L, Termamyl 300 L and Stainzyme 12 L, respectively. The concentration of each enzyme was 0.8% for Everlase 16 L, 0.6% for Termamyl 300 L and 0.6% for Stainzyme 12 L. The concentrated enzymatic solution contained 0.5% fatty alcohol ethoxylates and pH was adjusted to 9.5 with sodium hydroxide. The enzymatic solution was made with this concentrated enzymatic solution. The PAA solution was obtained by diluting Oxygerm (5% PAA product from Sani Marc) to the required test concentration.

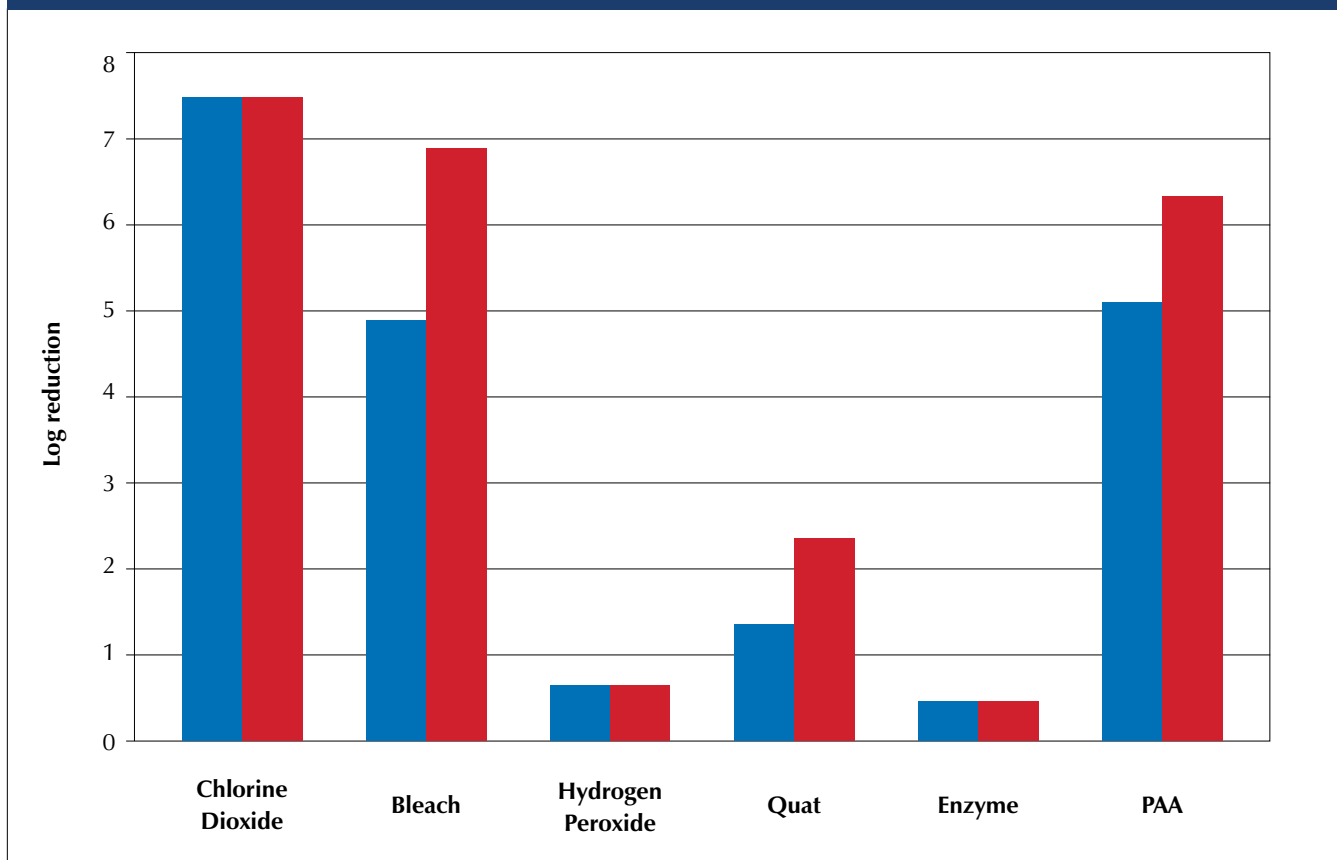
All disinfectants tested were aseptically diluted at appropriate concentrations with sterile water or hard water (hardness expressed as CaCO<sub>3</sub> concentration) just before performing the assays. Disinfectant samples were diluted and kept at room temperature.

**FIGURE 1: Concentration curve for killing bacteria within the biofilm (chlorine dioxide◆, sodium hypochlorite■, hydrogen peroxide▲, quaternary ammonium compound×, enzyme\*, peracetic acid●)**





**FIGURE 2: Effect of time on the efficacy of common disinfectants: chlorine dioxide (250 ppm, 5 min and 15 min); bleach (2000 ppm, 5 and 60 min); hydrogen peroxide (2000 ppm, 5 and 60 min); quat (2000 ppm, 5 and 60 min); enzyme (2000 ppm, 5 and 60 min); PAA+surfactants (600 ppm, 5 and 10 min)**



### Reactor preparation

The reactor (CBR 90 Biofilm Reactor, BioSurface Technologies Corporation, Bozeman, USA) preparation and sterilization were done according to ASTM standard test method E2562-12. Borosilicate glass disc coupons were used for all tests and served as a carrier for the biofilm formation. A total of 24 coupons were used for each assay. The coupons were retained by height rods, each one holding three coupons.

### Culture preparation

*Pseudomonas aeruginosa* ATCC 15442 was grown on a TGEa (Tryptone glucose extract agar) slant from a frozen stock culture. The inoculum was incubated for  $24 \pm 1$  h at  $37 \pm 1^\circ\text{C}$  under aerobic condition. A minimum of three daily transfers on TGE slants were done prior to the preparation of the final test suspension. For the final test culture step, bacteria were transferred to TGE broth and the viable cell density was determined using absorbance at 600 nm. The standardized culture was maintained at  $4^\circ\text{C}$  for a maximum of 30 minutes until the reactor inoculation step.

### *Pseudomonas aeruginosa* biofilm production

*P. aeruginosa* biofilm was produced on borosilicate glass disc coupons according to ASTM standard test method E2562-12

with some deviations. 350 mL of TGE broth were added to the sterile reactor. The reactor was then inoculated at  $10^6$  CFU/mL with the standardized *P. aeruginosa* culture. The inoculated reactor was incubated in batch mode at room temperature for  $24 \pm 1$  h with a rotation speed of  $125 \pm 5$  rpm. At the end of the batch mode, a continuous flow of  $17 \pm 2$  L of TGE broth (flow rate around 702 mL/h) was started for another 24 h. The reactor was still maintained at room temperature under stirring at the speed of  $125 \pm 5$  rpm. The drain spout of the reactor was connected to an empty carboy to allow the medium to drain and to maintain a constant volume (350 mL) in the reactor during the continuous phase.

### Disinfectant efficacy evaluation

Disinfectant efficacy was tested according to ASTM standard test method E2871-12. Rods were aseptically removed from the reactor and rinsed in 30 mL of sterile phosphate buffer (pH 7.2) in order to remove planktonic cells. The coupons, on which the biofilm was grown, were aseptically removed from rods using a flame-sterilized Allen wrench. Each coupon was placed in a sterile 50 mL conical tube. Then, 4 mL of disinfectant were added to each tube. For the initial population control, the disinfectant was replaced by 4 mL of sterile phosphate buffer (pH 7.2). Tubes were left at room temperature during the

contact time targeted. At the end of the contact time, 36 mL of neutralizer (Lethen broth; 25.7 g/L, Tween 80; 100 g/L, L-alpha-lecithin; 11 g/L, sodium thiosulfate; 2 or 20 g/L depending on the disinfectant's concentration) were added to each tube. Tubes were then mixed with a vortex and placed at 4°C until the next step. For each disinfectant treatment, five coupons were used. The initial population control was made for each assay and four coupons were used.

In order to quantify the viable population on each coupon, the biofilm was removed with a sequence of vortex and sonication (30 seconds vortex, 30 seconds sonication at 40 ± 2.5 KHz, 30 seconds vortex, 30 seconds sonication at 40 ± 2.5 KHz and 30 seconds vortex). Tubes were then serially diluted with sterile phosphate buffer (pH 7.2). Quantification of the viable biofilm population was done using the spread plating method on TGEa. Plates were incubated at 37 ± 1°C for 24 h. The colony number was counted on each plate, and results were reported in log reduction (mean log of initial population control – mean log of population after disinfectant treatment).

### Microscopy

Rods were aseptically removed from the reactor and gently rinsed in 30 mL of sterile phosphate buffer (pH 7.2) in order to remove planktonic cells. Coupons were then stained with safranin solution, 0.6% w/v, for 1 minute. Coupons were visualized at a 200X magnification with an optical microscope (Nikon, Eclipse E2000, Mississauga, Canada).

### RESULTS

All the data collected are presented in Figure 1 for disinfectant efficacy evaluation and in Figure 2 for microscopic observations. Concentrations curves have been limited to 2000 ppm to keep standard conditions. The authors estimate that a good disinfectant should be able to reach a 5 log reduction at these concentrations without harming the surfaces. As we see in Figure 1, oxidizing products seem to be more effective than non-oxidizing products (enzyme and quat) at penetrating the biofilm and killing the bacteria. The only exception to this is hydrogen peroxide.

Hydrogen peroxide shows no efficacy in killing bacteria in a biofilm up to 2000 ppm, as shown in Figure 1. Even if

we increase the time to 60 minutes, there is no increase in killing ratio. However, microscopy results show that hydrogen peroxide is effective at removing the biofilm matrix from the surface. This indicates that hydrogen peroxide can penetrate the biofilm to remove the matrix from the surface, but is unable to kill bacteria within it. This is not surprising since tests conducted on planktonic data in our lab show that unformulated hydrogen peroxide is not a good bactericide [15]. Adding surfactant is needed to achieve the correct killing ratio.

Sodium hypochlorite shows good efficacy in killing bacteria in biofilms, achieving almost 6 log of reduction at 1000 ppm. However, this tends to diminish to 5 logs at 2000 ppm. It is reasonable to think that a protective layer is formed by oxidation of the matrix at higher concentrations, lowering the diffusion of hypochlorite ions through the biofilm. This can be corroborated with the total kill of the bacteria in the biofilm at 60 min. Contrary to peroxide, hypochlorite is unable to remove the polysaccharide structure on the surface. Consequently, treatment with bleach will produce excellent initial activity, but the biofilm can regrow very quickly on the surfaces.

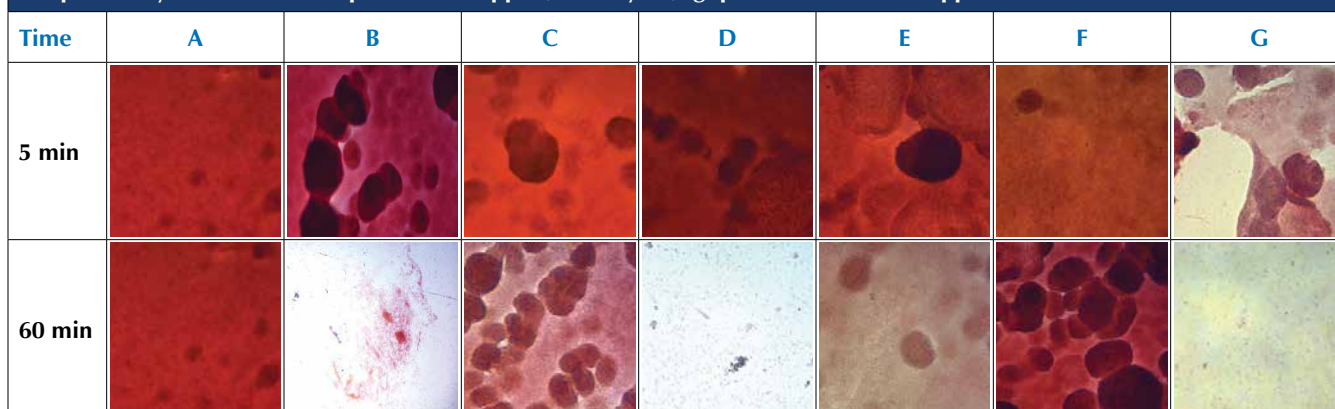
Chlorine dioxide shows a very strong effect on the biofilm killing ratio and on removal. Total kill has been observed for concentrations as low as 138 ppm. This could be explained by the fact that chlorine dioxide is a gas and could diffuse more readily in the matrix, accessing the bacteria more easily. Microscopy also shows a good removal of the biofilm at low temperature. The major problem with chlorine dioxide stems from its instability. It has to be produced at the same time and place where the treatment is done. This might be conceivable for large open spaces, but not for specific applications like hospital rooms.

Quat has shown a negligible effect on both killing and removal. Even if a reduction has been observed at 2000 ppm, efficacy is very low. This can be explained by the limited diffusion of Quat throughout the matrix, explaining the increase of log reduction with a prolonged time. This limited diffusion is due to the size and charge of the Quat. Quat is also unable to remove biofilm from the surface. A possible explanation for this is that positively charged quats create a layer on the negatively charged biofilm, leading to a repulsive interaction with other quat molecules.

**TABLE 1: Summary of Efficacy**

Product	Killing Efficacy	Biofilm Removal Efficacy
Hydrogen peroxide	Bad	Good
Bleach	Medium	Bad
Chlorine dioxide	Good	Good
Quat	Bad	Bad
Enzymes	Bad	Good
Peracetic acid	Good	Good

**FIGURE 3: Microscopy results for biofilm removal after treatment with (a) water (blank); (b) chlorine dioxide (250 ppm); (c) sodium hypochlorite (2000 ppm); (d) hydrogen peroxide (2000 ppm); (e) quaternary ammonium compound (2000 ppm), (f) enzyme, (g) peracetic acid (2000 ppm)**



The enzyme mix used showed no effect on antibacterial activity against biofilm. This result is not surprising since it is common knowledge that these kinds of enzymes are not harmful to bacteria [16]. However, microscopy reveals that the biofilm is completely removed from the surface of the coupon. We can therefore presume that the enzyme action will solely be on digesting the EPS matrix, liberating the bacteria in the liquid. Combining enzymes with a common sanitizer could do a good job of eradicating biofilm from a surface, as long as the disinfecting agent does not attack the enzymes.

Peracetic acid is the last product tested in this study. Results show that peracetic acid is very good at diffusing within the biofilm and killing bacteria in it. We observed a total kill of bacteria at 900 ppm of peracetic acid. Moreover, we noted that adding surfactant to the PAA allows us to reduce the concentration of PAA to 600 ppm. This is explained by the lowering of the solution's interfacial tension, which allows for better diffusion of the product in the biofilm. Microscopy also shows that the surfactant is effective at removing more of the biofilm structure.

## DISCUSSION

These results show that efficacy varies greatly depending on the technology used. However, there is a clear pattern. The more a molecule can diffuse into the biofilm, the higher the efficacy will be [17]. We have also noted that adding compounds, like a surfactant, to the solution will help disinfecting products reach the bottom of the biofilm more efficiently, allowing them to attack the microorganisms before they acquire resistance [13, 18]. These results are highly significant when having to choose products that can efficiently clean a highly contaminated area where biofilm will be present.

To help validate the theory that limited diffusion impairs the efficacy of products, we have tested two different contact times as shown in Figure 2. For chlorine dioxide, we see that diffusion is too rapid in the biofilm and that there's no change in results over time, since a total kill was achieved in 5 min. For PAA, however, we see an increase in efficacy over time,

with a total kill in 10 min. Quat and bleach at 2000 ppm also show a limited diffusion profile; efficacy increases between 5 and 60 min. Finally, enzymes and hydrogen peroxide show no change over time, revealing their low efficacy in killing bacteria within the biofilm.

## CONCLUSION

Table 1 shows a summary of all the results obtained for the efficacy of common disinfectants on biofilm. As we can see, peracetic acid and chlorine dioxide seem to be best at killing bacteria within a biofilm, followed by bleach. These three compounds are composed of small molecules that can easily diffuse in the polysaccharide matrix. They are also recognized as having great efficacy on planktonic bacteria. On the other hand, molecules like quat are bigger and positively charged. These two characteristics will lead to difficulty in diffusing through the matrix.

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# A double-blinded randomized controlled trial of incise-drapes in spine surgery: A feasibility study

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## ABSTRACT

**Background:** The use of an incise-drape, or plastic adhesive drape (PAD), to prevent surgical site infection is controversial with conflicting results in the existing literature. Testing the efficacy of PADs with traditional tissue cultures is expensive and invasive. With surgical site infection rates commonly below 5-10%, very large numbers would be required to assess this outcome. Through a double-blinded, randomized controlled trial (RCT), we investigated the feasibility of a novel, inexpensive, low-risk swabbing method to determine the effect of PADs on bacterial colony-forming-units (CFU) during elective spinal surgery.

**Methods:** Over 10 weeks, n=15 blinded elective spine patients were randomly assigned to iodine impregnated PAD versus no PAD. Bacterial CFUs per unit incision length were determined. A blinded team member collected surface specimens using flocked swabs on wounds at post-operation day (POD)-0 and POD-3 using a standardized technique. Specimens were plated for bacterial CFUs on blood and chocolate agar in triplicate serial dilutions. CFUs were manually counted. Secondary outcome measures included bacterial speciation and sample size calculations for future studies.

**Results:** There were no significant differences between groups in baseline characteristics. There was 100% recruitment rate, and complete adherence to the study protocol. With the numbers available, we were unable to detect differences in CFU counts between groups. There were no surgical site infections in either group at follow-up. Our new methodology using flocked swabs was feasible as a research tool and reliably yielded quantitative results for bacterial contamination of surgical incisions. PAD efficacy was not demonstrated in this pilot study.

**Conclusions:** Our findings via a double-blinded RCT demonstrated the feasibility of employing flocked swabs as a non-invasive tool for assessing surgical incision bacterial contamination. This tool can be used as a surrogate measure to assess the efficacy of interventions such as PADs for future research.

## KEY WORDS:

Adhesive drapes, surgery, incise drapes, surgical site infection, feasibility, swab

## INTRODUCTION

Surgical site infections (SSIs) are a major cause of morbidity and mortality amongst surgical patients. As a consequence, advances in medical and surgical knowledge and technology have led to necessary changes in peri-operative practices including prophylactic antibiotics, patient skin preparation, aseptic technique, surgical protective equipment and postoperative wound care.

Despite the intended dedication to practicing evidence-based medicine, some common practices continue without convincing evidence for reducing SSI risk. In particular, the use of a plastic adhesive drape (PAD) – with or without impregnated iodine products – has been a controversial topic for decades. Some studies have shown that the use of a PAD on the surgical site reduced the number of positive wound cultures

**Acknowledgements:** We would like to thank Marie Burelle for assistance with laboratory techniques, Venus Chirip for assistance with mass spectrometry, and Courtney Wilson for assistance with chart reviews.

**Conflict of interest:** Funding for this study was provided by the Division of Orthopaedic Surgery, Department of Surgery, University of Ottawa, The Ottawa Hospital. Surgical drapes were provided by Lyne Taylor on behalf of 3M Canada.

This study has been previously presented as a scientific poster at:

Canadian Spine Society Annual Meeting 2015, Halifax, Nova Scotia, Canada

Musculoskeletal Infection Society Annual Meeting 2015, Cleveland, Ohio, USA

postoperatively (1-3). This is concordant with other studies that have shown that PADs reduced migration of bacteria from the skin surface to the wound, are impermeable to bacteria, and are superior to cloth drapes (4,5). However, other studies have shown no improvement in SSI risk when comparing PADs to no drape at all (6-9). Additionally, a Cochrane systematic review including over 4,000 patients across seven published trials indicated that the use of a PAD without impregnated antibacterial agents (e.g., iodine) may increase SSIs, and that PADs with impregnated iodine showed no superiority compared to not using a PAD (10).

Given the conflicting findings, it is unclear why this discrepancy exists. To our knowledge, there have been no recent studies evaluating the effect of PADs on the bioburden of surgical wounds as reflected by colony-forming-unit (CFU) counts. Knowledge of this information may further elucidate the mechanism by which a reduction in positive wound cultures is observed.

Spine patients suffer SSIs more frequently than other orthopaedic patients; estimates suggest a rate of occurrence from 1.9% to 4.4% (11). Thus, we decided to study spine patients undergoing elective cervical, thoracic, and lumbosacral spinal surgery. The primary objective was to assess the feasibility and practicality of our novel and inexpensive measurement technique and to perform sample size calculations in preparation for a larger trial, with the ultimate goal of possibly replacing the invasive gold standard of tissue culture with our noninvasive technique. Secondary objectives included:

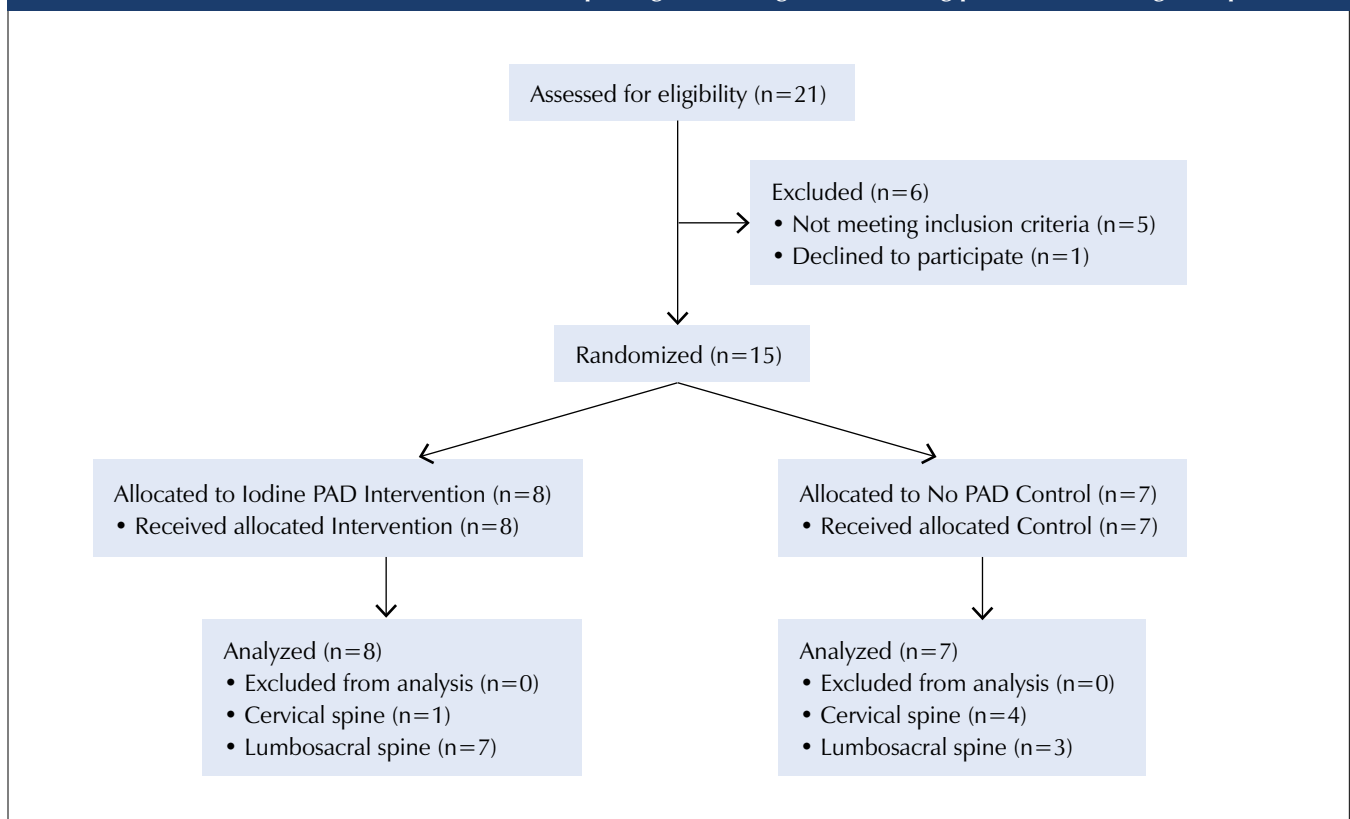
a) evaluating the effect of PADs on CFU counts of surgical wounds on post-operative days zero and three; b) determining if PADs affected the percentage of positive swabs obtained; and c) verifying which bacterial species could be isolated.

## METHODS

### Patient recruitment

A prospective, double-blinded, randomized controlled trial was performed over 10 weeks in 2013-2014 at a single centre tertiary academic hospital (January 28-March 10, 2014; June 3-June 30, 2014). During this time frame, all consecutive inpatient elective spinal surgery cases were screened for recruitment from within the practices of three fellowship-trained adult spinal surgeons. Recommendations set out in the CONSolidated Standards of Reporting Trials (CONSORT) Statement were followed in the study design (12). Elective adult spine surgery patients aged 18 years and over requiring post-operative inpatient admission of at least three days were eligible for inclusion. Patients were excluded if they met any of the following criteria: known iodine or adhesive allergy; active skin or soft tissue infections at the surgical site; undergoing surgery for fractures and tumours; refused participation. Ethics approval was granted by the Institutional Research Ethics Board. A random number generator was used to generate the random allocation sequence. Patients were blinded to their randomization. Figure 1 outlines the flow of patient recruitment in the study.

**FIGURE 1: CONSORT (Consolidated Standards of Reporting Trials) diagram illustrating patient flow through the protocol**



### Study protocol

Patients with hair at the surgical site were clipped with an electric clipper. All patients received a skin scrub with a 4% chlorhexidine gluconate brush followed by a wipe dry with a clean towel. Skin preparation was then performed with a sponge impregnated with chlorhexidine gluconate 2% and isopropyl alcohol 70% followed by a three-minute drying time. Standard operating room draping was performed with impermeable sterilized cloth drapes around the surgical site. Patients randomized to the PAD group had the 3M Ioban 2 PAD applied to the surgical site. The PAD covered all exposed skin within the surgical field. Perioperative antibiotics (i.e., weight-adjusted dosing of Cefazolin or Vancomycin) were administered prior to skin incision, at the four-hour intra-operative mark, and for 24 hours postoperatively.

At the end of surgery after skin closure, the PAD was removed and surgical site wiped dry with a sterile gauze sponge. At this point, a research team member blinded to the randomization was called into the operating theatre to collect the specimens from the closed incision. A flocced swab (Copan Diagnostics eSwab) was used to stroke a 5cm length of incision five times. This was repeated with two additional swabs on different 5cm segments of incision. All swabs were then immersed in the supplied 1mL of liquid Amies transport medium and transported to the laboratory within two hours of collection. Sterile dressing applied in the operating room consisted of a non-adherent layer against the skin followed by an absorbent layer, and then secured with adhesive tape occluding all sides of the wound. Specimen collection was repeated by the same blinded team member using the same protocol on POD-3, during the patients' first routine dressing change on the orthopaedic ward.

### Laboratory protocol and measurement technique

The blinded team member performed all of the laboratory processing of the specimens. Each swab was vortexed in its

transport medium to completely elute the bacteria. Then 10uL and 100uL aliquots were directly inoculated via micropipettor onto two different solid media in triplicate: tryptone soya agar with 5% sheep blood (Oxoid tryptone soya agar with 5% sheep blood) and chocolate blood agar (Oxoid chocolate agar enriched). All plates were streaked for enumeration with a 10uL wire loop using standard aseptic technique and incubated at 35°C in 5% CO<sub>2</sub> for 48 hours. Colonies were then counted manually. Phenotypically different colonies were isolated and streaked again for isolation onto tryptone soya agar with 5% sheep blood in preparation for speciation. Utilizing a MALDI TOF (matrix-assisted laser desorption ionization – time of flight) mass spectrometer (Bruker MALDI Biotyper), all subcultured colonies were prepared as per the manufacturer's instructions and analyzed by the device, generating the most probable species match.

Colony counts were converted into colonies per unit length of incision swabbed expressed as a number of colony-forming-units per centimetre (CFU/cm). The percentage of swabs showing any bacterial growth was also noted. CFU counts were selected as a surrogate outcome measure to directly evaluate the ability of the PAD to reduce surface bacterial contamination irrespective of the ultimate clinical outcome of SSI.

### Statistical analysis

Demographics and risk factor assessment was done by a combination of questionnaire and chart review for all participants based on previously published risk factors in the literature (11). Student's T-test of Fisher's Exact Test was used to compare baseline characteristics and SSI risk factors between groups. Fisher's Exact Test was used to compare the proportion of positive cultures between groups. As there was significant skewing of the CFU counts, a Mann-Whitney U test was used to test the null hypothesis that the two groups came from the same population.

**TABLE 1: Baseline characteristics and SSI risk factors**

Variable	No PAD (n=7)	With PAD (n=8)	P-value
Age (years ± SD)	62.1 ± 11.1	61.5 ± 8.9	0.90
Male Gender (%)	42.9	37.5	1.00
BMI (kg/m <sup>2</sup> ± SD)	30.7 ± 9.0	25.2 ± 5.0	0.23
Lumbar Surgery (%)	42.9	87.5	0.12
Posterior Approach (%)	71.2	87.5	0.56
Surgery Duration (minutes ± SD)	341.5 ± 148.9	279.1 ± 109.6	0.37
Incision Length (cm ± SD)	11.4 ± 5.6	11.5 ± 4.1	0.98
Diabetes (%)	28.6	25.0	1.00
Smoker (%)	0	12.5	1.00
Medical Comorbidities (Total n ± SD)	2.4 ± 1.4	2.4 ± 2.7	0.96

BMI: body mass index; PAD: plastic adhesive drape; SSI: surgical site infections

**TABLE 2: Medical comorbidities**

Medical condition	No PAD (n=7)	With PAD (n=8)	P-value
Type 1 diabetes	0	12.5	1.0
Type 2 diabetes	28.6	12.5	0.57
COPD	0	0	1.0
Hypertension	85.7	37.5	0.12
Previous MI	0	0	1.0
Angina	0	25	0.47
Hypercholesterolemia	42.9	37.5	1.0
Osteopenia	0	12.5	1.0
Osteoporosis	28.6	25	1.0
Rheumatoid arthritis	0	12.5	1.0
OSA	0	0	1.0
Insulin use	0	12.5	1.0
CHF	0	0	1.0

\* Values indicate percentage of patients (%).

COPD: chronic obstructive pulmonary disorder; MI: myocardial infarction;

OSA: obstructive sleep apnea; CHF: coronary heart failure

## RESULTS

A total of 15 patients were included (with PAD: n=8; no PAD: n=7). There were no significant differences between the two groups in terms of baseline demographics and SSI risk factors (Table 1). The control group (i.e., no PAD) trended towards having a higher BMI and greater numbers of cervical spine surgery ( $P>0.05$ ). One patient had previous radiation to the surgical site in the control group. No patients had previous SSIs. Medical comorbidities were highly diverse among this spine population and for the purposes of analysis, were simplified to a quantity of different diagnoses for each patient; a detailed breakdown is provided in Table 2.

On POD-0, five of the eight operative sites demonstrated positive cultures in at least one medium in the PAD group, compared to five of the seven operative sites in the no-PAD group ( $p=1.0$ ). On POD-3, seven of the eight operative sites demonstrated positive cultures in at least one medium in the PAD group, compared to five of the seven operative sites in the no-PAD group ( $p=0.57$ ). With the data available, we were unable to detect any significant between-group differences in terms of median colony counts per unit length of incision swabbed on POD-0 and POD-3 on either growth medium (Table 3). The percentage of swabs showing bacterial growth was also not significantly different when compared for each growth medium. Isolated bacterial species determined by mass spectrometry are shown in Table 4.

Post-hoc power analysis demonstrated that with the observed 71% baseline contamination rate (i.e. chocolate agar results without PAD) and the following assumptions:

$\alpha=0.05$ , power=0.80, n=28 subjects per group would be required to demonstrate a 50% reduction in contamination, and n=114 subjects per group would be required to demonstrate a 25% reduction in contamination rates (13).

## DISCUSSION

Our novel measurement technique employed flocked swabs as a key instrument. These are commercially designed to elute all bacteria from its swab tip into the transport medium once immersed and vortexed. Although designed for other laboratory purposes, we harnessed this property for quantitative analysis of bacteria collected from surgical incisions. Used in conjunction with a standardized swabbing protocol performed by the same blinded team member for every patient, we maximized the consistency of the samples and the reliability of the results. Importantly, this technique is much more cost-effective and minimally invasive than the current gold standard of tissue cultures for bacterial enumeration. There is virtually no foreseeable risk or morbidity to the patient from collecting a sample from a closed incision using a sterile swab, compared to surgically excising a tissue sample. Our novel measurement technique yielded reliable quantitative results, indicating that it is a technically feasible method as well. Because data has not been collected previously using this technique, we caution against interpreting the colony counts at face value as they may not reflect the true bioburden. However, with the consistency observed, it is reasonable to use the colony counts for relative comparison to one another.



TABLE 3: Wound bacterial load results				
Post-Operative Day-0		No PAD	With PAD	P value
Blood Agar	% Positive culture	42.9	50	1.0
	Median CFU/cm (range)	0 (0-7.69)	0.04 (0-4.18)	>0.2
	Mean CFU/cm $\pm$ SD	1.17 $\pm$ 2.88	0.68 $\pm$ 1.55	0.70
Chocolate Agar	% Positive culture	57.1	62.5	1.0
	Median CFU/cm (range)	0.06 (0-7.51)	0.09 (0-4.8)	>0.2
	Mean CFU/cm $\pm$ SD	1.18 $\pm$ 2.80	0.81 $\pm$ 1.77	0.77
Post-Operative Day-3		No PAD	With PAD	P
Blood Agar	% Positive culture	57.1	50	1.0
	Median CFU/cm (range)	0.08 (0-1.87)	0.22 (0-4.53)	>0.2
	Mean CFU/cm $\pm$ SD	0.37 $\pm$ 0.74	0.82 $\pm$ 1.55	0.48
Chocolate Agar	% Positive culture	71.4	75	1.0
	Median CFU/cm (range)	0.04 (0-2.22)	0.04 (0-4.56)	>0.2
	Mean CFU/cm $\pm$ SD	0.41 $\pm$ 0.89	0.80 $\pm$ 1.51	0.56

PAD: plastic adhesive drape; CFU: colony forming units

Two different growth media for bacterial culture were selected for use in our study: tryptone soya agar with 5% sheep blood (BA), and chocolate blood agar (CA). BA is widely used in the medical microbiological setting as a general-purpose differential medium suitable for growth of pathogenic aerobes and anaerobes (14). CA was selected to allow growth of less common fastidious organisms sometimes implicated in SSI, such as *Neisseria* and *Haemophilus* species (14). Mass spectrometry for bacterial speciation, the current technique used at our institution, only takes a few minutes for dozens of samples to be analyzed, and is very inexpensive per use.

Skin antiseptics agents such as chlorhexidine are designed to eliminate the organisms on the skin surface to create a sterile field. However, the duration of effect varies depending on the product, and over time the skin will recolonize with the bacteria within the deeper layers of skin and hair follicles originally missed by the antiseptics (5). For this reason, we elected to collect a post-operative day three specimen at the first routine dressing change. At this point, enough time has elapsed such that normal flora will be able to recolonize the skin, and the dressing will not have been opened prior to this point. This strategy also allowed us to remain consistent with our current post-operative protocol so as to not deviate from the standard of care.

Our results did not demonstrate statistical difference intra-operatively between PAD use and no PAD use, both in terms of colony counts and percentage of positive swabs. Thus, there is no evidence supporting the use of a PAD for the purpose of bacterial load reduction at the surgical site, and the theoretical benefit of reducing contamination at the skin under the PAD was also not observed. Note that the power analysis demonstrated an insufficient sample size to show a meaningful

TABLE 4: Bacterial species isolated using novel technique	
No PAD	With PAD
<i>Acinetobacter radioresistens</i>	<i>Bacillus thuringiensis</i>
<i>Bacillus thuringiensis</i>	<i>Moroxella osloensis</i>
<i>Kocuria kristinae</i>	<i>Pseudomonas luteola</i>
<i>Micrococcus luteus</i>	<i>Staphylococcus capitis</i>
<i>Rothia amarae</i>	<i>Staphylococcus epidermidis</i>
<i>Staphylococcus capitis</i>	<i>Staphylococcus hominis</i>
<i>Staphylococcus epidermidis</i>	<i>Streptococcus oralis</i>

difference in contamination rates, and thus these results are underpowered. However, our results are in alignment with the previous inconsistent findings in the literature, in that there are studies which do not show any change in positive wound culture incidence (15,16). A more recent study showed an increase in positive wound swabs with the use of PADs in hip fracture surgery (17) without a change in SSI rate, while others showed a beneficial effect on SSIs (4,5,18,19). A recent large review of anterior cervical discectomy and fusion patients observed no SSIs (20).

One must question if demonstrating a statistically significant difference in contamination rates or colony counts is sufficient to demonstrate any clinical relevance. For this reason, some authors advocate using SSI as an endpoint rather than colony counts (10,21). However, given baseline SSI rates of 2-4%, substantially more patients would be required to adequate power a study (13).

A recent Cochrane review (10) including over 3,000 patients with regular PADs and over 1,000 patients with iodine-impregnated PADs indicated an increase in SSIs with the use of regular PADs and equivalency of iodine-impregnated PADs to no PADs. However, the quality of the included studies was limited for several reasons: i) The studies spanned all surgical disciplines and were published over a long time period (1977-2002) during which there have been countless advances in surgical technique; ii) The studies were reported to be at high risk of bias from poor blinding and unclear randomization strategies, which may explain why both regular and iodine-impregnated PADs are still frequently used in surgery today. Therefore, a well-designed randomized controlled trial of adequate power may be necessary to prove or disprove the use of PADs. Employing CFU counts as an outcome measure can directly evaluate the ability of the PADs to reduce bacterial load at surgical sites, although future studies would benefit from measuring both clinical SSIs and CFU counts simultaneously as demonstrating reduction in bacterial load in isolation is unlikely to change practice.

There is a paucity of literature directly linking SSI rates to CFU counts. As it stands, the concept of increased bacterial quantity yielding higher risk of SSI is controversial but there exists evidence supporting it (22-24). Among microbiological literature, pathogens have an infective dose, defined as the number of pathogen cells required to infect a host (25). These doses are determined largely by epidemiological studies, outbreak data, and studies on healthy human volunteers. The infective dose varies depending on organism, host factors and route of infection (25). It does suggest though that infection is a dose-dependent phenomenon. Given the low basal rate of SSI and the large number of study participants required to demonstrate even a small change, using CFU counts as a surrogate measure of SSI risk remains common practice. Reduction of contamination at the wound site immediately after surgery may be a useful surrogate in addition to a worthy goal with regards to reducing wound infection rates. Studies evaluating this outcome would likely be easier to conduct and moreover important to pilot before considering studies evaluating actual infection rates given the large number of patients per group that would be required to show a 50% reduction.

The use of a PAD in surgery is fraught with practical issues. They can restrict motion of the surgical limb, adhere to unwanted objects, and potentially create plastic debris that can unknowingly remain within surgical incisions (and are invisible to radiographs). They often peel back at the incision edges as the surgical case progresses due to prolonged retraction of the skin. It is rare for a PAD to remain completely adhered to the skin and incision edges for the entire duration of the surgical case. Unfortunately, lifting off of the incise drape has been reported to increase the infection rate by six-fold (26). Another study suggested that using Duraprep can decrease the probability of the incise drape lifting (27). Current infection control guidelines from the American Centres for Disease Control do not make specific recommendations regarding the use of PADs (28).

Notably absent in our findings is the lack of detection of *Staphylococcus aureus*, one of the most commonly implicated

bacterium in SSIs. Given the small sample size, this is not surprising as the rate of colonization in the general population is between 25%-40% in the literature (29). Coagulase negative *Staphylococcus* species such as *S. epidermidis* was found, and these organisms are also common culprits in SSI while also being highly prevalent in normal human skin flora.

One limitation of this study is that the use of this novel measurement technique to detect CFUs may not be representative of the true bacterial load compared to the current standard. Although we demonstrated that our novel technique is feasibly performed and can produce reliable results, we do not have comparison data to the gold standard of tissue cultures, and thus cannot draw conclusions regarding its accuracy in detecting bacterial contamination. However, this can be addressed in a future larger, adequately powered study that also includes a simultaneous comparison of flocced swabbing to tissue culture results. Another limitation is the use of bacterial load as a surrogate measure for clinical infection. The evidence linking bacterial contamination to confirmed infection is controversial, and our methodology may not directly translate to clinical utility. In addition, the small sample size and inadequate power means we cannot draw conclusions regarding the efficacy of PADs.

## CONCLUSIONS

Our study demonstrates feasibility of study design. We successfully carried out a randomized double-blinded surgical trial with a novel low-cost and low-risk methodology to quantitatively analyze bacterial burden at surgical sites. We cannot recommend for or against the use of a PAD for the purposes of SSI reduction in elective spine surgery cases. However, we were able to determine the necessary sample size for future studies. Further research is required to increase our understanding of PADs and a detailed cost-analysis is necessary to determine overall cost-efficacy. Future investigations of the utility of PADs would benefit from measuring the outcomes of clinical infection as well as bacterial load via the gold standard of tissue culture.

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# Antimicrobial stewardship with once-weekly follow-up reduced carbapenem prescriptions in an acute care hospital

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**Background:** Antimicrobial stewardship programs (ASP) should be performed daily. However, it is often difficult for smaller hospitals. Thus, we started an ASP for carbapenem therapy with once-weekly follow-up by ward pharmacists in 2015.

**Methods:** To assess the outcomes of the ASP with once-weekly follow-up by a ward pharmacist, we assessed three groups of patients in 2014, 2015, and 2016. Additionally, we measured the following outcomes: number of de-escalations, number of intravenous to oral conversions, duration of therapy, susceptibility of *P. aeruginosa*, carbapenem consumption, and death within 30 and 60 days.

**Results:** Defined daily doses (DDD) (3 and 2 DDD per 100 patient-days (PD) in 2014 and 2016,  $P < 0.01$ ) and days of therapy (DOT) (6 and 4 DOT per 100 PD in 2014 and 2016,  $P < 0.01$ ) in carbapenem decreased with interventions. The death rates within 30 and 60 days were not significantly different between the three groups. Multivariate regression analysis showed that de-escalations were associated with interventions by both AST and ward pharmacists (OR, 2.63; 95% CI, 1.34–4.93). AST interventions had a negative association with the duration of carbapenem therapy (adjusted  $R^2$  of 0.006).

**Conclusions:** ASP with once-weekly follow-up by a ward pharmacist is a simple and beneficial method that can be adopted by smaller hospitals with limited human resources.

## KEY WORDS:

Antimicrobial stewardship programs, once-weekly, ward pharmacist Abstract

## INTRODUCTION

A systematic review and meta-analysis reported that antimicrobial stewardship programs (ASP) can improve the quality of antimicrobial use, reduce the use of antibiotics, and shorten the length of hospital stay without increasing mortality rates (1,2). Additionally, ASP interventions safely reduce the unnecessary use of antibiotics in hospitals, although the most effective behavior change techniques are not generally used (1).

Prospective audits and feedback should be performed daily in ASP (3). However, it is often difficult for smaller hospitals to achieve this, mainly due to limited human resources (4). Therefore, an alternative, simpler method would be beneficial for many local clinical settings. Vettese et al. (5) reported that a thrice-weekly, pharmacist-driven ASP can reduce antimicrobial expenditure, shorten the duration of therapy, and decrease the use of carbapenems, vancomycin, and levofloxacin. We started

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a once-weekly prospective audit and feedback program in October 2010. However, de-escalation was not recommended in some cases, because bacterial susceptibility had not yet been identified. In these cases, we suggested de-escalation the following week, although we thought that a once-weekly ASP was insufficient. However, it is difficult for busy physicians to maintain an ASP twice weekly or more frequently. Hence, we started an ASP with once-weekly follow-up by ward pharmacists in 2015. In this program, when de-escalation was not proposed by the ASP, the ward pharmacist could follow-up and subsequently suggest de-escalation to the patient's physician. Thus, this study aimed to assess the outcomes of the ASP with once-weekly follow-up by a ward pharmacist.

## METHODS

A three-year retrospective study was conducted involving all patients admitted to Kaetsu Hospital (Niigata, Japan), a 261-bed hospital with six wards, who were administered intravenous carbapenems (imipenem, meropenem, doripenem, and biapenem) between 1 January 2014 and 31 December 2016. The study protocol was approved by the Ethics Committee of Kaetsu Hospital.

The antimicrobial stewardship team (AST) consisted of four healthcare providers: a respiratory physician, board-certified infection control pharmacist, microbiology laboratory technician, and board-certified infection control nurse. A once-weekly ASP was commenced from October 2010. The AST pharmacist reviewed the medical charts of patients to whom carbapenems were administered every Monday excluding holidays, and identified cases that might need changes to the antimicrobial regimen and those with complicated infections. The AST gave feedback (e.g., de-escalation, withdrawal of antimicrobials, performance of bacterial screening, and other suggestions) based on the medical charts. In addition, the AST was notified of carbapenem use from November 2014. Specifically, physicians voluntarily informed the AST of the diagnosis and reason for carbapenem use. An ASP with once-weekly follow-up by ward pharmacists commenced from January 2015. Specifically, when the AST could not suggest de-escalation in a case because the susceptibility of the bacteria had not been identified, the AST pharmacist provided information about the patient to the ward pharmacist. Subsequently, the ward pharmacist monitored the susceptibility of the bacteria by daily review of medical charts. When bacterial susceptibility was identified, the ward pharmacist would suggest the possibility of de-escalation to the patient's physician.

To determine the efficacy of an ASP with once-weekly follow-up by the ward pharmacists, we assessed three groups in 2014, 2015, and 2016. Outcome measures were the number of de-escalations, number of intravenous to oral conversions, duration of carbapenem therapy, antimicrobial susceptibility of *P. aeruginosa*, carbapenem consumption, and death within 30 and 60 days. Intervention was defined as feedback to the patient's physician by the AST or ward pharmacist. Interventions were recorded via electronic medical charts. Interventions by the ward pharmacists were performed by talking directly or sending

the electronic medical chart to the physician. De-escalation was defined as a change in prescription from a carbapenem to another antimicrobial, including antipseudomonal penicillins and cephalosporins (6). To determine the susceptibility of *P. aeruginosa* to carbapenems, we collected data about the number of isolates and the number of susceptible isolates of *P. aeruginosa* to imipenem and meropenem. Data on bacteria isolated within <48 h of hospitalization, bacteria obtained from stool, and duplicate isolates from the same patient were excluded. Antimicrobial susceptibility testing was performed using broth microdilution according to the guidelines for MIC testing from the National Committee for Clinical Laboratory Standards. MICs of <2 µg/mL indicated *P. aeruginosa* susceptibility to imipenem and meropenem. Information about the total intravenous antimicrobial and intravenous carbapenem doses was collected, and the defined daily dose (DDD) and days of therapy (DOT) were assessed using the Japan Antimicrobial Consumption Surveillance system (7). The DDD was based on the WHO recommendation for each year. DOT was defined as the administration of a single agent on a given day regardless of the number of doses administered. DDD and DOT was normalized to 100 patient-days (PD). All data were recorded in electronic medical charts.

JMP v.9 software (SAS Institute Inc., Cary, NC) was used for all statistical analysis. Continuous variables were reported as means and standard deviation, and categorical variables as frequency and percentage. Univariate analysis was performed using one-way ANOVA followed by Tukey-Kramer post-hoc test or  $\chi^2$ -test. Multivariable analysis was performed using logistic regression analysis with a stepwise backward-forward selection ( $P < 0.25$ ) procedure to identify the independent factors associated with de-escalation and intravenous to oral conversion. Additionally, multivariable analysis was performed using multiple regression analysis with a stepwise backward-forward selection ( $P < 0.25$ ) procedure to identify the independent factors associated with the duration of carbapenem therapy. Interventions by the AST or ward pharmacists and notifications to the AST were subjected to multivariate analysis. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated in logistic regression analysis. Moreover, the partial regression coefficient (standard error) and adjusted  $R^2$  values were calculated in multiple regression analysis.  $P < 0.05$  was considered statistically significant.

## RESULTS

The three groups in this study comprised 417, 415, and 361 patients administered carbapenems in 2014, 2015, and 2016, respectively. Patient backgrounds, number of interventions, and outcomes are shown in Table 1; the proportion of males was significantly different between the groups. Unknown infections were also included during diagnosis. The duration of carbapenem therapy in both 2015 and 2016 decreased by one day compared with 2014 (significant difference between 2014 and 2015,  $P < 0.01$ ). Moreover, the number of deaths within 30 and 60 days were not significantly different between the three groups. In contrast, although notifications to the AST, total

**TABLE 1: Patient backgrounds, number of interventions, and outcomes**

	2014 n = 417	2015 n = 415	2016 n = 361	P*
<b>Sex male, n (%)</b>	245 (59)	277 (67)	211 (58)	0.02
<b>Age, years (SD)</b>	79 (13)	80 (13)	79 (13)	0.78
<b>Body weight, kg (SD)</b>	46 (13)	48 (12)	47 (14)	0.11
<b>Diagnosis</b>				
Respiratory, n (%)	125 (30)	114 (27)	117 (32)	0.32
Urinary, n (%)	61 (15)	43 (10)	42 (12)	0.16
Digestive, n (%)	58 (14)	56 (13)	48 (13)	0.97
Other, n (%)	172 (41)	202 (49)	153 (42)	0.07
<b>Notifications to AST, n (%)</b>	82 (20)	324 (78)	310 (86)	< 0.01
<b>Total interventions, n (%)</b>	16 (4)	74 (18)	63 (17)	< 0.01
Interventions by AST, n (%)	16 (4)	38 (9)	56 (16)	< 0.01
Interventions by ward pharmacists, n (%)	0 (0)	42 (10)	10 (3)	< 0.01
<b>Number of de-escalations, n (%)</b>	48 (12)	68 (16)	48 (13)	0.11
<b>Number of intravenous to oral conversions, n (%)</b>	12 (3)	14 (3)	18 (5)	0.27
<b>Duration of carbapenem therapy, days (SD)</b>	10 (6)	9 (6) <sup>†</sup>	9 (6)	<0.01
<b>Death within 30 days, n (%)</b>	79 (19)	80 (19)	84 (23)	0.26
<b>Death within 60 days, n (%)</b>	108 (26)	105 (25)	99 (26)	0.79

Continuous variables are reported as mean and standard deviation, and categorical variables as frequency and percentage.

Abbreviations: AST, antimicrobial stewardship team; SD, standard deviation.

\* One-way ANOVA followed by Tukey–Kramer post-hoc test or  $\chi^2$ -test.

† Significant compared with 2014.

**TABLE 2: Susceptibility of *P. aeruginosa* to imipenem and meropenem**

	2014 n = 57	2015 n = 41	2016 n = 41	P*
<b>Imipenem susceptibility of <i>P. aeruginosa</i> isolates, n (%)</b>	39 (68)	28 (68)	34 (83)	0.21
<b>Meropenem susceptibility of <i>P. aeruginosa</i> isolates, n (%)</b>	41 (72)	30 (73)	34 (83)	0.42

\*  $\chi^2$ -test.

interventions, interventions by the AST, and those by the ward pharmacists increased in 2015 and 2016 compared with 2014 (significant difference between the three groups by  $\chi^2$ -test,  $P < 0.01$ ), the number of de-escalations and intravenous to oral conversions were no different between the groups. Furthermore, interventions by both the AST and ward pharmacists occurred in a few cases. The number of interventions by ward pharmacists was lower in 2016 compared with 2015 because the number of AST interventions was higher.

The susceptibility of *P. aeruginosa* to both imipenem and meropenem is shown in Table 2, and showed an increase from 70% in 2014 to 80% in 2016; however, there was no significant difference between the three groups.

Antimicrobial consumption is shown in Table 3. The DDD of all antimicrobials in 2015 and 2016 was significantly increased compared with 2014 ( $P < 0.01$ ); however, the DDD of carbapenems was significantly lower in 2015 (2 DDD per 100 PD,  $P = 0.02$ ) and 2016 (2 DDD per 100 PD,  $P < 0.01$ ) compared with 2014 (3 DDD per 100 PD). Additionally, although the DOT of all antimicrobials was not significantly different between the three groups, fewer carbapenem DOT were recorded in 2015 and 2016 (4 DOT per 100 PD,  $P < 0.01$ ) compared with 2014 (6 DOT per 100 PD). In this study, 80% meropenem was the carbapenem that was mainly used for DDD and DOT.

The results of multivariate regression analysis of the factors associated with de-escalations, intravenous to oral conversions,

**TABLE 3: Antimicrobial consumption**

	2014	2015	2016	P*
DDD of all antimicrobials per month, DDD per 100 PD	15 (3)	23 (4) <sup>†</sup>	20 (2) <sup>†</sup>	<0.01
DDD of carbapenem per month, DDD per 100 PD	3 (0.6)	2 (0.4) <sup>†</sup>	2 (0.5) <sup>†</sup>	<0.01
DOT of all antimicrobial per month, DOT per 100 PD	23 (2)	22 (2)	22 (2)	0.45
DOT of carbapenems per month, DOT per 100 PD	6 (1)	4 (0.8) <sup>†</sup>	4 (0.9) <sup>†</sup>	<0.01

Values are reported as means with standard deviation in parentheses.

\* One-way ANOVA followed by Tukey–Kramer post-hoc test or  $\chi^2$ -test.

<sup>†</sup> Significant compared with 2014.

DDD=defined daily dose; DOT=days of therapy; PD=patient-days

**TABLE 4: Multivariate logistic regression analyses of factors associated with de-escalations**

	Odds ratio	OR (95% CI)	P
Interventions by AST or ward pharmacists -	1.00		
Interventions by AST or ward pharmacists +	2.63	1.34-4.93	< 0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

AST=antimicrobial stewardship team

**TABLE 5: Multivariate logistic regression analyses of factors associated with intravenous to oral conversions**

	Odds ratio	OR (95% CI)	P
Intervention by ward pharmacists -	1.00		
Intervention by ward pharmacists +	2.29	0.67-5.99	0.13

Abbreviations: OR, odds ratio; CI, confidence interval.

and duration of carbapenem therapy are shown in Tables 4–6. Interventions by both the AST and ward pharmacists showed a positive association with de-escalations (OR, 2.63; 95% CI, 1.34–4.93), and interventions by ward pharmacists tended toward a positive association with intravenous to oral conversions (OR, 2.29; 95% CI, 0.67–5.99). Interventions by the AST had a negative association with duration of carbapenem treatment (adjusted  $R^2$  values of 0.006).

## DISCUSSION

The total number of interventions significantly increased after the ASP with once-weekly follow-up by ward pharmacists commenced. In multivariate analysis, interventions by the AST and ward pharmacists were associated with de-escalations and decreased duration of carbapenem therapy. However, interventions by the AST and ward pharmacists affected only 20% of all carbapenem regimens. Furthermore, the number of de-escalations and intravenous to oral conversions did not increase after interventions by ward pharmacists started. Nevertheless, the number of patients, consumption of

carbapenem, and duration of carbapenem therapy decreased after increasing the total number of interventions. These results suggest that our interventions strongly affected carbapenem therapy because they promoted re-consideration of the regimen by the physicians. Additionally, these effects are supported by a systematic review (1). Moreover, the duration of carbapenem therapy decreased by one day from 10 days in 2014 to 9 days in 2015 and 2016. In a systematic review, the duration of antibiotic treatment decreased by 1.95 days from 11.0 days by ASP, which is similar to our result (1). In addition, mortality rates within 30 and 60 days were not changed by the interventions. Therefore, ASP with once-weekly follow-up by a ward pharmacist might improve the performance of the ASP and optimize carbapenem therapy without changing patient outcomes.

The susceptibility of *P. aeruginosa* to imipenem and meropenem increased from 70% to 80%, additionally, the DDD and DOT of carbapenems decreased by one-third after the total interventions increased. Goldstein et al. reported that the susceptibility of *P. aeruginosa* to imipenem increased from 61% to 81% after decreasing the amount of imipenem usage, which resembles our

**TABLE 6: Multivariate regression analysis of factors associated with the duration of carbapenems**

	Duration of carbapenems	P
Interventions by AST	-0.07 (0.28)	< 0.01
Notification to AST	0.05 (0.17)	0.10
Adjusted R <sup>2</sup>	0.006	

Values are shown as the partial regression coefficient (standard error).

results (1,8). Therefore, ASP with once-weekly follow-up by a ward pharmacist might improve the susceptibility of *P. aeruginosa* to carbapenems by reducing carbapenem consumption.

Voluntary notifications to the AST by physicians regarding the diagnosis and reason for prescribing carbapenems commenced in November 2014. The AST was notified of approximately 80% of carbapenem therapy cases after 2015. However, multivariable analysis revealed that these notifications tended toward a positive association only with the duration of carbapenem therapy. However, the AST gained an understanding of the focus of the infection and the physician's diagnosis via these notifications. Therefore, we considered that notifications from physicians to the AST might offer several advantages other than the direct effects of antimicrobial use.

The DDD and DOT of all antimicrobials were 15 to 20 per 100 PD in this study, which is similar to a previous Japanese study (7). However, in the United States and France, the average DDD and DOT among all antimicrobials were reported to be 60 to 70 per 100 PD (9,10), which is contrary to our results. On the other hand, the average DDD among all antimicrobials were reported to be 16 per 100 PD in French local hospitals (10) and resembled those of our study. Thus, the consumption of antimicrobials in our hospital was lower than that in large hospitals and resembled the rates in local general hospitals. Alternatively, The DDD of all antimicrobials in 2015 and 2016 were significantly increased compared with 2014. This reason for this is that, although the consumption of carbapenems decreased, that of ampicillin/sulbactam and ceftriaxone increased. Additionally, the DDD of ampicillin/sulbactam and ceftriaxone was close to that recommended by the WHO, contrary to the DDD of other antimicrobials in Japan.

A once-weekly ASP targeting only carbapenems is not a standard procedure. Ideally, the ASP should target all antimicrobials and be performed daily. However, it is often difficult for smaller hospitals to manage a daily ASP because of limited human resources (4). Thus, a once-weekly ASP and follow-up by a ward pharmacist would be easier for smaller hospitals to introduce and manage, but the ultimate goal should be daily ASP targeting all antimicrobials.

Our study has some limitations because of its retrospective design, lack of a control group, and small sample size. Moreover, a once-weekly ASP targeting only carbapenems is not a standard procedure.

ASP with once-weekly follow-up by ward pharmacists improved carbapenem therapy and susceptibility of *P. aeruginosa* to carbapenem in our 261-bed Japanese hospital. These interventions are simple and beneficial methods that could be introduced to smaller hospitals with limited human resources.

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# Microbial contamination on cell phones used by undergraduate students

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## ABSTRACT

**Background:** Undergraduate students handle their cell phones in several places, getting them exposed and contaminated with a variety of microorganisms, which may include pathogenic and non-pathogenic microorganisms.

**Objective:** We investigated the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. and counts of microbial groups on the surface of undergraduate students' cell phones.

**Methods:** A total of 304 cell phones used by undergraduate students were sponge sampled to detect the presence of pathogenic bacteria and to enumerate yeasts and molds, aerobic plate count, *Enterobacteriaceae*, coliforms and *Escherichia coli*. A questionnaire was applied to users of the cell phones sampled to obtain information on phone usage habits.

**Results:** All undergraduate students use their cell phones at home, school, public and private sites. All sponge samples tested negative for the presence of the investigated pathogens. The intervals of counts (Log CFU/cell phone) were 1.7-6.7 for aerobic plate count, 1.7-5.4 coliforms, 1.7-5.2 yeasts and molds, 1.7-4.6 *Enterobacteriaceae*, and 1.7-3.3 *E. coli*. **Conclusions:** Cell phones used by undergraduate students are a source of microbial groups in variable levels. Despite the fact that bacterial pathogens were not isolated from tested samples, usage habits and presence of *E. coli* suggest that cell phones could be a potential source of enteric pathogenic bacteria.

## KEY WORDS:

phones; microorganisms; pathogen; students; cross-contamination

## INTRODUCTION

The number of cell phones used worldwide grew from fewer than 1 to around 6 billion between 2000 and 2012 (1). In Mexico, 77.7 million people used cell phones in 2015; 66% users have a smartphone, while the rest own a device enabled to make/receive calls or messages without internet access (2). The use of this mobile communication technology in healthcare and higher education (3) has increased and generated interest in evaluating their role as reservoir of pathogenic and opportunist bacteria, and as source of contamination to our foods or to ourselves (4,5). Several investigations in hospitals have demonstrated the presence of *Staphylococcus aureus*, *Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* and coliforms on mobile phones used by medical staff (4,6,7), students (7) and patients (8). In contrast, studies exploring the quantitative

levels of microbial groups such as yeasts and molds, aerobic plate count, *Enterobacteriaceae* (9), coliforms and *E. coli* on cell phones are scarce. The enumeration of microbial groups could be useful in estimating the cell phones potential as reservoir of microorganisms including enteric bacteria, particularly when populations of pathogenic and opportunist microorganisms are below detectable levels. Populations of microbial groups may differ on cell phones according to their usage under different conditions and environments.

Cell phones are common among undergraduate students, which can be used to communicate for social or academic purposes, according to the technological features of device and Internet connection. Students related to health sciences majors use their cell phones while performing internships at hospitals or clinical laboratories, either to access information on their field of expertise, answer calls, text messages, or take pictures

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during their practices (10,11). On the other hand, students in social sciences majors use their cell phones when practicing or working at offices where a large number of people attend. The frequent use of cell phones in a diversity of sites raises the opportunity for cross-contamination, especially if no hygienic measures and safety practices are common among students (12). If pathogens are present on the surface of a cell phone, they could be transferred to the user skin, other surfaces, or foods, where survival and growth is possible. Two disease outbreaks were associated with exposure of students and employees after manipulating *Salmonella* Typhimurium in clinical and teaching microbiology laboratories in the United States (13). In this report, laboratory directors, managers, and faculty involved with clinical and teaching microbiology laboratories were advised to comply with biosafety guidelines that prohibits food, drinks or personal items like car keys, cell phones and music players use while working in the laboratory or placed on laboratory work surfaces as they may act as fomites.

The purpose of this study was to investigate the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp., and to enumerate yeasts and molds, aerobic plate count, *Enterobacteriaceae*, coliforms and *Escherichia coli* on the surface of cell phones used by undergraduate students in three University Campuses. In addition, a survey was conducted among the cell phone users to collect data on factors that might contribute to the microbial levels found.

## MATERIAL AND METHODS

### Study context

A total of 304 cell phones belonging to undergraduate students attending three campuses of the University of Guadalajara (Jalisco State, Mexico) were sampled. Students in each campus are enrolled in different majors, and were divided into two groups depending on whether or not they were registered in courses that include visits to hospitals and/or clinical and microbiology laboratories. The first group named "health sciences" included students from medicine, nursing, pharmacy and biology or biology-related majors who attend different courses that include visits to hospitals and clinical or microbiology laboratories. The second group was called "non-health-related sciences", and included students from engineering and social sciences, who do not attend classes in hospitals or laboratories. All students were selected at different locations of each campus including main entrances, classrooms, laboratories, libraries, and restroom entrances. Each student was asked for his/her consent to respond a questionnaire about his/her cell phone characteristics and usage habits, and to allow the sampling of their device's surface. Personnel in charge of sampling visually verified that participants did not clean their phone before sampling. The questionnaire was filled out by each participant and inquired about age, gender, educational background, technical characteristics of the cell phone, usage habits, and cleaning and disinfection practices on the device. The protocol was previously approved by the Bioethics Committee of each campus.

**TABLE 1: Characteristics and use of cell phones by undergraduate students at a university in Jalisco State, Mexico (n= 304)**

Characteristic	No. students (%)
<b>Type of cell phone</b>	
Touch-screen phone	197 (65)
Keyboard phone	107 (35)
<b>Use of cover protector</b>	148 (49)
<b>Location of usage</b>	
Home	304 (100)
Public and private transportation	304 (100)
School	304 (100)
Other places (park, restaurants and supermarket)	133 (44)
<b>Cell phone use</b>	
Calls and texting	304 (100)
Surf the Internet	208 (68)
Play audios and/or videos	206 (68)
Take pictures and/or videos	178 (59)
View or download electronic documents	109 (36)
Other (access calendar, clock, Global Position System, play games)	304 (100)
<b>Cleaning or disinfection of cell phone</b>	183 (60)

The whole surface of cell phone (including the front, back and lateral sides) was swabbed using a sterile sponge (3M™, St. Paul, MN, USA) aseptically hydrated with 50 ml of lactose broth (Becton, Dickinson and Company, Sparks, MD, USA). The sponge was then returned to the sterile bag and placed in an insulated cooler with refrigerant packs. Samples were transported to the laboratory and analyzed within 2 h.

### Microbiological analysis

Cell phone sponge samples were homogenized using a peristaltic blender for 1 min; decimal dilutions in 0.1% peptone diluent (Becton, Dickinson de México, Estado de México, México) were prepared for enumeration of aerobic plate count (APC), yeasts and molds (Y/M), *Enterobacteriaceae*, coliforms and *Escherichia coli* on Petrifilm plates (3M™, St. Paul, MN, USA). *Enterobacteriaceae*, coliforms and *E. coli* plates were incubated at 35°C for 24 h, APC at 35°C for 72 h, and Y/M at 25°C for 120 h, before counting.

An aliquot of each sponge rinse liquid was streaked on trypticase soy agar supplemented with 5% sheep blood (Becton, Dickinson and Company) for isolation of *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. at 35°C for 24 h. Single typical colonies were selected and tested for Gram stain, catalase, mannitol fermentation, coagulase and esculin hydrolysis.

The remaining volume of the sponge rinse liquid was incubated at 35°C for 24 h for *Salmonella* spp. isolation (14). Aliquots of 0.5 and 0.1 ml were transferred to 10 ml of tetrathionate broth (TT, Becton Dickinson and Company) and 10 ml of Rappaport-Vassiliadis R10 broth (RV, Becton Dickinson and Company), respectively. The broths were incubated at 35°C and 42 ± 0.5°C for 18-24 h, respectively, in a water bath (Thermo Fisher Scientific Precision 2868, Marietta, OH). Aliquots of 10 µl from TT and RV were individually streaked onto xylose lysine deoxycholate agar (XLD), *Salmonella-Shigella* agar (SS), enteric Hektoen agar (HE) and bismuth sulfite agar (SB). XLD, SS and HE plates were incubated at 35 ± 2°C for 18-24 h, and SB plates for 48 h. Two typical colonies from each plate were biochemically confirmed on triple sugar iron agar and lysine iron agar at 35 ± 2°C for 24 ± 2 h, and into urea broth at 35 ± 2°C during 48 h.

### Data Analysis

Data obtained from the questionnaires were used to perform descriptive statistics. Counts obtained for each microbial group were reported in Log CFU/cell phone prior to data analysis. The significance of differences among the counts of five microbial groups was assessed using an analysis of variance (Statgraphics Centurion XV ver.15.2.06; Statpoint Technologies, Inc., Warrenton, USA). When significant differences ( $P < 0.05$ ) were observed, separation of means was carried out using LSD (least difference statistical) multiple range test.

## RESULTS

A total of 304 students participated in the study, 137 (45%) females and 167 (55%) males, ranging from 17 to 35 years old. One hundred and fifty-one students (49.7%) corresponded to the “health science” group and 153 students (50.3%) to

the “non-health-related sciences” group. Sixty-five percent of students interviewed owned a cell phone with a touch screen and 35% had keyboard phones; 49% of cell phones had a protecting cover (Table 1).

All students (100%) reported using their cell phones at home, in places including their bedroom, bathroom and kitchen; also during their commute when using either public or private transportation, and at school. Forty-four percent said they use their cell phone at public sites such as parks, restaurants and supermarkets (Table 1). All students (100%) responded that they use their cell phone for making calls and send text messages, 68% use it to surf the Internet and to play audio and video, 59% to take pictures and/or videos, 36% to view or download electronic documents, and 100% to use software applications (Apps) like calendar, clock, Global Position System and/or games.

Approximately 72% ( $n = 109/151$ ) of students in the “health sciences” group said they use their phones in hospitals and/or laboratories. A 54% of these students said used to make phone calls and send text messages while providing health care for patients under professors’ supervision in hospitals. In addition, 52% of students indicated that they have taken pictures in teaching laboratories during handling of *Salmonella* spp., *Shigella* spp., *Escherichia coli*, Gram positive cocci, *Listeria monocytogenes*, or gastrointestinal helminths and protozoans, despite the biosafety rules in place and the warning from professors and technicians about this hazardous practice. A 6% students said used it in hospitals and laboratories.

When students were asked if they perform cleaning or disinfection procedures to their cell phones, only 183 of 304 (60%) students answered that they clean or disinfect their device (Table 1). A variety of open responses on this topic were collected among students, who seem to be more familiar with the concept of cleaning than that of disinfection. Only 78% ( $n = 142/183$ ) of respondents said they clean their device and from those, 97% ( $n = 138/142$ ) described the cleaning procedure as rubbing the surface with damp clothes, personal clothes, hands, baby towels, toilet paper or cotton pads. An example of the lack of knowledge on proper cleaning practices is that 3% ( $n = 4/142$ ) of students said they clean their phones by breathing on the surface of the device and rubbing it on their clothes. Knowledge about disinfection procedures was also poor. Although 41 of 183 (22%) students said they disinfect their cell phones, only 27 of them (66%) use antibacterial substances (70% ethanol, isopropyl alcohol or sodium hypochlorite); 14 students (34%) said they use detergent or a cosmetic cream to disinfect the surface of their device. This illustrates the lack of information on cleaning and disinfection concepts among respondents.

All sampled cell phones tested negative for the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. The frequency of isolation of microbial groups was 99.8% for APC, 53% Y/M, 31% coliforms, 29% *Enterobacteriaceae*, and 5% *E. coli* (Table 2). Mean APC counts were significantly higher ( $P < 0.05$ ) that those of Y/M, coliforms, *Enterobacteriaceae* and *E. coli*. The APC counts ranging from 1.7 to 6.7 Log CFU/phone, from those, 87% of cell phones ranged from ≥3.0 to 5.0 Log CFU/phone, whereas 5% of

**TABLE 2: Counts of microbial groups on the surface of cell phones used by undergraduate students at three university campuses**

Microbial group	No. samples with enumerable levels (%)	Mean Log CFU/cell phone <sup>a</sup> ±SD	Minimum-maximum count (Log CFU/cell phone)
Aerobic plate count	303 (99.8)	3.8 ± 0.64 A <sup>b</sup>	1.7 - 6.7
Yeasts and molds	161 (53)	2.2 ± 0.62 C	1.7 - 5.2
Coliforms	94 (31)	2.6 ± 0.80 B	1.7 - 5.4
<i>Enterobacteriaceae</i>	87 (29)	2.5 ± 0.70 B	1.7 - 4.6
<i>Escherichia coli</i>	16 (5)	2.2 ± 0.43 C	1.7 - 3.3

<sup>a</sup> Minimum detection limit was 1.7 Log CFU/phone

<sup>b</sup> Means with the same letter within columns (A, B, C), are not significantly different (P > 0.05)

the sampled devices contained  $\geq 5.0$  to 6.7 Log CFU/phone, which belonged to students in the “health sciences” group. The samples with enumerable levels of Y/M showed counts ranging from 1.7 to 5.2 Log CFU/phone, from those, 91% had counts between 1.7 and 3 Log CFU/phone and 3% contained  $>4.0$  to 5.2 Log CFU/phone corresponding to cell phones of students in the “health sciences” group. The distribution of *Enterobacteriaceae* and coliforms counts was similar, as these microbial groups were isolated from 100% and 99% phone samples respectively, with counts between  $\geq 1.7$  and 5.0 Log CFU/phone. *Enterobacteriaceae* counts  $>4.0$  Log CFU/phone were observed on 3% the surface of cell phones; two of them belonging to students in the “health sciences” group. Similarly, high coliform counts from 4.3 to 5.4 Log CFU/phone were found on 6% of sampled devices, which corresponded to six samples, from those, five belonged to students in the “health sciences” group. *Escherichia coli* was present only in 5% of phone samples with counts from  $\geq 1.7$  to 3.3 Log CFU/phone (Table 3). Of those samples, 14 belonged to students of the “health sciences” group and two to not health sciences group.

No statistical differences (P>0.05) were observed for APC, Y/M, coliforms and *Enterobacteriaceae* mean counts between groups of students (Table 4). No statistical comparison was performed for *E. coli* mean counts because of the low number of samples showing enumerable levels of this indicator. Except for *Enterobacteriaceae*, the number of cell phone samples with enumerable levels of microbial groups was higher in the “health science” group when compared to the “non-health-related sciences” group.

#### DISCUSSION

Undergraduate students commonly use their cell phones for academic, recreation and/or communication activities, almost everywhere where they are. Our findings indicated that health sciences students use their phones in microbiology laboratories and while attending patients in clinics and hospitals. Usage of cell phone in these sites could lead to convert the devices as reservoir and source of pathogenic and non-pathogenic microorganisms and favor cross-contamination (15). The potential of cell phones to transfer microorganisms can be reduced through the use of cleaning and disinfecting practices (16). However, the students’

**TABLE 3: Distribution of mean counts for microbial groups enumerated from the surface of cell phones used by undergraduate students at three university campuses**

Mean Log CFU/cell phone <sup>b</sup>	No. of samples (%)				
	Aerobic plate count (n= 303) <sup>a</sup>	Yeasts and molds (n=161)	Coliforms (n=94)	<i>Enterobacteriaceae</i> (n=87)	<i>Escherichia coli</i> (n=16)
$\geq 1.7 - 2.0$	3 (1)	90 (56)	34 (36)	26 (30)	8 (50)
$\geq 2.0 - 3.0$	22 (7)	57 (35)	36 (39)	40 (46)	7 (44)
$\geq 3.0 - 4.0$	190 (63)	10 (6)	18 (19)	18 (21)	1 (6)
$\geq 4.0 - 5.0$	72 (24)	3 (2)	5 (5)	3 (3)	0 (0)
$\geq 5.0 - 6.0$	12 (4)	1 (1)	1 (1)	0 (0)	0 (0)
$\geq 6.0 - 7.0$	4 (1)	0 (0)	0 (0)	0 (0)	0 (0)

<sup>a</sup> Number of samples with enumerable levels of microbial group

<sup>b</sup> Minimum detection limit was 1.7 Log CFU/phone

**TABLE 4: Counts of microbial groups on the surface of cell phones used by undergraduate students at three university campuses**

Microbial group	No. samples with enumerable levels (%)		Mean count (Log CFU/phone) <sup>a</sup>		Minimum-maximum count (Log CFU/phone)	
	Health sciences group	Non-health-related sciences group	Health sciences group	Non-health-related sciences group	Health sciences group	Non-health-related sciences group
Aerobic plate count	152 (50.2)	151 (49.8)	3.9 ± 0.71 A <sup>c</sup>	3.9 ± 0.60 A	1.7-6.7	2.7-5.3
Yeasts and molds	96 (59.6)	65 (40.4)	2.2 ± 0.64 A	2.2 ± 0.62 A	1.7-5.2	1.7-3.7
Coliforms	62 (66)	32 (34)	2.7 ± 0.86 A	2.6 ± 0.81 A	1.7-5.4	1.7-4.3
<i>Enterobacteriaceae</i>	34 (39)	53 (61)	2.6 ± 0.71 A	2.5 ± 0.68 A	1.7-4.6	1.7-4.4
<i>Escherichia coli</i>	14 (87.5)	2 (12.5)	2.2 ± 0.43	1.7 ± 0.00	1.7-3.3	1.7 <sup>b</sup>

<sup>a</sup> Minimum detection limit was 1.7 Log CFU/phone

<sup>b</sup> Both samples showed 1.7 Log CFU/phone

<sup>c</sup> Within rows, the means with the same letter are not significantly different ( $P > 0.05$ ). No statistical comparison was performed for mean counts of *E. coli* because of the low number of samples showing enumerable levels of this bacterium

responses evidenced the lack of information on cleaning and disinfection concepts for cell phones. Therefore, we suggest involving to students in a program of sanitary education at early stage of academic training to increase their knowledge about transmission and control of microorganisms.

In this study, the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. was investigated on the cell phone surface, which may be related to fecal and human contamination. None of these pathogenic bacteria were isolated from the students' cell phones, probably because the low levels in which pathogens are present on the surface of these devices. Also, it is likely that some devices were cleaned and/or disinfected sometime before our sampling; however, in our questionnaire we did not inquire when was the last time each device was cleaned and or disinfected, and we only made sure that it was not done immediately before sampling. Other researchers have reported a low isolation frequency of *Salmonella*, 1% on cell phones from university students in Nigeria (17) and 3% in Ghana (18). Most studies have included cell phones from medical science students in hospital setting, a site where the use of mobile phones raises the risk of cross-contamination, especially if effective disinfection are not enforced. A study performed at the Queen Elizabeth Hospital, in Barbados, showed that *Staphylococcus epidermidis* was isolated from 50% of mobile phones from medical staff, including students (6). Nwankwo et al. (7) reported the isolation of *S. epidermidis*, *S. aureus* and *Streptococcus* in 24, 14.8 and 11.1% of students' mobile phone swabs in Ghana. *Enterococcus* spp. was detected in 3% of cell phones belonging to food science students, but not in those devices from students on health and computer science in Slovenia (9).

The knowledge about quantitative levels of non-pathogenic organisms such as APC, Y/M, coliforms, *Enterobacteriaceae* and *E. coli* on the surface of cell phones was useful to estimate the

potential of devices as reservoir of microorganisms, given the lack of isolation of pathogenic bacteria in our study. The counts of these microbial groups may be used to evaluate handling and hygiene practices, as well as exposure of the cell phones surface to contamination sources.

Studies on the distribution and enumeration of microbial groups on cell phones are scarce, and comparison of findings should be cautious due to differences in methods and reporting units. Most of reported studies include the enumeration of APC (9, 18, 19), *Enterobacteriaceae* (9, 19), Y/M (9, 19), and coliforms (19), with counts ranging from 0.9 CFU/100 cm<sup>2</sup> to 6.9 Log CFU/cm<sup>2</sup>. Counts for these microbial groups in our study were higher than those reported in the previously cited studies (9, 18, 19). The high counts of microbial groups found on the surface of cell phones may be related to their constant handling in diverse sites, in which non-pathogenic and pathogenic microorganisms could be present. Cell phones do not possess conditions that favor microbial growth; therefore, high microbial counts may be originated from contact with heavily contaminated surfaces. The high variability observed for APC counts on cell phones of undergraduate students reflects a large diversity of contamination sources and handling conditions. Likewise, APC and Y/M are widely distributed in the environment and can contaminate the cell phones surface through contact with non-sanitized surfaces or as airborne contaminants. *Enterobacteriaceae* and coliforms enumeration could be useful to indicate general hygiene conditions of the devices, and the high counts found for these microbial groups could be a result of the direct or indirect exposition of cell phones to surfaces, persons, foods, and the environment, or could be related to the lack of proper hygienic measures of users. However, the presence of either of these groups does not necessarily imply fecal contamination or the presence of pathogens on the devices. Some *Enterobacteriaceae* and

coliform bacteria are common in human and animal feces, but others are commonly found in soil, water, and raw foods. From those sources, these microbial groups can be transferred to the surface of cell phones and their significance depends upon the conditions to which the device has been exposed (20). On the other hand, the presence of *E. coli* on a cell phone surface may indicate the possibility that fecal contamination has occurred and that other microorganisms of fecal origin, including pathogens, may be present. So, this bacterium may be used as an indicator of cell phone sanitation. The use of microbial groups as indicators of contamination of cell phones requires a thorough understanding of the handling and hygiene practices to which this device is subjected and the effect of these practices on microbial groups.

Results of this investigation show the potential of cell phones to participate as fomites and be a vehicle of different types of microorganisms. It is important to provide information not only to undergraduate students but also to general population on preventive strategies to reduce cross-contamination, as well as on hygiene measures to properly clean and disinfect these devices. We did not find evidence to support the hypothesis that these devices could be a reservoir for pathogens like *Salmonella*, *Staphylococcus*, *Streptococcus* or *Enterococcus*, however, information collected on usage habits evidences practices that increase the risk of microbial contamination of cell phones with pathogenic microorganisms.

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# Expanding central line care bundle to address line manipulations

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## ABSTRACT

**Background:** Central Line-Associated Blood Stream Infections (CLABSI) are serious healthcare-acquired conditions associated with high morbidity and mortality. Nationally the CLABSI incidence has reduced with the implementation of Centers for Disease Control and Prevention recommended prevention bundles. However, central line manipulation by healthcare workers and patients continue to cause CLABSIs and has not been adequately addressed in prevention bundles.

**Project Aim:** To evaluate line manipulations as CLABSI risk factor and describe prevention strategies.

**Methods:** The study evaluated CLABSIs during 2013-2015 from five hospital units admitting medicine service patients. CLABSI data were provided by the infection control division of the department of medicine. Data included demographics (age, gender, race), LOS, CLABSI information (date of event, pathogen, line type and location), and mortality. Additional chart reviews were conducted to obtain information on indication for line insertion, duration of line, manipulation and patient behaviors. Demographics and risk factors are reported as frequencies and percentages. CLABSI incidence per 1000 line days are reported over time.

**Results:** Thirty CLABSI events were reported during the study period. Line manipulation was noted within 48-72 hours prior to first documentation of symptoms of infection in 16 (53%) instances of CLABSI. Of these 16, nine (56%) line manipulations were for thrombolysis of blocked catheters, five (31%) CLABSI followed patient accession of lines for IV drug abuse, two (13%) patients had opioid dependence and received parenteral opioids at frequent intervals. Two of the patients who had thrombolysis also had line accession, one by the patient and one by healthcare worker for frequent IV medications prior to developing CLABSI.

**Conclusions:** Fifty-three percent of CLABSI occurred following line manipulations by healthcare worker or the patient. More intensive line care and strategies to avoid line manipulations by patients are needed to effectively further reduce CLABSIs.

## KEY WORDS:

Central venous access; bloodstream infection; healthcare-associated infections; hygiene; infection control; infection prevention; intravenous use; misuse; non-adherence

## BACKGROUND

Central Line-Associated Blood Stream Infections (CLABSIs) are healthcare-acquired conditions (HACs) associated with high morbidity and mortality. A total of 250,000 bloodstream infections (BSI) occur annually in hospitalized patients and about one third are CLABSI in ICU settings (1). One in four patients who develop CLABSI will die within 30 days as per 2014 report from the Centers for Disease Control and Prevention (CDC) (2). CLABSIs are known to increase median length of hospital stay by 24 days (3). Implementation of CDC recommended prevention bundle has significantly reduced CLABSI incidence across the United States by 50% in 2013 and 2014 from the

2008 baseline (4). The common mechanisms of developing CLABSIs are pathogen migration along external surface of the catheter which usually occurs in first seven days of insertion, or hub contamination from the handling of equipment causing intraluminal colonization and infection within ten days of insertion. Less common mechanism is hematogenous seeding of pathogens from infection source elsewhere in the body and contaminated infusion fluids. The CLABSI risk factors present at the time of insertion are well established such as selection of site, type of catheter (non-antibiotic impregnated), number of lumens of the port, lack of aseptic precautions, multiple attempts, skill and experience of the person inserting line (5). Factors during line

**Conflict of interest:** The authors have no financial conflicts of interest to declare.

This project was a part of hospital initiatives to reduce line infections, a quality improvement project.

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maintenance phase causing contamination at the puncture site as well as intravenous fluids and equipment; are frequent handling and manipulations of the catheter, line days, and inadequate hand hygiene and barrier precautions (4-7).

The recommended prevention bundle mainly incorporates strategies to reduce risk at insertion and maintenance of central lines (8). Healthcare providers manipulate central lines as part of usual line care. They flush the lines, administer fluids, pharmaceuticals and thrombolytic therapy. It has been known that soiled dressings and local contamination leads to line infection (9). Frequent access of lines causes colonization and can lead to bacteremia and sepsis (10). Some patients may tamper with the central line due to underlying delirium or behavioral health issues, or they may inject illicit substances. This also leads to contamination and subsequent BSIs (11,12).

However, the CDC prevention bundle does not specifically address line manipulations. Lately, BSIs have not been counted as central line-associated if there is clear documentation of access or high suspicion of access of central lines by the patients (13). Apart from mandatory surveillance and reporting, line manipulation is a safety concern associated with very high

risk of BSI which needs to be addressed. Our study evaluates the risk factors and discusses interventions aimed at preventing line manipulations.

**METHODS**

As part of national surveillance CLABSIs are reported to National Health Safety Network (NHSN). A CLABSI is a laboratory-confirmed bloodstream infection where central line was in place for more than two calendar days on the date of event, with day of device placement being Day 1, and the line was also in place on the date of event or the day before. A BSI is also considered CLABSI if it develops on or within 24 hours of removal of central line.

If the patient is admitted or transferred into a facility with an implanted central line (port) in place, and that is the patient’s only central line, the day of first access in an inpatient location is considered risk Day 1. “Access” is defined as line placement, insertion of needle into the port, infusion or withdrawal through the line. Such lines continue to be eligible for a CLABSI once they are accessed until they are either discontinued (i.e., removed from body) or the day after patient discharge. The CLABSI

**FIGURE 1: CLABSI Risk Factors**

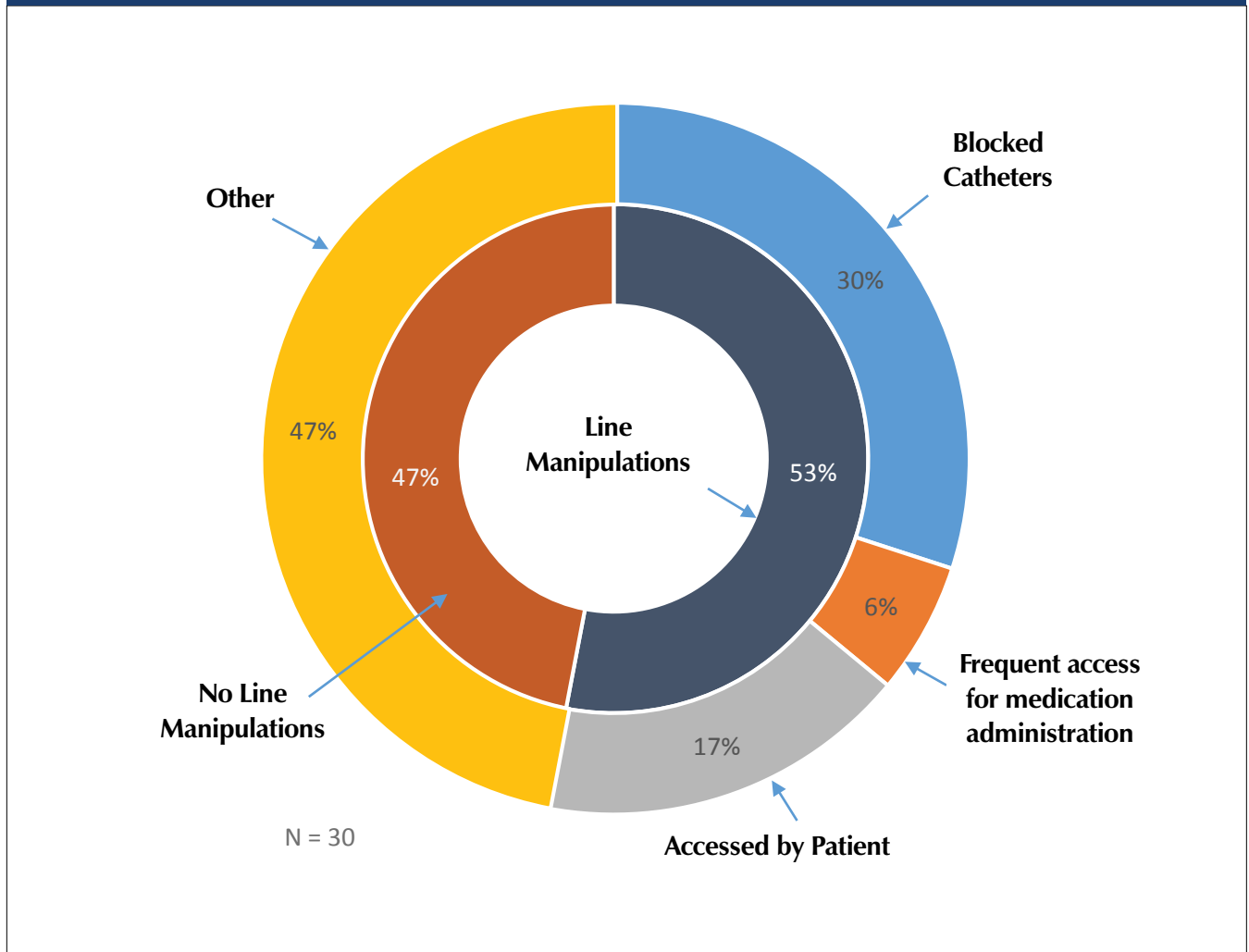
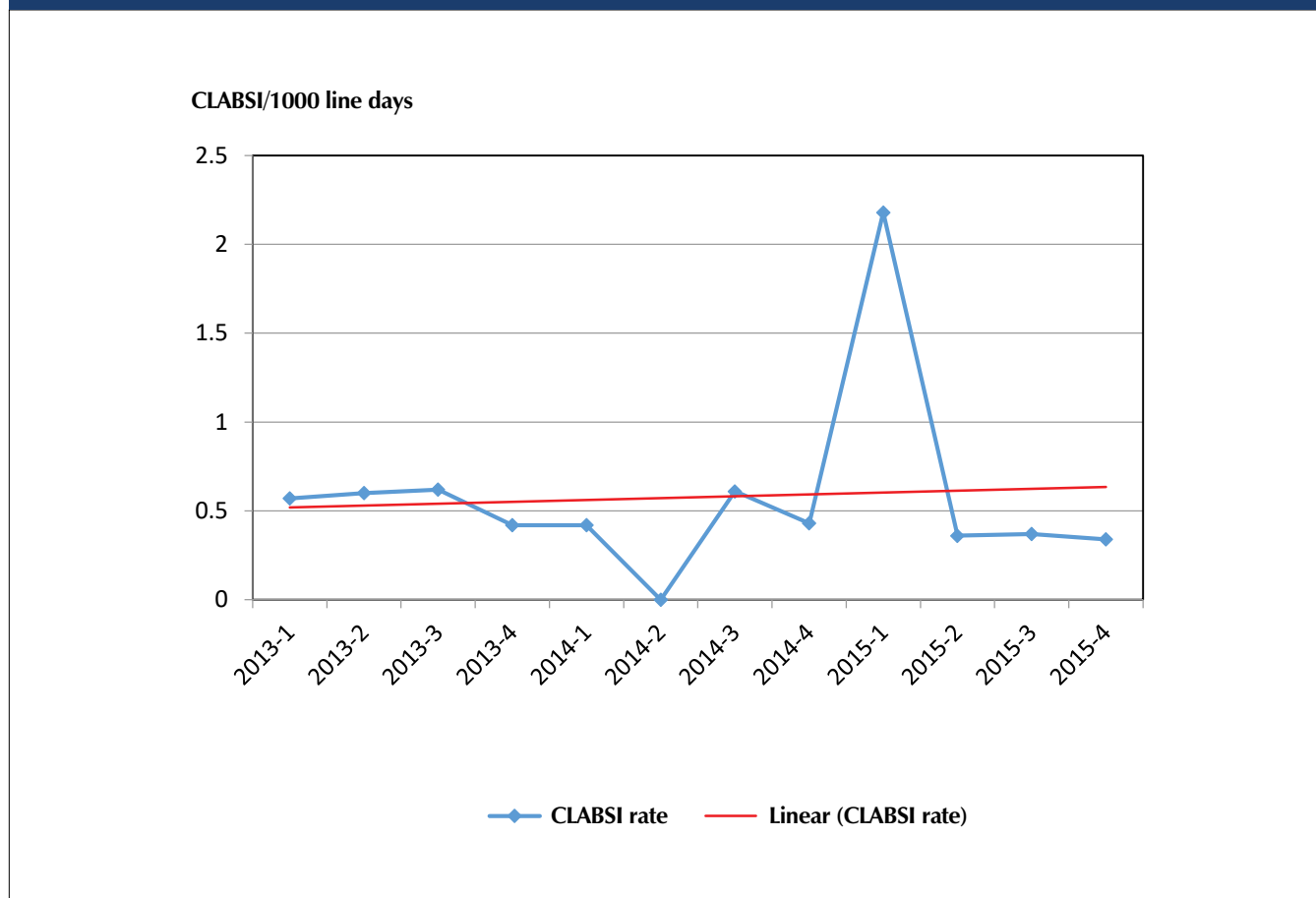




FIGURE 2: CLABSI Rates on medicine units over time



defining and reporting also follows the rules for repeat infection timeframe, transfer rule for attribution to the unit, and secondary infection due to other site infection or commensal organisms. Additional details can be found at the NHSN website (13).

Our study evaluated CLABSIs reported to NHSN during 2013-2015 from hospital units admitting medicine service line patients at a tertiary care academic center. CLABSI data was provided by the infection control division of department of medicine. Data included demographics (age, gender, race), hospital length of stay (LOS), CLABSI information (date of event, pathogen, line type and location), and mortality. Additional chart reviews were conducted to obtain information on indication for line, line insertion details, duration of line, line manipulation and patient behaviors. Study results are reported descriptively as frequencies and percentages for the demographics and risk factors. The CLABSI incidence are reported as events per thousand line days.

## RESULTS

There were 30 CLABSIs reported in 29 patients on medical units from 2013-2015. Patient demographic characteristics were: 10 females (33.3%), age range 26 to 88 years with median age of 42 years. Twenty-three (76.7%) patients were younger than 65 years. Four (14%) patients were African-American,

23 (79%) Caucasian and two (7%) did not have race identified in the charts. Two (7%) patients died in the hospital.

Thirteen (45%) of these patients developed CLABSI during readmission within 30 days and seven (24%) were transferred from outside hospital. The LOS ranged from eight to 189 days with 29 days being median duration. The medicine study units CLABSI incidence rates for 2013, 2014 and 2015 were respectively 0.57, 0.36 and 0.64 per 1000 line days.

The central line types included twenty (66.7%) peripherally inserted central catheters (PICC) and 10 (33.3%) central venous catheters (CVC). The CVC insertion sites included two subclavian, one femoral and seven internal jugular veins. Twenty-four (80%) lines were inserted on right side. All lines were elective procedures except for one emergency femoral line insertion. Lines were placed by interventional radiology (12, 40%), IV team (12, 40%) and by physicians on the floor or ICU (6, 20%). Seventy-nine (23) percent of line placements involved a single puncture.

Indications for central lines were total parenteral nutrition (1, 3%), monitoring (1, 3%), dialysis (4, 14%) and antibiotics, fluids and medications (24, 80%). Insertion sites appeared normal in 23 (77%) lines while swelling or bleeding was documented in seven (23%) lines. Time to infection ranged from 2 to 80 days, with a median of 11 days after insertion of lines.

**TABLE 1: Demographics: 29 patients had 30 episodes of CLABSI**

Patients	N=29
Age, Median (Range) years	42 (26-88)
Patients <65 years age, N (%)	23 (76.7)
Female, N (%)	10 (33.3)
African American, N (%)	4 (14)

A total of 16 (53%) instances of line manipulations were noted within 48-72 hours of infection (Figure 1). Nine (56%) of these manipulations were by the IV team for thrombolysis of blocked catheters, six (31%) episodes of CLABSI followed patient accession of lines, five for IVDA and one was tampering by patient pulling out the line causing bleeding and exposure. Two (13%) patients were opioid-dependent requiring IV opioids at frequent intervals for pain management. Two of the patients who had thrombolysis also had line accession, one by the patient and one by healthcare worker for frequent IV medications prior to developing CLABSI.

During the study period, a total of 2053 doses of alteplase were used for thrombolysis in 1033 medical patients which demonstrates large number of patients are exposed to line manipulation.

## DISCUSSION

In the last few decades there have been several initiatives to improve patient outcomes and curtail cost of healthcare in United States. Institute of Medicine reported high number of adverse events and hospital acquired conditions that prove costly and result in poor outcomes (14). CLABSIs were recognized as a priority for prevention, well studied, with risk factors identified and management standardized.

However, line manipulations as a risk factor is not adequately addressed for CLABSI prevention (5,15).

Our CLABSI cohort did not reveal any specific demographic characteristics except that 65% of patients were younger than 65 years. There were no management factors identified such as multiple attempts at insertion or poor line care. In our study 53% of CLABSI developed following line manipulations by healthcare worker or the patient. Elsewhere it has been reported that catheter manipulations in neonatal care unit were significantly associated with CLABSI in newborn children (16). The study implemented strategies to reduce line access by the nurses which resulted in reduction in CLABSI. The blocked catheters when flushed or accessed for thrombolysis also cause CLABSI by pushing colonized organisms into the blood. Similar mechanism is observed for line accession by the patients (3,4). Thus patients with intravenous drug abuse (IVDA) history are at risk for CLABSI. Many require outpatient intravenous antibiotics which makes PICC lines use necessary as an outpatient. Patients misuse the lines and a home central line is unsafe in this group of patients (17). If the physician knows or suspects that the patient will misuse the site then it is recommended not to discharge patient with central line (6,18,19). The authors explain

the risk in these patients is four folds due to IVDA, frequent manipulation, long time CVC colonization and sepsis, air embolism, drugs may contain thrombogenic materials leading to thrombosis and related complication.

Our study also identified 14% patients as having IVDA history and opioid dependence. There has been introduction of tamper resistant devices that makes it difficult for the patient to access the lines (19). This may reduce the risk somewhat; however, tampering may still occur by a patient injecting drug into tubes or trying to break the caps.

Our study is limited in that we did not use controls; the data is retrospectively collected which carries documentation bias of missing data and misinterpretation. However, the frequency of line manipulations is striking and as a safety and quality initiative appropriately resulted in prevention strategies.

In 2015 the NHSN reporting requirements excluded BSIs as CLABSI if there was documentation of line manipulation or high suspicion of line access such as syringe or drugs found in patient room. This was appropriate since hospitals and providers should not be held accountable for the patient actions. However, the risk of BSI and sepsis needs to be addressed in these patients. To effectively reduce CLABSI in these situations more intensive line care during and after line manipulations are required.

Currently at our institution besides the recommended line care following efforts are implemented 1. Removing blocked line if possible or replacing if indicated. 2. Identifying patient behaviors IVDA, opioid dependency and avoiding lines in these patients. 3. Nurse reviews medication regimen carefully to reduce the frequency of accessing lines. 4. Reinforcing line care bundle and personal hygiene. These measures will benefit patients and outcomes.

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## CONCISE REPORT

# How much do beds and mattresses sleep around? Automated measurement of bed frame and mattress movement in an acute care hospital

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## ABSTRACT

Hospital mattresses and bed frames are potential vectors for transmission of pathogens; however, data are lacking on the relative contributions of bed frames and mattresses to transmission or the magnitude of risk associated with bed and mattress movement. This proof of concept study describes the use of a real-time location system to track both bed frame and mattress movement in an acute-care hospital. The results provide a basis for future research to determine the associated risks of infection transmission.

## KEY WORDS:

bed frame, mattress, RTLS, contamination, nosocomial

## INTRODUCTION

Hospital mattresses and bed frames are potential vectors for transmission of antibiotic-resistant organisms (AROs) and other pathogens (1). Organisms that have been found to colonize bed components include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Clostridium difficile*, *Acinetobacter baumannii*, and norovirus (2,3).

Contamination may occur because of suboptimal cleaning or disinfection practices, resilient pathogens, and because mattress covers are permeable and susceptible to damage (4,5).

Little work has been done to study the capacity for beds and mattresses to contaminate each other, nor the relative role of bed frames and mattresses in ARO transmission as they are moved between patient rooms. Mattresses may be moved to different bed frames for a variety of reasons, including a need to repair or replace one but not the other, and a patient requiring a specialized mattress. Reasons for moving beds between rooms may include transferring patients between units, patient isolation for infection control, and other bed management or patient flow issues. However, there are limited data on the frequency of

mattress and bed frame movement. The gap in existing research may be partly due to difficulties in continuously tracking the movement and exchange of mattresses and beds.

This study used a real-time location system (RTLS) in one ward of an acute care hospital to study the rate of exchange of mattresses between beds and the rate of movement of beds between patient rooms.

## METHODS

A RTLS was installed in the multi-organ transplant ward of an acute care hospital in southern Ontario. The system used small transponders (see Figure 1) attached to equipment that emitted ultrasound pings at regular intervals, which were heard by a network of wireless receivers situated in patient rooms and hallways. Signals were processed by a geographical information systems engine, which computed movement, location, and proximity of tags to each other. A total of 59 bed frames and mattresses were outfitted with tags, as part of a larger pilot project to study the movement of patients, staff and equipment, and their implications for infection control policies and practices.

**Conflicts of Interest:** Dr. Furness discloses that he was an employee of Infonaut Inc., maker of the software used to track beds and mattresses in this research.

**TABLE I: Bed and mattress tag attrition during study period**

	<b>Beds</b>	<b>Mattresses</b>
Initial tagged inventory	+59	+59
Tag absent entirely during study period (all causes)	-11	-29
Tag absent for >5 days during study period (all causes)	-4	-2
Untagged mattresses that were able to be included		+18
Totals used in analysis	44	46

After verifying reliability of the system, data were collected for a 32-day period. Bed frame tags that never appeared or that disappeared were excluded from analysis. Any bed that was absent from the ward for more than five days (15%) of the study period was also excluded. Beds stationed in hallways were excluded from analysis.

Measurement was done by counting the number of mattresses each bed frame held during the study period. Mattresses without tags were included in analysis if they were never swapped, or if they were swapped before or after a tagged mattress. However, no instances of untagged mattresses being replaced by another untagged mattress could be tracked.

To measure the number of rooms that a bed visited during the study period, the number of room changes was counted rather than the number of unique rooms. For example, if a bed moved from one patient room to another and then back, this was counted as two changes (i.e., three rooms visited). If a bed left the ward and then returned to the same patient room, this was not counted as a room change.

## RESULTS

Tag attrition data appear in Table I. Tag attrition had two main causes: first, several tagged bed frames and mattresses were moved off the ward and were replaced by non-tagged beds and/or mattresses. Second, several tags either fell off or were inadvertently removed by staff. Because fallen tags could not be re-attached to the same mattress with certainty, no re-attaching was done.

**FIGURE 1: Ultrasound transponder (Sonitor Corp., Oslo, Norway)**

There were 41 bed frames (93.2%) that had only one mattress. One bed frame (2.3%) had two mattresses, and two bed frames (4.5%) had three mattresses. No bed frame had more than three mattresses during the study period.

Data for the rate of movement of beds between patient rooms are shown in Table II. A majority of beds (65.9%) visited more than one room during the study period (mean = 2.39; SD=1.26).

## DISCUSSION

By tracking bed and mattress movement using a novel RTLS, we found that 93.2% of bed frames never had more than one mattress, but 65.9% of beds were found in more than one patient room over a 32-day period.

Beds and mattresses have been established as vectors of infection. They have been found to be colonized by microorganisms in experimental studies and during outbreaks, and colonization may persist despite cleaning (2). One study of hospital bed frames and mattresses found that 56.4% of bed frames and 84.6% of mattresses contained a variety of organisms after terminal cleaning (4).

Hospitalized patients have been shown to have a higher risk of acquiring MRSA or VRE if the previous occupants of their room were carriers of those organisms (6). However, the absolute risk was small, with MRSA transmission increasing from 2.9% to 3.9% and VRE from 2.8% to 4.5%. Prior room occupants accounted for few transmission events, with a population attributable risk of less than 2% for both organisms. It is possible that previous occupants of the bed and/or mattress rather than the room may be more predictive of transmission events. A more recent study found that patients were more likely to develop *C. difficile* infection in hospital if the prior bed

**TABLE II: Frequency of rooms visited by bed frames**

<b>Number of rooms</b>	<b>Bed count</b>	<b>Percent of total</b>
1	15	34.1
2	9	20.5
3	10	22.7
4	8	18.2
5	2	4.5
<b>TOTALS</b>	<b>44</b>	<b>100.0</b>

occupant had received antibiotics, with an incidence of 0.72% compared to 0.43% ( $p < 0.01$ ) (7). However, there was no information provided on the relative contributions of bed frames and mattresses to infection transmission or the magnitude of risk associated with bed and mattress movement.

To our knowledge, this is the first report describing bed and mattress movement in an acute care hospital and provides the basis for future research incorporating environmental sampling and patient data to determine the associated risks of infection transmission.

There are several limitations to this study. First, patient outcomes were not monitored during this pilot stage so it was not possible to link bed and mattress movement to infection transmission. Second, the study period was relatively short due to logistical issues, and there may be fluctuations in rates of movement that would be evident in looking at longer time periods. Finally, several mattress tags fell off, and it is possible that those mattresses were moved more frequently than those that retained their tags.

In conclusion, beds were observed to move frequently between rooms, but frames and mattresses tended to stay together. The RTLS system was able to monitor bed frame and mattress movement, which suggests it is a useful measurement tool for future time and motion studies in hospital wards.

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## CONCISE REPORT

# Can the use of assistive technology and interactive therapeutic robots in nursing homes contribute to the spread of infectious disease?

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## ABSTRACT

**Background:** There is an increasing use of assistive technology and interactive therapeutic robots in nursing homes. However, little is known about the possible risks for transmitting infectious diseases through the use of such devices.

**Methods:** Representative surface samples of two multipurpose hygiene chairs and two interactive therapeutic robots were collected on a weekly basis at two nursing homes over a period of two months.

**Results:** We found that both robots and hygiene chairs may contribute to pathogen transmission.

## KEY WORDS

Assistive technology, interactive therapeutic robots, HAI, multipurpose hygiene chairs, nursing home

## BACKGROUND

Norwegian municipalities are increasingly using assistive technology and interactive therapeutic robots in their nursing homes [1]. Some of these products come in close physical and protracted contact with several patients and might constitute a source of infection. Little is known about the possible risks for transmitting infectious diseases through these devices. In this study we focused on multipurpose hygiene chairs and PARO interactive therapeutic robots.

Multipurpose hygiene chairs are used for washing and cleaning routines that require assistance from nursing staff (Figure 1).

PARO robots (Figure 2) are used in dementia care [2] to stimulate patients and cleaning done by the nursing home staff can only be done in a superficial way. Washing the interactive robot is not possible so that the artificial fur needs to be replaced by the distributor.

We collected representative surface samples of two hygiene chairs and two robots on a weekly basis over a period of two months at two nursing homes and analyzed the samples for the presence of clinically relevant microorganisms.

## MATERIAL & METHODS

### *Nursing homes*

Two nursing homes of approximately the same size, but located in different municipalities and with slightly different management structures took part in the study. Both nursing homes have implemented infection control programs.

### *Multipurpose hygiene chairs and PARO robots*

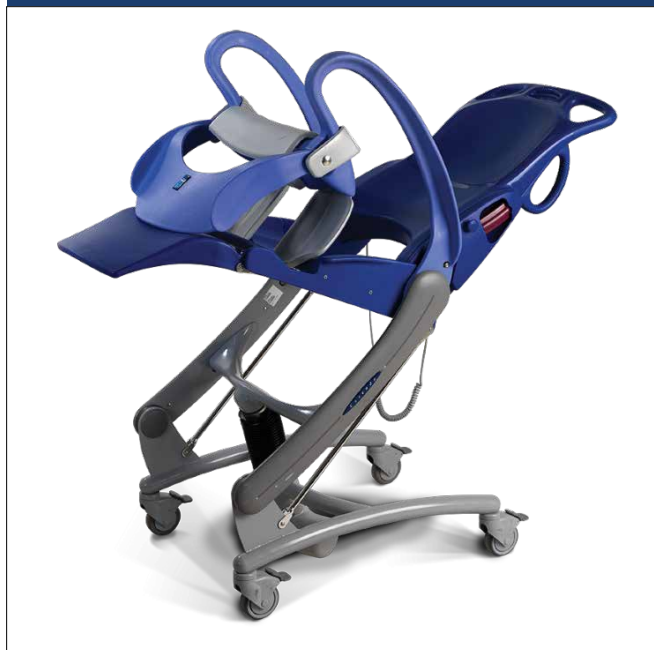
Four hygiene chairs (Carendo, ArjoHuntleigh, Sweden), two in each nursing home, were labeled according to the following scheme NxCx (N for nursing home 1 or 2, C for chair 1 or 2). N1C1 was not in use, due to necessary maintenance, but served as reference. N2C2 had been used by one resident only. N1C2 and N2C1 were in use by more than one resident, and no special precautions other than visible cleaning have been done. All hygiene chairs were visible clean according to applied standards [3] before sampling.

Four PARO robots, two in each nursing home, labeled NxPx (N for nursing home 1 or 2, P for robot 1 or 2). N1P1 and N1P2 were in sporadic use during the sampling period. For all PARO robots, there was no cleaning performed between the use by different residents.

**Conflicts of interest:** None to report.

**Acknowledgement:** We would like to thank Larvik and Nøtterøy municipalities, HSN Innovatoriet and the regional research fund "Oslofjordfondet" for financing this project.

**FIGURE 1: Multipurpose hygiene chair Carendo, ArjoHuntleigh [from: <http://www.arjohuntleigh.com/products/hygiene-systems/showering/shower-chairs/carendo/> ]**



### Swab sampling

Sterile flocked swabs were moistened in sterile water prior to surface swabbing of approximately 100 cm<sup>2</sup>. Two duplicate samples were taken each time and stored in either sterile water for bacterial cultivation or RNAlater for PCR analysis respectively.

Duplicate surface samples were taken with the M40 Transport system for bacterial cultivation.

### ATP analysis

Duplicate ATP surface samples were taken according to the manufacturer's protocol (Hygiene, UltraSnap™ surface test).

### Contact sampling with dry nutrient medium plates

Duplicate surface contact samples were taken with Rida®Count test plates for total bacteria and *Staphylococcus aureus* counts.

### MRSA

*Staphylococcus aureus* colonies from Rida®Count *Staph. aureus* test plates were transferred to MRSASelect™ agar (BioRad).

### Bacteriology from swab samples

Duplicates swab samples were pooled and transferred to the following selective media:

- **E. coli/coliform and ESBL detection:** Brilliance E.coli/coliform selective Agar and ESBL agar (Oxoid).
- **Enterococci and VRE:** HiCrome™ Rapid Enterococci agar, VancoScreening Brain Heart Infusion agar (NordicAST).
- **MRSA:** Samples were grown for 48 hours in PHMB enrichment broth without ceftoxitin, [4] and screened for MRSA. Results have been validated by PCR [5].

**FIGURE 2: PARO interactive therapeutic robot in close contact with resident**



- **Clostridium difficile:** Samples were grown anaerobically in CCFT-broth [6] and plated on Braziers *Clostridium difficile* selective agar, after two and ten days. Results have been validated by PCR [7].

Antibiotic resistant strains were identified by MALDI-TOF MS Biotyper (Bruker Daltonik, Germany).

### PCR

qPCR was performed for influenza A and norovirus 1 and 2 were done as described in [8].

### RESULTS

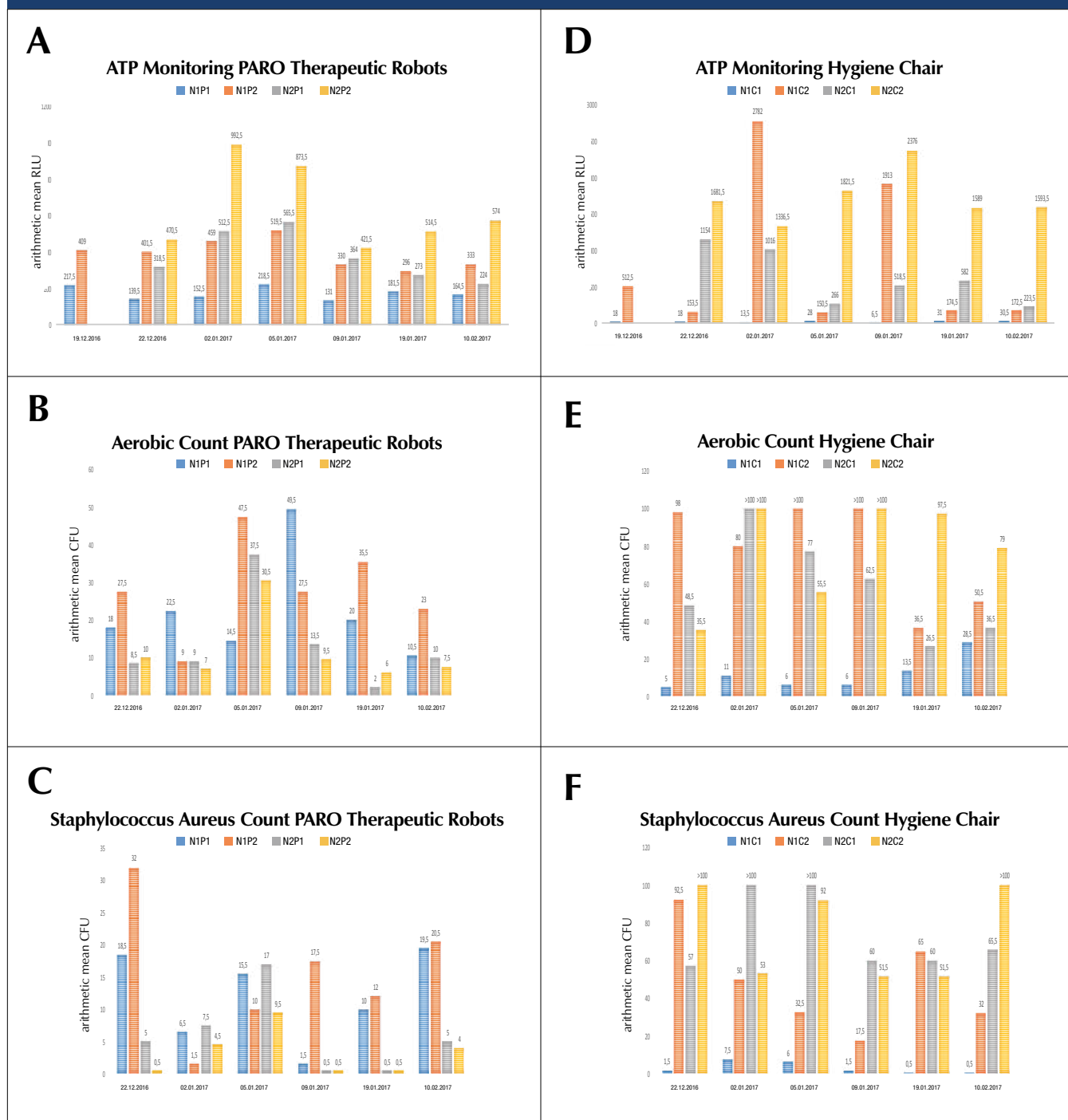
PARO robots N1P1 and N1P2 were not in daily use, which could explain the lower arithmetic mean relative light units (RLU) values, i.e., luciferase activity, compared to the frequently used N2P1 and N2P2 (Figure 3, a). By contrast, N1P1 and N1P2 gave higher arithmetic mean CFU for both aerobic (Fig. 3, b) and *Staphylococcus aureus* counts (Fig. 3, c).

One sampling of N2P2 gave a single atypical colony on the MRSASelect™ agar. This colony was subsequently identified as methicillin resistant *Staphylococcus epidermis* by MALDI-TOF.

The hygiene chair N1C1 was not in use at the time of testing which may explain the results of the ATP monitoring. However the aerobic count had an arithmetic mean CFU/ml up to 28.5 (Fig. 3). All other Hygiene chairs were sampled after standard cleaning. These showed both a high aerobic count (Fig. 3, e), as well as a higher degree of contamination with *Staphylococcus aureus* (Fig. 3, f). Furthermore, the ATP monitoring (Fig. 3, d) revealed that biological contamination in nursing home two was higher overall than in nursing home one. One Rida®Count sampling of N2C1 gave typical colonies for MRSA on the MRSASelect™ agar. The latter was, however, not validated by other methods. Coliform bacteria (Table 1) were found on all four robots. N1P1 tested positive for *Enterobacteriaceae* at one sampling (Table 1, N1P1).



**FIGURE 3: Serial measurements (mean values) of ATP (a,d), aerobic counts (b,e) and *Staphylococcus aureus* counts (c,f) for PARO robots (a,b,c) and hygiene chairs (d,e,f).**



Except for N1C2, all hygiene chairs showed little or no *E. coli* or other coliform bacteria (Table 1). N2C2 tested positive for *Enterobacteriaceae*. Cefoxitin resistant *Pseudomonas fulva* and *Pseudomonas putida* were found on samples obtained from N2C1 and N1C2. N2C1 tested positive for a vancomycin-resistant enterococci (VRE), namely *Enterococcus casseliflavus* (Table 1).

None of the samples tested positive for viral nucleic acid or *Clostridium difficile*.

## DISCUSSION

This study has several limitations, such as the sample size, duration and number of participating nursing homes. However, the authors believe that this study gives an indication of the possible role that assistive technology and interactive therapeutic robots have in the transmission of microorganisms and that further research in this field is required to increase patient safety in nursing homes.

PARO robots are often used by several residents and shared between different nursing home sections. It is difficult to clean the artificial fur; it can only be removed and washed by the distributor. However, based on ATP monitoring and aerobic count (Fig. 3, a and b) it seems that bacteria do not long remain viable on the PARO. Nevertheless, it seems also that the PARO robot may be a beneficial environment for *Staphylococcus aureus* (Fig. 3, c). Further studies are needed to confirm this.

The finding that biological contamination in nursing home two was higher overall than in nursing home one, may be due to different managerial structures of the cleaning services. Cleaning in nursing home one is done by municipal employees only working in this particular nursing home, whereas cleaning personnel in nursing home two is done by employees working in different municipal institutions.

**TABLE 1: Result of selective bacterial cultivation. (-) no growth, (\*) no growth of *Enterobacteriaceae*, but cefotaxitin resistant *Pseudomonas fulva* and *Pseudomonas putida*, (\*\*) *Enterococcus casseliflavus***

#### N1P1

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
19.12.16	-	-	-	-	-
22.12.16	-	-	-	+	-
02.01.17	-	-	-	-	-
05.01.17	-	-	-	-	-
09.01.17	-	-	-	-	-
19.01.17	-	-	-	-	-
10.02.17	-	+++	-	-	-

#### N1P2

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
19.12.16	-	-	-	-	-
22.12.16	-	+++	-	-	-
02.01.17	-	+++	-	-	-
05.01.17	-	++	-	-	-
09.01.17	-	+	-	-	-
19.01.17	-	-	-	-	-
10.02.17	-	-	-	-	-

#### N2P1

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
22.12.16	-	-	-	-	-
02.01.17	-	++	-	-	-
05.01.17	-	++	-	-	-
09.01.17	-	-	-	-	-
19.01.17	-	+	-	-	-
10.02.17	-	-	-	-	-

#### N2P2

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
22.12.16	-	-	-	-	-
02.01.17	-	+++	-	-	-
05.01.17	-	++	-	-	-
09.01.17	-	-	-	-	-
19.01.17	-	-	-	-	-
10.02.17	-	++	-	-	-

#### N1C1

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
19.12.16	-	-	-	-	-
22.12.16	-	-	-	-	-
02.01.17	-	-	-	-	-
05.01.17	-	-	-	-	-
09.01.17	-	-	-	+	-
19.01.17	-	-	-	-	-
10.02.17	-	-	-	-	-

#### N1C2

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
19.12.16	-	+++	-*	+++	-
22.12.16	-	++	-*	+++	-
02.01.17	++	++	-*	+++	-
05.01.17	-	-	-	-	-
09.01.17	-	+++	-*	+++	-
19.01.17	-	-	-	-	-
10.02.17	-	-	-	-	-

#### N2C1

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
22.12.16	(+)	-	-*	-	-
02.01.17	-	-	-	-	-
05.01.17	-	(+)	-*	+	+**
09.01.17	-	-	-	+	+**
19.01.17	-	-	-	-	-
10.02.17	-	-	-	-	-

#### N2C2

Dato	<i>E.coli</i>	Colif.	ESBL	Entero	VRE
11.12.16	-	-	-	++	-
02.01.17	-	-	-	-	-
05.01.17	-	-	-	-	-
08.01.17	-	-	-	-	-
09.01.17	-	-	-	-	-
19.02.17	-	-	-	+	-
10.02.17	-	-	-	++	-

The presence of coliform bacteria (Table 1) on the fur of the PARO robot may be due to inadequate hand hygiene [9], and could indicate that the robot is contributing in the transfer of microorganisms between different patient zones.

The hygiene chairs showed a high level of bacterial contamination, even after standard cleaning. Interestingly, N1C1 which was not in use showed an increase in the aerobic count. This may indicate that the rough surface structure of the hygiene chairs may accumulate airborne bacteria. In general, this study has shown that current cleaning procedures for hygiene chairs are not adequate. One of the chairs in this study, N1C2, used by several residents, tested positive for cefotaxim-resistant *P. fulva* and *P. putida*.

That influenza virus, norovirus and *Clostridium difficile* were not found may be due to unrelated factors. The national peak of influenza virus infections in Norway was in week 51 [10], three weeks before the first samples were taken. Furthermore, there were no ongoing infections in the nursing homes related to these agents and there have been only 46 clinical CDI cases in 2016 in the municipalities where the two nursing homes are located.

This study demonstrate the need for further research on the role of assistive technology and interactive therapeutic robots in pathogen distribution and the need for new cleaning procedures, a constant evaluation of infection control systems, as well as improved product design.

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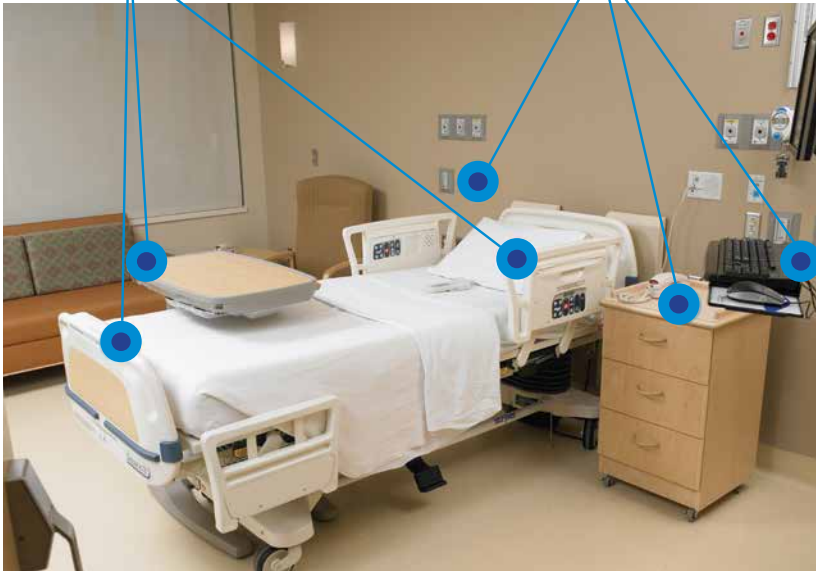
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Vernacare Canada Inc.	233	800-268-2422	<a href="http://www.vernacare.com">www.vernacare.com</a>
Virox Technologies Inc.	IFC	800-387-7578	<a href="http://www.virox.com">www.virox.com</a>

To reach infection control professionals across Canada through the ***Canadian Journal of Infection Control*** and its targeted readership, please contact me at

**Al Whalen, Marketing Manager 1-866-985-9782 [awhalen@kelman.ca](mailto:awhalen@kelman.ca)**





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<sup>1</sup> 3M has not found an organism that could survive and grow in 3M™ SoluPrep™ 2% CHG 70% IPA Solution. (Based on test organisms identified by Health Canada for professional healthcare use<sup>2</sup> using test method EN13727 and EN 13624 and 3M internal challenge studies using 3M Canada test methods based on current USP and cGMP Health Canada).

<sup>2</sup> Health Canada Guidance Document: Human-Use Antiseptic Drugs, Ottawa, 2009/11/27. [https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt\\_formats/pdf/prodpharma/applic-demande/guide-ld/antiseptic\\_guide\\_ld-eng.pdf](https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/dhp-mps/alt_formats/pdf/prodpharma/applic-demande/guide-ld/antiseptic_guide_ld-eng.pdf)

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