

# CJTIC

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

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ISSUE#01

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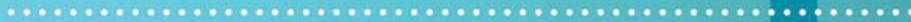


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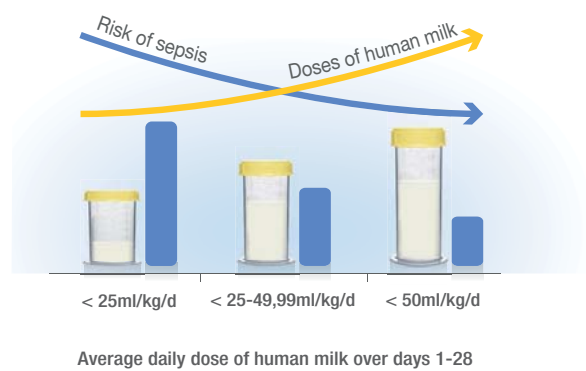
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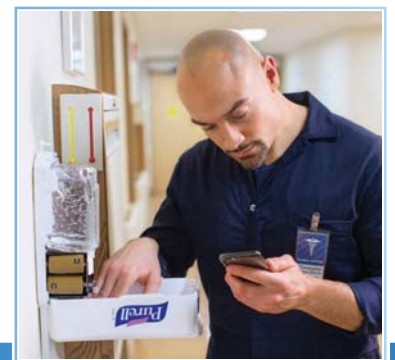
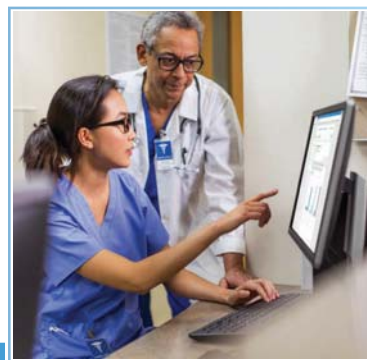
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# Taking your work home with you: Potential risks of contaminated clothing and hair in the dental clinic and attitudes about infection control

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## ABSTRACT

**Background:** Microbial contamination of clinic clothing is a potential source of infectious organisms spreading to the environment and susceptible people. The goal of this study was to educate dental professionals about the levels of bacterial contamination on clinic clothing and hair following dental clinic sessions.

**Methods:** Surveys of 30 dental and dental hygiene students assessed attitudes regarding microbial contamination on clinic clothing. Bacterial samples were isolated from a sterilized swatch of clinic clothing (scrubs) attached to the pants below the coat-line or to a hair band and processed for bacterial enumeration and identification.

**Results:** We found nearly all dental and dental hygiene students perform errands in their contaminated clinic clothing, but almost all felt they would be more likely to take better infection control precautions if they were aware of how much bacteria contaminate their clothing after a day in the clinic. Microbial analysis of swatches from scrubs showed a range from 250-60,000 bacteria/swatch (median=5,400), while hair samples contained 130-84,800 bacteria/swatch (median=19,300), including some potential pathogens like *Staphylococcus aureus* and *Enterococcus faecalis*.

**Conclusion:** These findings demonstrate the importance of changing out of clinic clothing and washing one's hair as soon as possible after a clinic session.

## KEY WORDS

bacterial contamination, infection control, *S. aureus*, pathogens, clinic clothing

## INTRODUCTION

Most dental professionals are aware of infection control in a dental office. However, many are unaware of the amount of bacteria that is transferred to their clinic clothing (scrubs) and hair during a day in the clinic. A study by Nordstrom *et al* found that 79% of unwashed operating room clothing (23/29) was contaminated with gram-positive cocci bacteria, 10% including *Staphylococcus aureus* [1]. In that same study 69% of clothing samples (20/29) contained gram-negative coliforms, in some cases including *Escherichia coli* [1]. In another study where nurses were provided with sterilized scrubs prior to a 12-hour shift, the average bacterial load was 1246 or 5795 bacteria/inch<sup>2</sup> for day and night shifts, respectively and 70% of scrub samples contained methicillin-resistant *Staphylococcus aureus* (MRSA) an important nosocomial pathogen [2, 3]. The importance of proper glove removal and hand washing in a clinical setting was demonstrated in a study by Munoz-Price *et al* where they

demonstrated that potential bacterial pathogens present on health care workers hands often leads to contamination of lab coats which can then serve as a source for recontamination [4].

Since contaminating bacteria remain on scrubs in hospital settings, it is likely that bacteria, including potential pathogens, are transferred to dental professionals' scrubs and hair after a day in clinic (hair coverings are not typically worn in the dental clinic). These microbes could then be a source of cross contamination to the environment since many dental professionals wear their scrubs home and launder them themselves. The purpose of this investigation was to improve the knowledge of dental professionals on the amount and potential species of bacteria that they are unknowingly bringing home with them after a day in clinic in order to prevent cross contamination to the environment.

**Acknowledgements:** We thank Dr. Joshua Thomson for many helpful comments on this study and Eric Jacobs for photographing the blood agar plates with optochin disks (Fig. S1).

## MATERIALS AND METHODS

### Survey of attitudes towards infection control

A survey was conducted by a judgmental sample of dental and dental hygiene students (n=30) at the University of Detroit Mercy School of Dentistry (IRB approval #1516-29). The survey consisted of five questions: Three multiple-choice questions to measure participants' infection control protocols utilized after a day in clinic. Two questions in the Likert scale format to measure awareness of bacterial cross contamination of scrubs to the environment.

### Microbial analysis

Autoclaved (sterile) scrub swatches (12 inch<sup>2</sup>, 3" x 4") were pinned on clinic scrubs on the thigh area (n=12) or attached to a hair band (n=10) to collect bacteria during a typical clinic day. After one or two clinic sessions (3 hours/session) scrub swatches were submerged in 10ml of sterile phosphate buffered saline (PBS), minced with sterile scissors and gently vortexed to elute adhered bacteria from the fabric. 50µl of bacteria were plated along with 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> (ten-fold) dilutions onto Blood Agar Base (Oxoid CM0055) + 5% defibrinated sheep blood (BD 211947), 1 mg/ml Vitamin K (MP Biomedicals 102259) and 0.5 mg/ml hemin (ACROS Organics 345960050) and grown at 37°C in an anaerobic chamber (Coy Laboratory Products) for 48-72 hours to culture facultative and obligate anaerobic microbes often associated with dental procedures. Colonies were enumerated to determine the level of contamination. Numerous colonies with distinct colony morphologies were subjected to culturing, DNA isolation, PCR and DNA sequencing analysis to determine the species.

### Distinguishing Streptococcal species

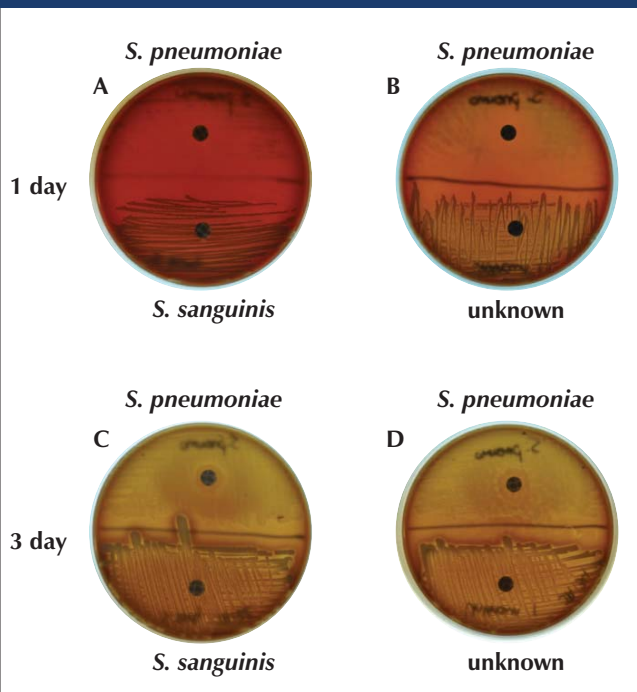
In one instance the DNA sequencing results could not distinguish *Streptococcus mitis* from *S. pneumoniae*. We employed the use of the optochin test to distinguish these two species. The undetermined *Streptococcus* strain was struck onto half of a Blood Agar Base plate (Oxoid CM0055) with 5% sheep blood (Hemostat DSB500) with 1 mg/ml Vitamin K (MP Biomedicals 102259) and 0.5 mg/ml hemin (ACROS Organics 345960050) while a lab isolate of *Streptococcus sanguinis* (in the Mitis group of oral streptococci) or *S. pneumoniae* was struck on the other half of the plate. Mitis streptococci are resistant to growth inhibition by optochin, while *S. pneumoniae* is sensitive to optochin. Optochin-impregnated disks were purchased from Fisher Scientific (Oxoid DD0001) and applied to each half of the plate with the use of sterile forceps. The plate was incubated at 37°C for 24 or 72 hours in a 5% CO<sub>2</sub> incubator. No observed zones of inhibition around the disk were noted for either *S. sanguinis* or the unknown strain (*S. mitis*, Table 1 and Fig. S1).

### DNA sequencing analysis

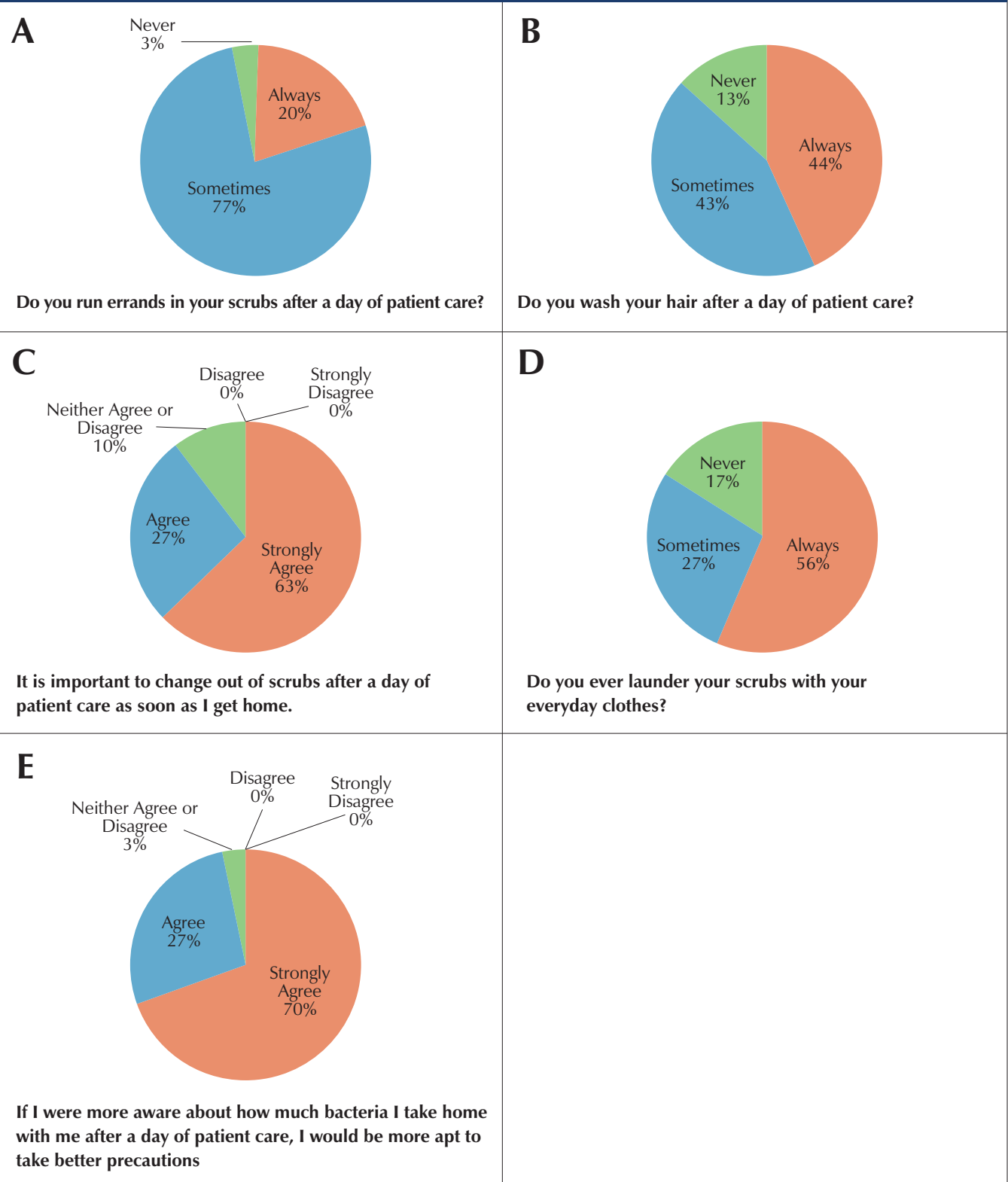
Distinct bacterial species (based on colony morphology) were isolated on blood agar plates, re-struck to a fresh blood agar plate and a single colony was inoculated into a microcentrifuge tube containing 100µl of autoclaved ultrapure H<sub>2</sub>O. The sample was boiled for 5 minutes then centrifuged at 13,000 rpm

for 5 minutes in a microcentrifuge. 70µl of the lysate was moved to a fresh microcentrifuge tube and used as the DNA template for PCR. The 16S rRNA gene was amplified using the primers 16S 5' GAGAGTTTGATYMTGGCTCAG and 16S 3' GAAGGAGGTGWTCCARCCGCA. We performed 30 rounds of PCR amplification using an annealing temperature of 50°C and elongation time of 1 minute/cycle with Phusion High-Fidelity DNA Polymerase (Thermo Scientific F530S). After purification of the 1.5 kb PCR product using a Qiagen PCR clean-up kit, the PCR product was quantified using a Qubit spectrophotometer and sent for sequencing by Genewiz using the 16S 5' GCAACGCGAAGAACCTTACC 3' primer to read the V6 variable region or 5' CCAGACTCCTACGGGAGGCAG 3' to read the V3 variable region 16S [5]. 16S sequences were aligned to known bacteria 16S rRNA genes using BLAST (NCBI) and results are reported in Table 1. This allowed for identification of the types of bacteria that typically contaminate scrubs and hair after working in the dental clinic.

**FIGURE S1: Discriminating between a streptococcal isolate as *S. mitis* or *S. pneumoniae*.** The unknown streptococcal isolate was struck onto blood agar base with 5% sheep blood and an optochin disk was placed in the center of the streak. Cell were grown for 1 (A and B) or 3 days (C and D) in 5% CO<sub>2</sub> incubator at 37°C and the appearance of a zone of inhibition around the optochin is indicative of a *S. pneumoniae* isolate. A previously characterized lab isolate of *S. pneumoniae* was used as a positive control for sensitivity to optochin (A-D, top) and a previously acquired lab isolate of *S. sanguinis* (also in the Mitis Group of streptococci) was used a strain resistant to optochin (A and C, bottom). The unknown (B and D, bottom) grew similarly to *S. sanguinis* (resistant to optochin). Thus, based on DNA analysis of the 16S gene, this strain is *S. mitis*.

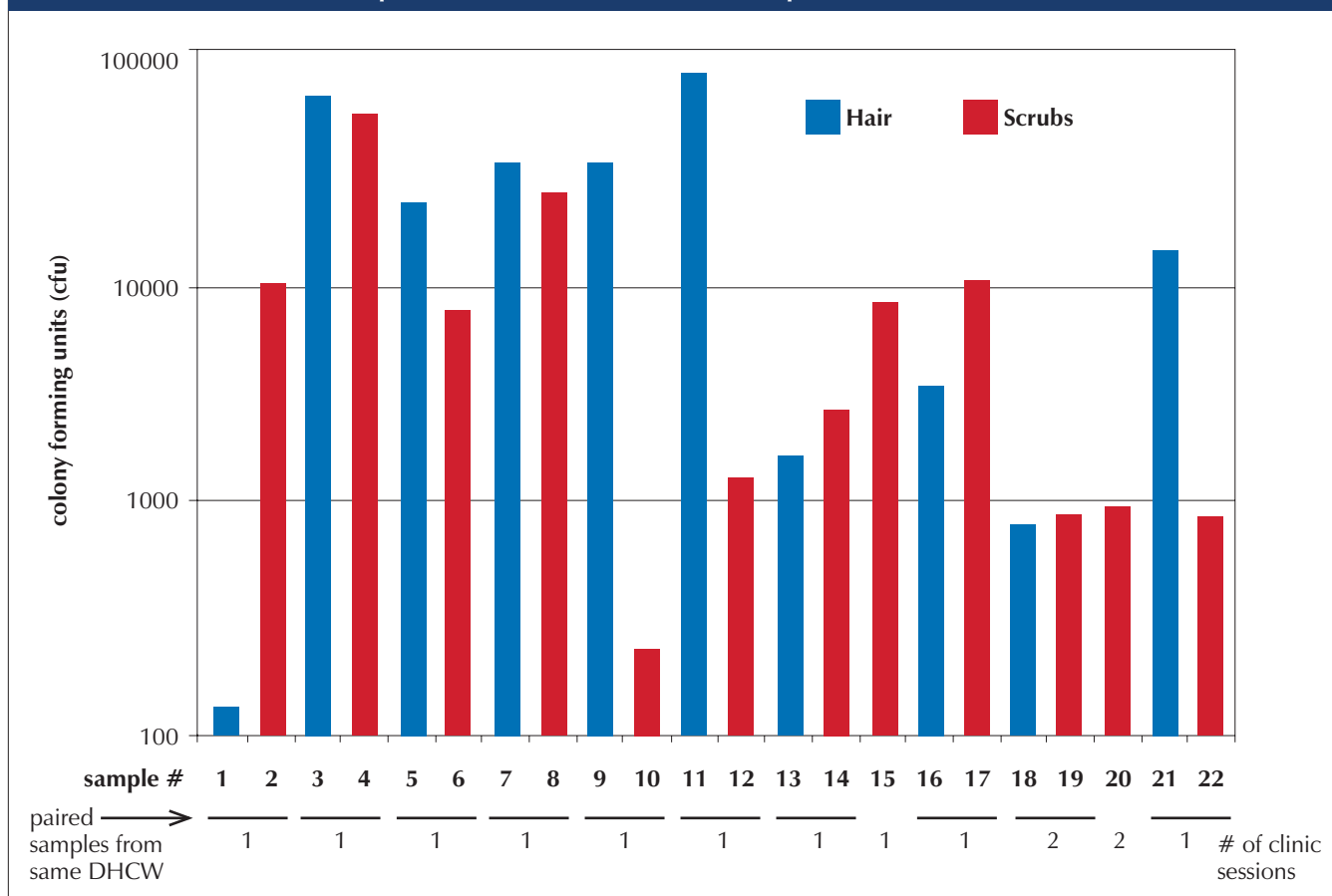


**FIGURE 1:** Survey questions reveal potential sources of environmental contamination from clinic clothing and hair after a day treating patients. 30 dental or dental hygiene students filled out a questionnaire assessing habits after a day treating patients in the clinic including whether students performed errands while still in clinic clothing, whether they felt it important to change out of clinic clothing as soon as they arrived at home and whether they washed their hair after a day treating patients in the clinic. The impact on changing behaviors upon knowing whether bacterial contaminants are brought home was also assessed.





**FIGURE 2:** Levels of bacteria on scrub swatches from clothing and hair. Sterile swatches were removed from scrubs or hair-bands after 1 or 2 clinic sessions (3 or 6 hours) and minced in sterile PBS prior to plating on blood agar plates and grown for 48-72 hours at 37°C under anaerobic conditions. Colonies were counted and a total bacterial count was determined based on the dilution plated and the total volume of the sample.



## RESULTS

### Attitudes and awareness concerning infection control

Based on our survey, 97% of dental healthcare workers (DHCWs) sometimes or always perform errands in their clinic scrubs after treating patients (Fig. 1A). Furthermore, only 44% of DHCWs always wash their hair after a clinic session (Fig. 1B). While most DHCWs (90%, Fig. 1C) recognized the importance of changing out of scrubs as soon as they get home, 83% sometimes or always wash their clinic clothing along with other garments (Fig. 1D). In some cases the DHCWs indicated they use cool water for washing clinic clothes to prevent the color from bleeding (personal communication). While laundry machines do not typically kill all microorganisms in a contaminated load, the use of cold water would result in higher levels of survival of some bacteria (and viruses) than using hot water with bleach [6-9]. Furthermore washing garments together can lead to transfer from one garment to another (cross-contamination, [10, 11]).

Encouragingly, 97% of DHCWs also indicated they would take better precautions if they were more aware of the bacteria they may acquire on their clothing and take home from the dental clinic (Fig. 1E).

### Levels of contamination acquired in the dental clinic

To assess the level of bacteria that may travel home with DHCWs on their clothing if they do not change out of scrubs at work, 12-inch<sup>2</sup> (3" x 4") swatches of sterilized scrub material were pinned onto the DHCWs clinic clothing just below the clinic coat line. While clinic coats remain in the dental clinic and are washed by a professional clinical clothing laundry service, DHCWs often wear their underlying scrubs home and launder them on their own (alone or mixed with other garments, Fig. 1D). By placing a 12-inch<sup>2</sup> scrub swatch just below the clinic coat line we obtained contaminants that might normally travel home with the DHCWs. In addition, some DHCWs wore a hairband with a sterile scrub swatch attached as well to capture bacteria that may land on one's hair during dental procedures. Typically DHCWs do not wear hair coverings in the clinic.

In 12 scrub swatches attached to clinic clothing, the level of bacterial contamination on the scrub swatch varied from 250-60,000 colonies/sample (median=5400; Fig. 2). Of the 10 samples from hair, the level of bacterial contamination on the swatch varied from 130-84,800 colonies/sample (median=19,300; Fig 2). Thus, both clothing and hair are potential sources of contamination after DHCWs leave the clinic for the day.

### Specific bacteria associated with clinic samples

To identify specific bacterial species that contaminated scrub swatches after a dental clinic session, single colonies from bacteria plated on blood agar plates were isolated, re-struck to fresh plates and lysed by boiling for DNA analysis. The 16S rRNA gene is typically used for bacterial identification by sequencing the variable regions, which provide a unique DNA sequence fingerprint for each species [5]. For our analysis, the 16S rRNA gene was amplified by PCR for each strain isolated (41 total strains) and then subjected to DNA sequencing. Results are provided in Table 1.

In multiple instances we identified *Staphylococci* and *Propionibacterium* species common on the skin and hair from both hair and scrubs samples (Table 1). In other cases we identified common oral bacteria such as *Streptococcus sanguinis*, *Streptococcus mitis*, *Veillonella parvula*, *Micrococcus species*, and *Granulicatella species* likely from contamination of scrubs during dental procedures (Table 1, Fig. S1). Potential pathogens like *Bacillus cereus*, *Enterococcus faecalis*, *Corynebacterium pseudodiphtheriticum* and *Dolosigranulum pigrum* were also identified, raising concerns about what species could be spread to the environment if one were to wear scrubs home from the clinic or not wash one's hair after a clinic session (Table 1). Finally, *Staphylococcus aureus*, a common nosocomial pathogen and major threat in the battle against multiple drug resistance [3] was also identified on scrubs (Table 1). A number

of the species isolated were  $\beta$ -hemolytic, indicating virulence factors capable of lysing host cells (Table 1).

### DISCUSSION

The goal of this study was to make DHCWs aware of the numbers and types of bacteria they may be unwittingly bringing home to their families or to the community after a day of treating patients in the clinic. While clinic coats are worn to prevent the transmission of microbes from patients to clinic clothing, organisms may still get transmitted to other areas of the clothing (such as below the coat line) or to the DHCW's hair. It is encouraging that based on our survey 97% of DHCWs agreed or strongly agreed that if they were more aware about how many bacteria were taken home after a day of patient care, they would be more apt to take better precautions (Fig. 1E).

Based on the often large numbers of bacteria isolated on swatches attached to dental scrubs or DHCWs' hair (as high as 7000 bacteria/inch<sup>2</sup>), it is concerning that 97% of DHCWs in our survey sometimes or always perform errands on their way home from a clinic session in their scrubs (Fig. 1A) and only 63% strongly agree that it is important to change out of clinic clothing as soon as they get home from a day of treating patients (Fig. 1C). Finally, given that in >50% of our swatches from hair samples, the levels of bacterial contamination were >1,000 bacteria/inch<sup>2</sup>, the fact that only 44% always wash their hair after a day in the clinic was concerning (Fig. 1B).

**TABLE 1: Bacteria isolated and identified by 16S rRNA gene sequencing on scrubs and hair swatches**

Genus and Species	$\beta$ -Hly	Times Isolated	Source	Reservoir
<i>Bacillus cereus</i>	+	1	Scrubs	Soil/Food
<i>Bacillus thuringiensis</i>	+	1	Scrubs	Soil/Pesticides
<i>Corynebacterium pseudodiphtheriticum</i>	-	1	Scrubs	URT
<i>Dolosigranulum pigrum</i>	-	1	Scrubs	URT
<i>Enterococcus faecalis</i>	-	1	Scrubs	GI Tract/Oral Cavity
<i>Granulicatella sp.</i>	-	1	Hair	Oral Cavity/GI Tract
<i>Micrococcus sp.</i>	-	2	Scrubs	Skin/Hair/Oral Cavity
<i>Neisseria perflava</i>	-	1	Scrubs	Oral Cavity/URT
<i>Propionibacterium acnes</i>	-	4	Hair and Scrubs	Skin/Hair
<i>Propionibacterium avidum</i>	+	1	Hair	Skin/Hair
<i>Staphylococcus aureus</i>	+	1	Scrubs	Skin/Hair/Nose
<i>Staphylococcus capitis</i>	-	4	Hair and Scrubs	Skin/Hair
<i>Staphylococcus epidermidis</i>	-	12	Hair and Scrubs	Skin/Hair
<i>Staphylococcus hominis</i>	-	4	Hair and Scrubs	Skin/Hair
<i>Staphylococcus pasteurii</i> or <i>S. warneri</i>	-	1	Scrubs	Skin/Hair
<i>Streptococcus sp. VT 162</i>	-	1	Scrubs	Oral Cavity
<i>Streptococcus mitis</i>	-	2	Scrubs	Oral Cavity
<i>Streptococcus sanguinis</i>	-	1	Scrubs	Oral Cavity
<i>Veillonella parvula</i>	-	1	Scrubs	Oral Cavity

$\beta$ -Hly =  $\beta$ -hemolytic; URT=upper respiratory tract, GI=gastrointestinal tract

The large range in colonies isolated could be a result of the specific procedures taking place in clinic, the number of clinic sessions during which the sterilized scrub swatch was worn, the frequency with which a DHCW brushed their hands against their scrubs or hair or a variety of other factors. Although it should be noted we found no correlation between the number of clinic sessions attended and the levels of contamination (Fig. 2). Due to the quantity and types of species found on the samples the results clearly demonstrated that there is potential for cross-contamination from the dental clinic to the environment, including the transmission of pathogens (Fig. 2 and Table 1). A comparison of species found on the hair swatches and previous studies in the normal microbiota of hair [12, 13] showed many of the isolates we identified are normally present on human hair, yet some isolates (*Granulicatella* species) are not typically found on hair and may have been acquired while providing patient care (Table 1). Thus, human hair is not considered a sterile surface and a limitation of this study is that we did not document any transmission from DHCW hair or scrubs to patients. One may presume the risk of such transmission to be fairly low given the absence of documented cases of such transmission. However, previous reports have demonstrated potential pathogens such as *S. aureus* present in HCW's hair leading to the suggestion that head coverings be worn while performing certain medical procedures where the chance of cross-contamination or wound infections are present [14-16].

While most of the species found on the samples were common environmental microbes that can be found in soil or on the skin, a few samples revealed potential pathogens were present such as *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Corynebacterium pseudodiphtheriticum* [17] and *Dolosigranulum pigrum* ([18]; Table 1). It should be noted that in general healthcare workers have a higher risk of nasal carriage of *S. aureus* than the general population [19] although in a recent study dental students had a lower rate of carriage as compared to medical students [20].

We hope the findings presented in this work will highlight the issue of clothing contamination for DHCWs and help prevent cross contamination to the environment. There is a growing body of data implicating healthcare workers' uniforms as a potential reservoir of pathogenic organisms [1, 2, 4]). This study suggests the importance of using in-house laundry services at one's dental facility or at least being sure to change out of clinic clothing as soon as arriving at home as well as washing clinic clothing in hot water with bleach to facilitate decontamination. Additionally, if laundry service for scrubs is not provided by one's dental care facility, one may want to change out of scrubs before leaving work and carry the soiled items home separately. For those who don't routinely wash their hair after a clinic session, a head covering may also be advisable.

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## ORIGINAL ARTICLE

# PVL toxin-producing methicillin-resistant *Staphylococcus aureus* (MRSA) are predominant in a tertiary-care metropolitan teaching hospital

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## ABSTRACT

**Background:** There is increasing evidence that the clones of Panton-Valentine Leukocidin toxin, (PVL)-producing methicillin-resistant *Staphylococcus aureus* (MRSA) are replacing toxin non-producing methicillin-resistant *Staphylococcus aureus* in healthcare settings. Our study sought to characterize clinical isolates of MRSA and the prevalence of PVL toxin producing MRSA in our tertiary healthcare center in the United States during a one-year period.

**Methods:** A total of 5,497 clinical samples submitted to microbiology laboratory were processed for presumptive identification of MRSA with further confirmation by polymerase chain reaction (PCR) for the identification of *mecA*, *Staphylococcal* chromosome cassette *mec* (SCC*mec*) type, and Panton-Valentine Leukocidin Toxin (PVL) gene. The antibiotyping was performed using VITEK® 2 system, and disk diffusion method, and data graphed using Microsoft Office program.

**Results:** Of *Staphylococcus aureus* isolates 52.2% (n=617) were MRSA. The prevalence of MRSA was higher within the 40-64 year old age bracket (~50%). Panton-Valentine Leukocidin Toxin was identified in 60% of SCC*mec* Type IV positive MRSA isolates and 28% of SCC*mec* Type II positive MRSA isolates; but the isolates were susceptible to vancomycin and rifampicin.

**Conclusion:** Our findings suggest a high prevalence of PVL toxin-producing isolates of MRSA, and thus adding an increasing risk of virulent infection.

## KEY WORDS

*Staphylococcus aureus*, methicillin-resistance *Staphylococcus aureus*, MRSA, healthcare-acquired MRSA, HA-MRSA, community-acquired MRSA, CA-MRSA, Panton-Valentine Leukocidin toxin, PVL toxin, *mecA*, *Staphylococcal* chromosome cassette *mec*, SCC*mec*, skin and soft tissue infection.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive pathogen, causing illness in both healthy and immunocompromised patients, and leading to high morbidity and mortality (1). MRSA cause both, hospital- and community-acquired infections. Hospital acquired (HA)-MRSA strains cause nosocomial infections, and are associated with distinct molecular features and predisposition factors than community-acquired (CA)-MRSA strains. Usually CA-MRSA strains are more virulent and likely to infect those without predisposition factors found associated with HA-MRSA (2).

CA-MRSA, particularly the Panton-Valentine Leukocidin Toxin (PVL)-producing strain leads to invasive infections, often in the soft tissue, such as boils and abscesses (3). CA-MRSA can be distinguished from HA-MRSA by tissue tropism and the size of the *Staphylococcal* chromosome cassette *mec* (SCC*mec*) (1). This genetic element carries the *mecA* gene encoding resistance to beta-lactam antibiotics. There are five known SCC*mec*

cassettes with type I-III being associated with HA-MRSA. Type I, II, and III cassettes are traditionally larger and indicating gene transfer of additional drug resistance markers located within the cassette, giving HA-MRSA, the phenotype with a multi-drug resistant makeup (4). SCC*mec* type IV and V are associated with CA-MRSA and are significantly smaller in size, which usually do not confer MDR phenotypes, but appears to have resulted in increased mobility, and hence greater potential for horizontal spread to the species of diverse genetic background (4). CA-MRSA infections can still be susceptible to clindamycin, rifampin, levofloxacin, and vancomycin (5). Recent USA studies showed an increased incidence of CA-MRSA (about 21%) since 2011, and involved healthcare-associated infections (5). This is alarming for a number of reasons. The ability of the microbe to transfer the type IV SCC*mec* cassette so readily alludes to the idea that the resistant phenotype may be on the horizon. This is also disconcerting because research into the CA-MRSA phenotype is still in its infancy and will require extensive studies



to determine resistance and spreading patterns. The spread of CA-MRSA into the hospital setting will place compromised patients in an even more dangerous predicament.

Our study sought to characterize the prevalence of HA-MRSA and CA-MRSA in a tertiary healthcare center in the United States over a one-year period.

## MATERIALS AND METHODS

The samples included in this study were submitted to clinical microbiology laboratory between June 2011 and June 2012, to determine the prevalence of *S. aureus* infection and the prevalence of SCCmec types I – III (CA-MRSA) versus SCCmec types IV and V (HA-MRSA) in the sample collected from the patients at Hahnemann University Hospital, an urban teaching facility in Philadelphia, Pennsylvania. Glycerol stocks of isolates were revived in Tryptocase Soy Broth (TSB), an antibiogram performed using VITEK® 2 system, and disk diffusion method for retrospective analyses of phenotypically pre-identified MRSA, as per recommendation by the Clinical Laboratory Standards Institute (CLSI) (2). American Type Culture Collection (ATCC) MRSA strains of USA-300 (BAA 1680) and USA-400 (BAA 1683) were included as positive reference strains of CA-MRSA (2).

Isolates were tested for the presence of the *mecA* cassette (SCCmec) by Polymerase Chain Reaction (PCR) for confirmation of MRSA. The isolates were also tested for the presence of the PLVT gene, and for the type of SCCmec cassette each isolate was carrying. To isolate bacterial DNA, the isolates were grown overnight at 37°C in TSB. Cells were pelleted, washed, and subjected to DNA isolation as per instructions of the manufacturer (DNeasy Blood & Tissue Kit, Qiagen, Germantown, MD). DNA concentration of each isolate sample was determined and subjected to PCR using well-established primers for *mecA* and *PLVT* (6, 7). Multiplex PCR was performed to determine the type of SCCmec present in the isolates (8). The data was analyzed and graphed using Microsoft Office Program.

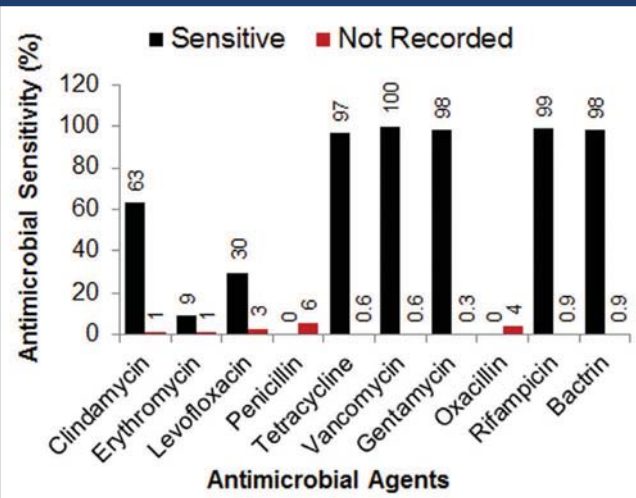
## RESULTS

Out of 5,497 isolates, 617 were identified as *Staphylococcus aureus*; of which 322 (52%) were identified as MRSA based on the presence of the *mecA* gene. Approximately 50% of the MRSA positive samples prevalence lied primarily within the 40-64 years old age bracket, followed by the patients of less than 40 years (27%) and above 64 years (23%) of age groups, infants had contributed about 1% of total MRSA isolates.

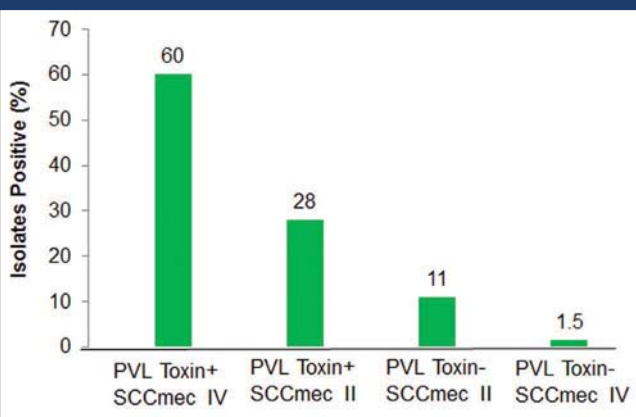
The antibiogram demonstrated decreased susceptibility to erythromycin, penicillin, and levofloxacin. A majority of the isolates were susceptible to rifampicin, gentamycin, tetracycline and co-trimoxazole. Oxacillin was included as a control for testing methicillin resistance. All tested isolates were susceptible to vancomycin (Figure 1).

Figure 2 is a graphical presentation, and demonstrates findings of molecular characterization of the isolates. The isolates were positive for *mecA* gene. We determined that 283 (87.6%) of the tested MRSA isolates were PVL positive. SCCmec cassettes data demonstrated that none of the isolates contained a type I or type III cassette and 126 (39.1%) had a type II cassette, the

**Figure 1. Antibiogram of MRSA isolates. The isolates demonstrated relatively low susceptibility to erythromycin, penicillin and levofloxacin. Isolates were prominently less resistant to tetracycline, gentamycin, rifampicin and bactrin. All isolates tested were notably susceptible to vancomycin.**



**Figure 2. Genetic characterization of MRSA isolates. About 87% and 61% of MRSA isolates were positive for PVL toxin gene and SCCmec Cassett Type IV, respectively, indicating that vast majority of isolates were having molecular features of community-acquired MRSA (CA-MRSA). The majority of the CA-MRSA isolates were PVL toxin-producers.**



prototypical cassettes contained in HA-MRSA. The majority of the isolates (198 of 322; 61.5%) from this study tested positive for type IV, the standard cassette found in CA-MRSA.

In order to correlate isolates of different cassettes types with the presence of PVL toxin gene, we used PCR analysis, and the showed that only 1.5% of the SCCmec type IV isolates and 11% of the type II cassette isolates were PVL toxin negative. Sixty percent (193 of 322) of the tested isolates were positive for both SCCmec type IV and the PVL toxin while 28% (90 of 322) of the SCCmec isolates that were identified as type II were positive for the PVL toxin. Taken together, this data suggests that this sampling pool is mostly type IV SCCmec and PVL positive,

indicating that a majority of these isolates are having molecular features of community-acquired MRSA.

## DISCUSSION

The situation that CA-MRSA would exceed the prevalence of HA-MRSA at this hospital is possible since horizontal spread allows for the simple transfer of resistance markers between microbes (1, 9). The SCCmec cassette characteristic of CA-MRSA and encoded with this cassette is the PVL toxin, suggesting that the presence of the leucocyte-toxic gene is favored.

Ninety percent of the isolates tested showed resistance to erythromycin, but only 34% were resistant to clindamycin, suggesting inducible clindamycin resistance. Previous work in our lab from 2008 suggested an increase in prevalence of clindamycin resistance from 2008 to 2011 (10). Interestingly, we found that, when comparing isolates from 2008 to 2011, there was a significant increase in susceptibility to tetracycline, gentamycin, and rifampicin. Although we did not use antibiogram as criteria to differentiate CA-MRSA from HA-MRSA, this finding supports our assumption that CA-MRSA prevalence is on the rise at our hospital as this particular type tends to be more susceptible to non-beta lactams.

The rapid emergence and spread of CA-MRSA also has a negative implication of how easily it can transpose the type IV cassette, and suggests that isolates could easily pick up resistance markers from other strains in the environment (4). MRSA isolates that contain the PVL toxin destroy leucocytes and skin and mucous membrane epithelium and confer increased virulence that can lead to life-threatening infections such as necrotizing hemorrhagic pneumonia with very high mortality rates (3). Therefore, the rates of PVL-positivity in MRSA of 87%, as in this study, are alarming for infectious disease specialists and infection control practitioners.

In conclusion, we suggest that the increased prevalence of CA-MRSA might be due to the small size of the IV cassette; however, there could be other mechanisms at play giving it a genetic advantage. This is one of the rare report detecting PVL toxin gene in majority of the MRSA isolates.

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# *Pseudomonas* bacteremia; in vitro susceptibility pattern in a tertiary care hospital

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## INTRODUCTION

*Pseudomonas* sp. is ubiquitously present worldwide of which *Pseudomonas aeruginosa* is a major nosocomial pathogen, which survives in moist environments and colonizes the respiratory tract of mechanically ventilated patients (1). *P. aeruginosa* bacteremia occurs most frequently in critically ill patients, particularly those who are immunocompromised, such as cystic fibrosis patients, burn victims and ICU patients (2).

*P. aeruginosa* has been implicated as the eighth most common pathogen causing nosocomial blood stream infections; alone it contributes to 10-20 % of nosocomial infections (2). *P. aeruginosa* has emerged as quite a challenging pathogen for clinicians. MDR, XDR, and PDR phenotypes elaborate inactivating enzymes, such as extended-spectrum beta-lactamases (ESBL) and metallo- $\beta$ -lactamases (MBL), that make beta-lactams and carbapenems ineffective (3). ESBL-producing *P. aeruginosa* was initially detected in Europe in the mid-1980s, and MBL-producing *P. aeruginosa* was first reported from Japan in 1991. Resistant strains of *P. aeruginosa* have become a growing concern worldwide (4). *P. aeruginosa* resistance to carbapenems has been reported to be emerging at a rate of 20% (5).

Multidrug-resistant *P. aeruginosa* (MDR) infection has been reported to be associated with increased morbidity which includes increased length of stay, invasive procedures (i.e., bronchoscopy, tracheostomy, catheter implantation), higher incidence of surgery and increased mortality rates, as compared to MDR *Pseudomonas* sp. Infections (6).

Antibiotic resistance has been shown to vary by location. The resistance profile of multidrug-resistant strains, therefore, requires enhanced monitoring, especially for empiric treatment. Obtaining regional resistance data is important for establishing guidelines for appropriate antibiotic use, and may help control the rate of antibiotic resistance. Aim of the present study was to determine the sensitivity pattern of *pseudomonas* sp. isolated from bloodstream infections, and the prevalence of multidrug resistance, extensive drug resistance and pan drug resistance.

## METHODS

This is a retrospective cross-sectional study of all the *Pseudomonas* isolates isolated from blood samples of patients with fever/sepsis, received at the department of microbiology of Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi over a period of 19 months.

Blood was collected 10 ml for adults and 5ml for paediatric patients and diluted in a ratio of 1:10 added to blood culture bottles with BHI broth. These blood culture bottles were then incubated aerobically at 37°C. The samples were subcultured after overnight incubation, day 3 and day 5. The samples were subcultured on blood agar and MacConkey agar and incubated at 37°C for a duration of 18 hours. *Pseudomonas* sp. was identified as per standard bacteriological methods (7).

The antibiotic sensitivity patterns of these isolates were studied by using the Kirby Bauer Disc Diffusion method on Mueller-Hinton agar, in accordance with CLSI Guidelines, and using Hi-media antibiotic discs (8). The antibiotics discs which were tested included piperacillin-tazobactam (100/10ug), amikacin (30 ug), gentamicin (10 ug), tobramycin (10 ug), netimicin (30 ug), cefepime (30 ug), ceftazidime (30 ug), ciprofloxacin (5 ug), levofloxacin (5 mcg) and meropenem (10 ug), imipenem (10 ug) and colistin (10 ug). For this study, multi-drug resistance was defined as resistance of a *Pseudomonas* isolate to at least three of the following four drugs: amikacin, imipenem, ceftazidime and ciprofloxacin. These antibiotics were chosen because they are representative of their antibiotic classes (9). Antibiotics with intermediate susceptibility CLSI were considered resistant in the study analysis.

Although definitions of MDR *Pseudomonas* in other studies were noted (10), it is worth mentioning that currently, no international consensus for the definition of multidrug resistance exists, making direct comparison of literature

very difficult. Extensively drug resistant (XDR) from the MDR *Pseudomonas* isolated were those isolates resistant to all the antibiotics except colistin (9). Pan drug resistance (PDR) is resistance to all antibiotics.

## RESULTS

From 3951 sets of blood cultures 676 significant isolates were grown. An isolate was considered significant when a recognized pathogen was isolated from one or more blood samples and the patient had at least one of the following signs and symptoms: fever ( $>38^{\circ}\text{C}$ ), chills or hypotension and for commensals (e.g., coagulase negative staphylococci) isolation on two or more blood samples drawn on separate occasions. Gram positive cocci were isolated in 178 (26.33%) while Gram negative rods were isolated in 498 (73.66%) cases. *Pseudomonas* sp. constituted 14.05% (95) of the total isolates. ICU, ward and outpatient department (OPD) contributed 73.8% (70), 24.21% (23), 2.10% (2) respectively of the total isolates. Figure 1 shows distribution of *Pseudomonas* sp. among medical and surgical ICUs and wards.

The susceptibility pattern of *Pseudomonas* sp. from ICU and wards was obtained as shown in Table 1. Among aminoglycosides, maximum sensitivity was reported to netilmicin in wards and amikacin in ICU. Most of the isolates were sensitive to levofloxacin in Fluoroquinolone group. Most of the strains were resistant to cepheims. *Pseudomonas* sp. isolated from wards and ICU were mostly sensitive to carbapenems (ward-meropenem, ICU-imipenem).

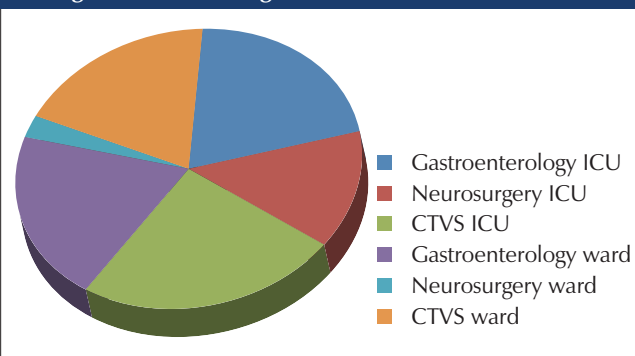
Carbapenem resistance was found to be more in ICU as compared to ward.

MDR, XDR and PDR *Pseudomonas* were analyzed and it was found that six MDR (10.75%), (four from Gastroenterology ICU and two from ward) and four (4.3%) extensively drug resistant (XDR) were isolated (Gastroenterology ICU). No PDR was observed. Five out of six MDR strains (83.3%) were found to be susceptible only to Imipenem, one MDR strain was found to be susceptible to Gentamicin and Amikacin, while all were sensitive to colistin.

## DISCUSSION

*Pseudomonas* sp. bloodstream infection is a serious infection with significant patient mortality and healthcare costs. There is a global emergence of multidrug resistant strains of *Pseudomonas*. In the present study Gram negative rods constituted 73.66%

**FIGURE 1: Distribution of *Pseudomonas* sp. among medical and surgical ICUs and wards**



**TABLE 1: Susceptibility pattern of *Pseudomonas* sp. in ICU and medicine wards**

Antibiotics	Breakpoints (zone diameter)	% Resistance ICU	Medicine wards
<b>Beta lactam/beta lactamase inhibitor</b>			
Piperacillin-tazobactam	21	66.1	65.3
<b>Cephems</b>			
ceftazidime	18	71.2	69.6
Cefepime	18	84.7	78.3
<b>Carbapenems</b>			
Imipenem	19	17.4	13.1
Meropenem	19	44.24	26.1
<b>Aminoglycosides</b>			
Gentamicin	15	61.6	56.6
Amikacin	17	46.2	47.8
Tobramycin	15	88.5	69.6
Netilmicin	15	57.7	43.5
<b>Fluoroquinolones</b>			
Ciprofloxacin	21	94.3	95.7
Levofloxacin	17	38.5	43.5
<b>Lipopeptides</b>			
Colistin	12	0	0



of total isolates from blood and *Pseudomonas* sp. constituted 14% of total. In a study by Lachhab Z et al Gram negative rods constituted 83.6% and *Pseudomonas* sp. was 10% of total blood culture isolates from ICU (5).

Of total of 93 strains of the *Pseudomonas* isolated from the hospital ICU and wards, Piperacillin -tazobactam (Betalactam/ betalactamase inhibitor) 66.1% resistance found in ICU and 65.3% resistance found in wards. Ceftazidime resistance has been found to be 71.2% in ICU and 69.6% in wards. In the research implemented by Van Elder and Ahani Azari et al, the high resistance to drugs of the  $\beta$ -Lactam group and sensitivity toward the Amino glycosides and have been observed (11,12).

Ceftazidime resistance is mainly mediated by production of  $\beta$ -lactamases such as ESBL (extended spectrum beta lactamases), MBL (metallo-beta lactamases), and occasionally AmpC- $\beta$ -lactamases (13). Besides production of various  $\beta$ -lactamases, other mechanisms such as the lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux pumps can also contribute for resistance to  $\beta$ -lactams (14). Horizontal gene spread is considered to be responsible for the high frequency of ESBLs detected in *P. aeruginosa* (15).

In the present study, cefepime resistance has been found to be 84.7% in ICU and 78.3% in wards. This is in stark contrast to findings by Patel et al who reported Cefepime to be 15.63% resistant in isolates of *P. aeruginosa* (16), whereas Endimiani et al reported that 10-35% of the isolates of the clinical population in North America are resistant to Cefepime (17).

Aminoglycoside resistance has been found to be 63.5% in ICU while 54.3% in ward. In a study by Teixeira B et al the frequency of resistant *P. aeruginosa* isolates was found to be higher for the aminoglycosides tobramycin and amikacin (30.7 and 29.9%, respectively). The enzymatic modification of aminoglycosides by aminoglycoside-acetyltransferases (AAC), aminoglycoside-adenyltransferases (AAD), and aminoglycoside-phosphotransferases (APH), is the most common resistance mechanism in *P. aeruginosa* and these enzymes can be coded on mobile genetic elements that contribute to their dispersion (18).

In another retrospective case-control study in Turkey, it was found that the major risk factors for infection or colonization with multi-resistant *P. aeruginosa* were prolonged stay in the ICU, previous and lengthy imipenem usage, and mechanical ventilation (19). Also, in our study, maximum isolates of *Pseudomonas* spp. were from the ICUs irrespective of the type of ICU. High consumption of the antibiotics has led to the increase of vulnerability of the hospitalized patients toward the opportunistic infections.

Prior fluoroquinolone use has been identified as a risk factor for the emergence of imipenem-resistant *P. aeruginosa* (20). Out of 17 imipenem-resistant *P. aeruginosa* isolated during the study period, 15 (90%) showed resistance to ciprofloxacin or levofloxacin, suggesting that cross-resistance may have developed for imipenem due to prior use of fluoroquinolones. Similar findings have been reported in a study by Rajkumari N et al (9). The widespread use of quinolones inevitably results in increasing cases of resistance.

Carbapenem resistance has been found to be 15.5% for imipenem and 35% for meropenem. Among the beta-lactam antibiotics, carbapenems with antipseudomonal activity are important agents for the therapy of infections due to *P. aeruginosa*. The development of carbapenem resistance among *P. aeruginosa* strains is multifactorial. Plasmid or integron-mediated carbapenemases, increased expression of efflux systems, reduced porin expression and increased chromosomal cephalosporinase activity have all been defined as contributory factors (21).

Colistin (polymixin E) was one of the first commercially available antibiotics. While toxicity concerns have limited its usage, its potent activity against multidrug-resistant strains of *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* in vitro suggests that it may be effective for drug resistant infections. All isolates including multi drug resistant *P. aeruginosa* were found to be susceptible to colistin in a study by Walkty et al (22).

Prevalence of MDR is found to 10.75%, XDR 4.43%. No PDR has been found in our study. In a study by Gill JS et al, they reported a prevalence of 50% for MDR (resistant to one or more than one antimicrobial agent in three or more antimicrobial categories), XDR 2.3% (resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less) and no PDR (3).

## CONCLUSION

The study suggests that MDR and XDR strains of *Pseudomonas* sp. are emerging. MDR strains are resistant to commonly used antibiotics and showed maximum sensitivity to carbapenems. For XDR strains, glycopeptides are the only resort. Careful monitoring and surveillance of antibiotic use and bacterial susceptibility, the detection of carbapenem-resistant strains, and the implementation of strict infection control measures become critical for limiting the spread of the underlying resistance mechanisms.

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# Engaging patients as observers in monitoring hand hygiene compliance in ambulatory care

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## ABSTRACT

**Background:** Hand hygiene in ambulatory care is important in minimizing the risk of transmission of disease; however, it is not well studied. Ambulatory care presents a unique challenge in auditing all four moments of hand hygiene due to many reasons, one of them being respecting patient confidentiality.

**Methods:** A quality improvement (QI) lens was used to assess the feasibility of engaging patients as observers to investigate hand hygiene compliance rates in an ambulatory care setting, and whether all four moments of hand hygiene were being met. This QI study was conducted over a three-year time frame and consisted of 1691 hand hygiene opportunities observed.

**Results:** The QI study demonstrated an overall hand hygiene compliance rate of 94-97% for all four moments of hand hygiene, maintaining the target of an overall compliance at 95%.

**Conclusions:** The results of this study suggest that involving the patient as the observer is a feasible and beneficial way to monitor hand hygiene compliance in an ambulatory care setting.

## KEY WORDS

Hand hygiene, ambulatory care, outpatients, patient as observer, patient as auditor

## INTRODUCTION

Hand hygiene (HH) in ambulatory care is important in minimizing the risk of transmission of disease; however, it is not well studied. The rate with which healthcare workers (HCWs) comply with best practice recommendations for HH compliance has been reported as approximately 40% and 5-10% of patients admitted into hospitals acquire at least one healthcare associated infection (HAI) (1). Effective HH has been shown to decrease rates of nosocomial infection and decrease the transient flora located on the skin (2). To maintain effective HH, research has shown that monitoring HH compliance and providing HCWs with feedback is considered an integral part of a successful HH program (3). The Ministry of Health and Long-Term Care (MOHLTC) in 2008 launched a multi-faceted evidence-based initiative, *Just Clean Your Hands* (JCYH), for the four moments for HH in hospitals across the

province of Ontario. This initiative uses an audit tool and trained auditors to observe the four moments of HH: Moment 1 (before initial patient/patient environment contact); Moment 2 (before aseptic procedures); Moment 3 (after body fluid exposure risk); and Moment 4 (after patient/patient environment contact) (4). This program was developed to help reduce HAIs through limiting the spread of infection by providing education and tools for promotion, evaluation and auditing for health care providers.

There is no shortage of widespread initiatives to address low HCWs' compliance including best practice guidelines, education campaigns and guidance on auditing compliance (5). Survey results conducted by the National Patient Safety Agency (NPSA) concluded that HAIs could be reduced if patients asked their health care providers if they had cleaned their hands before touching them (6). Inviting patients to remind HCWs

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**Conflicts of Interest:** None to declare.

**FIGURE 1: Patient survey tool used by patient observers to audit hand hygiene compliance**

## Clean Hands *Matter*

Your health care provider should clean their hands at the following times with either:

- soap and water, or
  - alcohol hand rub.
1. Before any contact with you
  2. Before doing a procedure for you  
e.g., giving a needle; drawing blood
  3. After doing a procedure (after removing gloves worn for the procedure)  
e.g., removing a baby diaper; doing a rectal exam or vaginal exam
  4. When leaving the room after contact with you

Please help us to help you.

How did your health care provider do?

After your visit, please complete this form and leave it in the box in the waiting room. [Although you may interact with more than one health care provider today, could you please choose only one for the purposes of this survey.](#) Thanks.

[fold here so provider does not see response]

My health care provider cleaned their hands:

- |                                 |                              |                             |  |
|---------------------------------|------------------------------|-----------------------------|--|
| 1. Just before contact with me: | <input type="checkbox"/> yes | <input type="checkbox"/> no |  |
| 2. Before a procedure:          | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> n/a - no procedure this visit |
| 3. After a procedure:           | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> n/a - no procedure this visit |
| 4. After contact with me:       | <input type="checkbox"/> yes | <input type="checkbox"/> no | <input type="checkbox"/> unable to observe             |

My health care provider was (**select one only please**):

- Staff physician
- Resident physician
- Nurse
- Other health professional

about HH through the provision of individual alcohol-based hand-rub containers and actively supporting an “It’s OK to ask” attitude were perceived as the most useful interventions by both patients and HCWs (6). Improving HH among HCWs is a simple and effective measure to reduce the burden of HAIs, but commitment and action at a national level is essential to ensure sustained improvement at the point of care (7). More efforts are also required to widen the scope to all facilities, including those primarily providing primary and ambulatory care (7).

To that end, there are overt and covert methods of auditing. Three main overt methods have been studied: 1. direct observation; 2. measuring product use; and 3. use of self-report or peer evaluation. The gold standard for HH compliance is direct observation, but there are associated costs, including training dedicated staff, time to perform observations and few audits performed at night or on weekends (8). A major drawback of direct observation is that it captures a tiny fraction of overall HH opportunities (0.1-0.2%). Also, this method is most human-resource-intensive of collecting HH data. Another drawback includes the Hawthorne effect – a change in a

person’s behaviour when they are being observed. However, routine auditing by direct observation has been shown to have the lowest cost compared to covert technological auditing and is the most feasible to perform (8).

Effective monitoring of HH compliance is challenging in an ambulatory care setting as direct observation is difficult due to availability of trained auditors, physical layout restrictions, and respecting patient confidentiality. Thus, several studies have started to explore the use of patients as observers in these types of settings (9, 10, 11). Le-Abuyen et al. (2014) found that the patient-as-observer approach appeared to be a viable alternative for hand hygiene auditing in an ambulatory care setting because it educated, engaged, and empowered patients to play a more active role in their own health care. Bittle and LaMarche (2009) found that it was feasible to use patients as observers in an ambulatory care setting and patients were willing to give healthcare providers feedback. Their study concluded that making patients active participants in their care process increased healthcare provider accountability (10). This also strengthens the patient-healthcare provider bond and improves



**TABLE 1: Patients as observer data in the Family Practice Unit from 2013-16**

	2013 (June) N=200 n (%)	2014 (April) N=215 n (%)	2015 (August) N=242 n (%)	2016 (August) N=137 n (%)
Surveys completed	198 (99)	215 (100)	242 (100)	137 (100)
Surveys completed correctly	188 (94.9)	206 (95.8)	237 (97.9)	109 (79.6)
Overall HH compliance	97%	95%	95%	94%

communication, which through joint efforts, will ultimately lead to minimizing the spread of potentially harmful organisms (2).

The purpose of this QI study at Sunnybrook Health Sciences Centre (Sunnybrook) Family Practice was twofold: 1. To investigate whether it was feasible to use patients as observers who could observe all four moments of HH as observation of moments 2 and 3 by auditors can be challenging and 2. To determine if target HH compliance rates of 95% were being achieved in the Family Practice Unit using said patients as observers.

## METHODS

According to the policy activities that constitute research at Sunnybrook, this work met criteria for operational improvement activities exempt from ethics review. The work reported here meets this criterion because hand hygiene is a universally recommended practice. Compliance with HH was evaluated using auditing and feedback to hospital staff. Patients were also informed that participation in this initiative was voluntary and that their care would not be compromised regardless of their response.

The Academic Family Health Team is located at Sunnybrook Health Sciences Centre in Toronto, Ontario. This unit provides comprehensive primary care services to patients and families including primary prevention, low-risk maternity care, chronic disease management and complex care of the elderly. The Family Health Team includes family physicians, resident physicians, medical students, nurses, pharmacists, social workers, a dietitian, an occupational therapist, diabetes educators, administrative staff and volunteers.

This QI study was conducted from 2013 to 2016 and involved four implementation cycles. Each implementation cycle occurred for a two and half week period. A convenience sampling strategy was used in order to obtain representative data by engaging a sample of participants presenting for regularly scheduled appointments. During the implementation cycles, all capable patients presenting to the Family Practice reception on arrival, were asked if they would be willing to participate in observing and recording their health care providers' HH compliance. The clinic office coordinators were responsible for recruiting patients and training patients agreeing to participate. Patients agreeing to participate were given a short one-page survey audit tool (see Figure 1) on which to record their observations. Verbal instructions and instructions found on the survey audit tool included asking patients to: 1) Observe the hand hygiene process for all four moments (before any contact with the patient,

before a procedure, after a procedure, and after any contact with the patient) and examples of procedures relevant to the family practice unit were provided; 2) Fill out the survey anonymously; and 3) Drop it off in a box located in the waiting room.

The contents of the drop boxes were collected at the end of each implementation period. The responses were entered into a spreadsheet for analysis and reporting. Reports were generated to illustrate overall moment-specific HH compliance and profession-specific HH compliance. The rate of compliance was calculated as the number of HH events (hand hygiene protocol was performed) during the implementation period divided by HH opportunities (the total observations during that same timeframe). A chi-square analysis was also completed to test if the proportions differed across years.

## RESULTS

Table 1 shows the total number of surveys distributed and completed by patient observers and the HH compliance for each implementation cycle (2013-2016). Almost all surveys were completed by patient observers in each implementation cycle (range 99-100%). The range for surveys being completed correctly by the patient observers were 79.6% (2016) to 97.9% (2015). Hand hygiene compliance rates for the Family Health Team since the start of the patients as observer program ranged from 94% in 2016 to 97% in 2013, exceeding the overall hospital target of 87%. When HH compliance was observed by profession, allied health professionals (outside of medicine and nursing) had the highest compliance. The chi-square analysis to test if the proportions differed across years was not statistically significant ( $p = 0.34$ ).

## DISCUSSION

The results of this QI study in the Family Health Team suggest that involving the patient as observer is a feasible way to monitor HH compliance in an ambulatory care setting. The patient observers in this study were able to successfully audit all four moments of HH in the clinic with most of the audit forms being correctly filled out. Hand hygiene compliance rates observed by the patient observers in the clinic are comparable to those observed by trained auditors in the same clinic for moments 1 and 4 (data not shown). This suggests that using patients as observers for HH compliance is feasible and may help to reduce costs, increase providers' accountability, engage patients in their healthcare and may

potentially improve communication between patient and provider. However, the rates are likely to be equally affected by observation bias and Hawthorne effect, inflating the compliance rates. Relatively small number of hand hygiene opportunities (HHOs) observed should also be recognized as a limitation of this study, as capturing ~1,600 HHOs over the course of three years just confirms that direct observation is capable of delivering only a very small fraction of the overall data. It should be noted the study team did not sustain recruitment and training of patients as observers post study. It is possible that during each data collection phase, patients previously could have been selected again however, we cannot confirm this. This QI study focused on feasibility of engaging patients as observers.

The results of this QI study are comparable to those of similar nature performed in the ambulatory care settings. For example, Le-Abuyen et al. (2014) found that the overall HH compliance before direct contact with the patient was 96.8% as reported by patient observers in an academic ambulatory care hospital in Ontario, Canada. Bittle and LaMarche (2009) found that the overall HH compliance as measured by the patient-as-observer process averaged 88% (range, 74%–100%) in an ambulatory care facility located in Baltimore, United States. The results of the study also provide insights to profession-specific HH compliance rates, something that has not been extensively reported on in the literature.

The results of this study must be considered in light of study limitations. Convenience sampling was selected due to feasibility, budgetary constraints and the availability and the quickness with which data could be gathered by the participants; although effort was taken to ensure a representative sample of patient observers was obtained. Other limitations to our approach include the reliance on volunteer resources, particularly the clinic coordinator to consent patients and provide instructions to those who agree to participate. This may prove to be a limitation when workload for this role increases substantially or during times when the clinic may be understaffed. Lastly, the study team is aware of the bias the Hawthorne effect creates and recognize this bias might have affected the compliance rates; however, the end goal was patient and provider safety and thus the team is willing to use implicit bias to improve the quality of care delivered.

The Family Health Team unit continues to collect data using patients as observers with consistently high HH compliance rates. This initiative emphasized the importance of patient communication as a novel way to evaluate programs that have direct patient care implications. Education sessions were conducted by the executive director with clinic coordinators and coordinated delivery of results to Infection, Prevention and Control (I, P&C). Limited financial resources were used to maintain this project and thus speak to its sustainability. The success of this initiative has encouraged expansion to other ambulatory areas of the hospital, including the cancer centre, and diagnostic imaging area. Expansion into other

programs is under consideration. Future directions for this study include a validation study of both trained observers and patient observers as well as a qualitative analysis of the impact of this initiative on patient engagement.

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# Recommendations for conjunctivitis prevention in ophthalmology/optometry clinical office practice

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Conjunctivitis is caused by a variety of bacteria and viruses, but adenovirus is a primary cause of outbreaks in healthcare settings, particularly in eye clinics/offices. Both patients and health care workers may acquire and transmit adenovirus during these outbreaks (5). Since adenovirus is shed before onset of symptoms, consistent application of infection prevention and control measures is necessary to protect patients and staff from infection.

## HAND HYGIENE

Perform hand hygiene according to the *4 Moments for Hand Hygiene* in Ontario's *Just Clean Your Hands* program:

1. Before contact with the patient or items in the patient's care environment.
  2. Before any clean/aseptic procedure.
  3. After any exposure risk to body fluids, including tears (even if gloves worn).
  4. On leaving the patient or patient's care environment.
- Alcohol-based hand rub or a handwashing sink with soap and water must be provided at the point-of-care, i.e., within arm's-length of the patient.

## PERSONAL PROTECTIVE EQUIPMENT

Gloves should be worn when examining a patient with conjunctivitis (4). Clean hands before putting on and immediately after taking off gloves.

## ENVIRONMENT AND EQUIPMENT CLEANING

Equipment/devices that come in contact with non-intact skin or mucous membranes, e.g., conjunctiva, are classified as semi-critical and require high-level disinfection as a minimum standard. Reusable tonometers and other ophthalmologic equipment (e.g., intra-ocular ultrasound probes, fundus contact lenses, gonioscopy lenses, rigid contact lenses) that touch the eye must undergo cleaning followed by high-level disinfection (e.g., hydrogen peroxide formulations) between patient use. Cleaning with alcohol is not sufficient (1,2,3). Semi-critical medical equipment/devices designated as single-use by the manufacturer must not be re-used on another patient (1).

For tonometry:

- Clean reusable components of the tonometer according to manufacturer's instructions following use with each patient.
- Use only tips and covers that are approved for use by the tonometer manufacturer.
- Where possible, use disposable/single-patient use devices (e.g., tonometer tips/tip covers).
- If disposable tips/tip covers are used, remove and discard tips/tip covers after use on a patient. A new tip/tip cover must be used for each patient.
- If reusable tips/tip covers are used, they must be high-level disinfected between each patient.
- When hand-held tonometers are used with tip covers, the tip does not require high-level disinfection between uses. Follow manufacturer's instructions for cleaning the tip. This is an exception to the usual practice of high-level disinfecting semi-critical devices following use of a sheath or cover (3).

Facilities must have a dedicated area for reprocessing these devices, trained staff, and sufficient supply of reusable and single-use instruments and devices to support these recommendations.

Adenovirus may survive on surfaces for prolonged periods (5). Items that may have been touched by the patient in the waiting room and examination room, e.g., arm rests on chairs, should be cleaned and low-level disinfected with a hospital grade disinfectant that has a virucidal claim. (These are available as convenient to use disinfectant wipes.)

## WORK RESTRICTIONS

Healthcare workers with adenovirus conjunctivitis must not provide patient care from the day of onset of conjunctivitis for a period of 14 days. If the second eye becomes infected, the period is extended to 14 days after onset in the second eye (4).

Healthcare workers with bacterial conjunctivitis should be restricted from patient care for the duration of symptoms and instructed on proper hand hygiene (4).

## RESOURCES

The following resources from the Provincial Infectious Diseases Advisory Committee's (PIDAC) *Best Practices for Infection*

*Prevention & Control in Clinical Office Practice* may be useful to you in evaluating your current practices related to infection prevention and control and/or medical device reprocessing:

- Appendix J: *Checklist for Office Infection Prevention and Control*
- Appendix M: *Checklist for Reprocessing*

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## OUTBREAK INVESTIGATION

# Investigation of salmonellosis outbreak following a hospital endoscopy: A public health case study

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## ABSTRACT

**Background:** The results of a public health investigation are presented. During 2014, a local public health unit in Ontario became aware that three cases of *S. enteritidis* may be related. One common factor was identified: all three had received endoscopy at the local community hospital prior to their diagnosis.

**Methods:** Infection prevention and control assessment of the procedures used in the operation of the endoscopy suite as well as reprocessing methods used for the equipment was completed. In addition, microbiological testing of the endoscope and epidemiological investigation techniques were used to try to confirm the hypothesis that the procedure was the most likely source of transmission for the three patients.

**Results:** No significant infection prevention and control lapses were identified at the endoscopy suite. Reprocessing methods and verification, including documentation, were found to be adequate. However, the epidemiological investigation implicated the endoscope as being the likely source of transmission of *S. enteritidis* for the three patients.

**Conclusions:** The question is proposed for future examination in the IPAC field: are current reprocessing guidelines for endoscopy equipment adequate to protect patients from exogenous infection? And, for public health investigations: should recent endoscopic procedures be included as a potential acquisition exposure question when interviewing lab-confirmed cases of salmonella?

## KEY WORDS

Salmonella, endoscopy

## BACKGROUND

Salmonellosis is caused by the bacterium salmonella, a Gram-negative non-spore forming bacillus that has more than 2,000 serotypes. This infection occurs worldwide and salmonellosis is the second most common enteric infection in Ontario, with an average of almost 2,500 cases occurring per year. *S. Typhimurium* and *S. Enteritidis* are the leading causes of salmonellosis in Ontario (1). The health unit jurisdiction where the investigation occurred averages 18 cases of Salmonella per year (2).

The majority of infections with salmonella are associated with the ingestion of contaminated food or contact with infected animals (3). Investigations of sporadic cases and outbreaks of salmonellosis focus on the ingestion of the organism in food, travel history or contact with animals. Once acquired, the established incubation period for salmonella ranges from 6 to 72 hours, although longer incubation periods have been reported. The period of communicability lasts throughout the course of infection, varying from several days to several weeks (4).

Infection prevention and control issues related to endoscopy procedure are well documented in the literature. Since the introduction of standardized guidelines for reprocessing of endoscopes in the early 1990s, the incidence of associated infections have dropped dramatically and disease transmission has been mainly associated with lapses in IPAC practices or

non-endoscopic procedure issues such as the contamination/improper use or care of intravenous lines and administration of anesthesia or other medications (5).

This paper describes a public health investigation of a small cluster of salmonella cases which appear to have been associated with endoscopy procedures at one hospital.

## METHODS

### Initial Investigation

The Infectious Disease Prevention and Control (IDPC) team at an Ontario public health unit received a telephone call late in the day on Thursday April 17, 2014 from Case A who had been diagnosed with salmonellosis on April 11, 2014 and who was concerned that, while hospitalized at Hospital A with symptoms of salmonellosis from April 7 to April 16 2014, she had met another patient, Case B, who was also diagnosed with salmonella on April 11 and hospitalized with symptoms from April 9 to April 25. Case A and Case B shared a hospital room at Hospital A (a community hospital with 102 beds) while they were both hospitalized with symptoms of salmonellosis. During a conversation, they discovered that they had both received endoscope procedures at the outpatient department of Hospital A in the same week, approximately three weeks prior to their in-patient stay. The complaint was reviewed and it was decided that further investigation was warranted.

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**Conflict of Interest/Disclosure:** None to declare

On Tuesday April 22, 2014 existing public health salmonella report investigation records of Case A and Case B were reviewed. It was noted that both patients had lab confirmation of *S. enteritidis*. A line list was reviewed of all *S. enteritidis* received by the health unit in 2014. Seven cases were identified. Three cases had a travel history during their incubation periods, and were, therefore, removed from the investigation. This left four remaining cases with no travel history, including Case A and Case B. Case C had a note written in the paper file that she had undergone an endoscope procedure at Hospital A prior to onset of symptoms. Infection Control at the hospital was notified. The infection control practitioner (ICP) checked hospital records and confirmed that three of the four cases of *S. enteritidis* identified with no travel history had colonoscopy procedures at the hospital and provided the dates of their procedures.

All four cases were re-interviewed by the IDPC team to confirm that they had no travel history, the onset date of their illness, and whether or not they had a recent colonoscopy procedure. Onset dates were confirmed for Case B and Case C. Case A remained unclear of an onset date, as there was a long history of diarrhea and gastro-intestinal upset, although did state that symptoms seemed to worsen about a week prior to hospitalization. Although these cases had been previously interviewed about possible sources of infection, this part of the interview was repeated for validation purposes. Public health inquires routinely about food, sources of water, animal contact and attendance at special events up to three days prior to the onset of salmonellosis symptoms. Three cases were confirmed as having a recent colonoscopy procedure and no travel history. The fourth case had no travel history and no recent colonoscopy procedure.

#### *Epidemiological analysis of existing case information*

Through the re-interviewing of Cases A, B and C it was established that no common food or other exposure could be identified in the usual incubation period for salmonella. While the usual incubation period of 6 to 72 hours did not support a point-source common cause for these three cases, re-interview exposure questions extended beyond 72 hours to include the previous month because salmonella has been known to have longer incubation periods. It is also possible that the same source of contamination was ingested by the cases on different days, such as when a contaminated food like poultry or produce is widely distributed in a community. Only one significant commonality was found among Cases A and C – Case C attended an event at a restaurant bar on March 28 and Case A worked at this same restaurant bar with the last

day worked being March 23. Case C denied eating food at the restaurant but consumed beverages. Case B was asked directly about this restaurant but denied ever going there.

Dates of colonoscopy were confirmed with the cases and matched the information provided by the hospital. This information in Table 1. The date of hospitalization minus seven days was used as the onset date for Case A, who could not clearly define the date of symptom onset.

It was hypothesized that these three cases may have had the same source of infection based on their common exposure of a colonoscopy at the hospital. However, the procedure date did not fit into the established incubation period for salmonella (6 to 72 hours, usually about 12 to 36 hours). Instead the incubation period, using the theory that the colonoscopy was the source, ranged from 4 to 8 days.

Public Health Ontario (PHO), the provincial agency responsible for providing scientific and technical advice and support to public health and health care including laboratory services, was contacted to inquire about phage typing results of the three isolates. One phage type was available (PT 8), while the other two were pending.

#### *Feasibility and risk*

On Wednesday April 23, the IDPC team requested that the PHO library services conduct a literature search on the transmission of salmonella during colonoscopy. The search was limited to articles published after 1980.

Reports of transmission of salmonella species' during colonoscopy have not been reported since endoscope reprocessing guidelines from the Association for Professionals in Infection Control and Epidemiology (APIC) were put into place in 1989 (6). Prior to 1989, Dwyer et al. (7) reported an outbreak of *S. Newport* transmitted by fiberoptic colonoscopy. This was the first outbreak reported where transmission followed a route that was not fecal-oral, but rather was hypothesized to have been transmitted via a contaminated colonoscope directly to a patient's lower gastrointestinal tract. Moreover, incubation periods in the Dwyer et al. outbreak were found to be longer than expected for two of the cases. One developed acute gastroenteritis on the seventh day after the procedure and one on the ninth day after the procedure. This suggested two new pieces of information about salmonella:

1) transmission of salmonella during colonoscopy directly to the lower gastrointestinal tract is possible and 2) this mode of transmission may result in a longer incubation period than the usual ingestion route.

**TABLE 1: Summary of dates used for establishing acquisition risk timelines**

	Onset Date	Lab Test Date	Usual Incubation Period to Identify Acquisition Source	Date of Colonoscopy	Attendance at Restaurant/Bar
<b>Case A</b>	April 1	April 7	March 29 to April 1	March 24 2014	March 23
<b>Case B</b>	April 6 2014	April 10	April 3 to April 6	March 31 2014	Did not attend
<b>Case C</b>	March 29 2014	March 31	March 26 to March 29	March 25 2014	March 28

### *Hospital investigation*

On Thursday April 24, the public health unit shared its findings with the hospital. Automated machines for disinfection are used after the scopes are manually brushed, cleaned and tested for leaks. While the “dirty” and “clean” areas are in the same room, a barrier exists between them (a counter) and policies and procedures are in place to attempt to keep the areas separate. It was noted that there is limited storage for clean endoscopes (they were short one hook for hanging), therefore, one randomly chosen, processed endoscope is always stored in the automated machine (with the lid open).

To identify any other potential sources of enteric bacteria to patients other than the endoscope itself, a discussion was held regarding process flow of patients undergoing an endoscope procedure and all actions and equipment that occur before, during and after a procedure. Patients receive the same instructions for preparation; however, specific preparation products varied and were obtained through different community pharmacies, ruling out preparation products as a source of infection. No other items or procedures were identified at that time as a potential source of enteric bacteria.

The hospital performed 15 to 20 colonoscopies per weekday and had 10 endoscopes used for colonoscopies. Each scope is stored with a printout from the disinfectant machine confirming the process was completed and when the scope is used. A copy of the printout is subsequently attached to the appropriate patient’s chart.

The hospital agreed to review patient records of Cases A, B and C to identify if any commonalities with staff or equipment were present and to confirm the quality control verification check of the scope cleaning.

At 5:45 p.m. that same day, the health unit received an email from the hospital that their record review revealed the same endoscope was used on Case A, Case B and Case C. No other commonality was found between cases, including no common staff among all three cases. The public health unit and the hospital agreed to remove this endoscope, identified as endoscope #17, from use until an investigation was complete.

### *Public health investigation*

At the request of the public health, the hospital generated a list of patients who received a colonoscopy at the hospital within a defined time period. The suspect case definition used to generate this list included patients who had a colonoscopy procedure at the hospital with endoscope # 17 between March 17 and April 3, 2014. The time period used in this case definition was informed by the longer than expected incubation period reported by Dwyer et al (1987) (8) for transmission through colonoscopy. This produced a list of 24 people, not including the three people identified as cases.

Results of the health unit and hospital investigation were shared with PHO. PHO provided a procedure from the Public Health Agency of Canada (PHAC) (9) for sampling an endoscope to test for bacterial contamination. Several limitations were identified in the methodology; however, public health and the hospital agreed to proceed with this testing.

On Tuesday April 29, confirmation was received from PHO lab that all three cases were of the same genotype (phage type 8) and that the PFGE patterns were identical. Based on this information, the public health unit moved forward with active case finding and officially declared a suspect outbreak. Initial calls were to be made to patients who were scoped the same day and on all the days in between the cases with endoscope #17. If interviews yielded suspect cases, then the calling would widen to include the original range of dates found within the case definition. The rationale for this was to lessen the number of patients who may become worried or anxious until additional information was discovered which would necessitate contacting all patients exposed during the period of concern. An initial list of 14 patients was identified to be contacted.

### **RESULTS**

The endoscope was sampled on May 1. Public health and the hospital performed the sampling. Sample results were received on May 7. No salmonella species was detected.

The patients were contacted and asked if they experienced symptoms of gastrointestinal upset before or after their procedure. It was anticipated that some patients would have been experiencing gastrointestinal symptoms before the procedure and these symptoms were the reason for the procedure. Since patients were required to do a pre-procedure colonic cleansing, the questions were also carefully worded to ask about any changes in the type or intensity of symptoms after the procedure. Patients were also asked if they would be willing to submit a stool sample for salmonella testing.

Of the 14 patients contacted, eight submitted a stool sample. All submitted samples were negative for salmonella.

None of the patients experienced fever after the procedure, which is a common symptom of salmonella infection. Of those that experienced abdominal pain and diarrhea, which are other common symptoms of salmonella infection, it was neither severe nor different from what they were experiencing before the procedure.

Based on these results, no further patients were contacted.

### **DISCUSSION**

This outbreak supports the suggestions of Dwyer et al that transmission of salmonella can occur during colonoscopy with contamination occurring directly to the lower gastrointestinal tract and that when this occurs, the incubation period may be longer than with the usual ingestion route.

No further evidence of symptomatic or non-symptomatic salmonella infection was found among colonoscopy patients from the hospital who had a procedure performed with endoscope #17 during the time frame of the affected individuals. In addition, eight patients were lab tested for salmonella, all with negative results. While lab testing of patients was performed some weeks after they would have been expected to have salmonella if infected, carriers can shed the bacteria for years and 5 % of patients recovering from non-typhoidal salmonellosis can shed the bacteria for 20 weeks (10).

While culture results from endoscope #17 were negative for salmonella, the scope would have been cleaned and disinfected many times between the time period of suspect transmission and sampling.

The following recommendations were made to the hospital regarding infection prevention and control:

1. If a renovation opportunity arises, have separate rooms for clean and dirty endoscopes.
2. Purchase an additional hanger so that one endoscope does not need to be stored inside the automated disinfection machine.
3. Do not put endoscope #17 back into service until the supplier/manufacturer is notified of the occurrence of three identical salmonella infections in patients that occurred after receiving a colonoscopy with endoscope #17. The hospital is to follow the direction of the supplier/manufacturer.
4. Infection control staff should consider complete infection prevention and control audit of the endoscopy area as soon as possible.

## CONCLUSION

While a definitive explanation was not found, it is clear that the occurrence of three cases of identical isolates of *Salmonella* enteritidis in three patients who all had a colonoscopy using the same endoscope within a period of eight days in the same hospital did not occur by chance. While Public Health Ontario laboratory data as of April 23, 2014 confirmed that PT8 was the most common PT seen in the year-to-date *Salmonella* enteritidis isolates, consisting of 32% of the 356 cases with PT results known, the availability of PFGE results which showed the isolates to be indistinguishable supports the conclusion that the similarity did not occur by chance alone.

Public health authorities investigating salmonella infections and infection prevention and control practitioners need to be vigilant for occurrences of salmonella infections following colonoscopy procedures so that the cause of these rare transmissions can be discovered and endoscope design and/or processing practices can be adjusted to prevent transmission of salmonella in the endoscope suite.

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## OUTBREAK INVESTIGATIONS

# *Serratia marcescens* outbreak causing septicemia in neonatal intensive care unit: Substantiation of single source

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## ABSTRACT

We investigated an outbreak of *Serratia marcescens* (*S. marcescens*) in NICU of our hospital and are reporting the characteristics of this outbreak along with interventions leading to its resolution.

In the month of September and November 2016, seven neonates were identified with blood cultures positive *S. marcescens* septicemia. To identify the source of the isolate surface swabs were taken from different environmental sources. All *S. marcescens* isolates were identified by Vitek automated identification system, API 20 E and their antibiogram pattern and further genotyping was done by pulse field gel electrophoresis.

During surveillance, 25 blood cultures of newborns were analyzed, 32 environmental samples along with hand swabs of 10 healthcare workers (HCWs) were taken. Seven neonates had blood culture positive *S. marcescens* sepsis. Only one environmental source (water flasks) yielded *S. marcescens* with similar antibiogram suggesting the same strain which was further confirmed by pulse field gel electrophoresis.

Timely delivery of culture and sensitivity results, good liaison and effective communication between neonatologist and microbiologist, targeted antimicrobial therapy helped in saving the life of six neonates suffering from *S. marcescens* septicemia.

## KEY WORDS

*S. marcescens*, neonatal septicemia, neonatal intensive care unit (NICU)

## INTRODUCTION

Low birth weight and pre-term neonates are at high risk for contracting healthcare associated infection (HAIs). Recent advances in NICU have allowed provision of care with increasingly higher acuity to preemies with lower gestational age. Despite these advances there is increased incidence of HAIs among neonates (1). These HAIs can be of different types but the most life threatening is late onset neonatal sepsis (LOS). LOS is difficult to diagnose clinically because of nonspecific signs and symptoms. *Serratia marcescens* has emerged as an important nosocomial pathogen in LOS (2). It is a ubiquitous pathogen that tends to colonize neonatal skin and alimentary tract and spreads via environmental dissemination and hands of healthcare workers (HCW) (3). We investigated an outbreak of *S. marcescens* in NICU of our hospital and report the characteristics of this outbreak along with interventions that led to its cessation.

## METHODOLOGY

In September 2016, within the span of a few days, two phenotypically similar. *S. marcescens* isolates were identified from blood cultures of neonates admitted in our NICU. This clustering warranted the need to investigate the occurrence of an outbreak in NICU. Diagnosis of LOS was made on the basis of clinical features, raised C-reactive protein (CRP), low platelet counts, and positive blood culture. All the isolates had a similar antibiogram.

### Patients

Before the start of surveillance in the affected unit, *S. marcescens* was isolated from the blood cultures of three neonates. The index case was identified as a baby girl referred from another hospital. She was delivered weighing 1560 gm. at 30 weeks of gestation by a lower segment cesarean section (LSCS) due to severe pregnancy induced hypertension with

**Acknowledgement:** We acknowledge the valuable input given by Omair Sattar and Dr. Ali Yasir during this study.

raised Doppler indices. She presented with respiratory distress soon after birth and was placed on nasal continuous pressure airway pressure (nCPAP) and transferred to our setup. A diagnosis of grade 2 respiratory distress syndrome (RDS) along with presumed sepsis was considered. First line antibiotics (Ampicillin and Amikacin) were started and a septic screen was done at 12 hours of age. Supportive care with attention to fluids, electrolytes and temperature management was ensured. Initial CRP was 27g/dl. On day 4 after birth, 24 Sep 2016, the baby expired. Her blood culture yielded growth of *S. marcescens* one day before the death of baby.

During the next 10 days, blood culture from two other neonates had growth of *S. marcescens*. One of them was a late preterm born at 36 weeks of gestation and admitted for establishing feeds. He had episodes of frequent desaturations on day 5 of life. A presumed diagnosis of late onset sepsis was made and later cultures yielded growth of *S. marcescens* in his blood. The other one was admitted to NICU with the history of

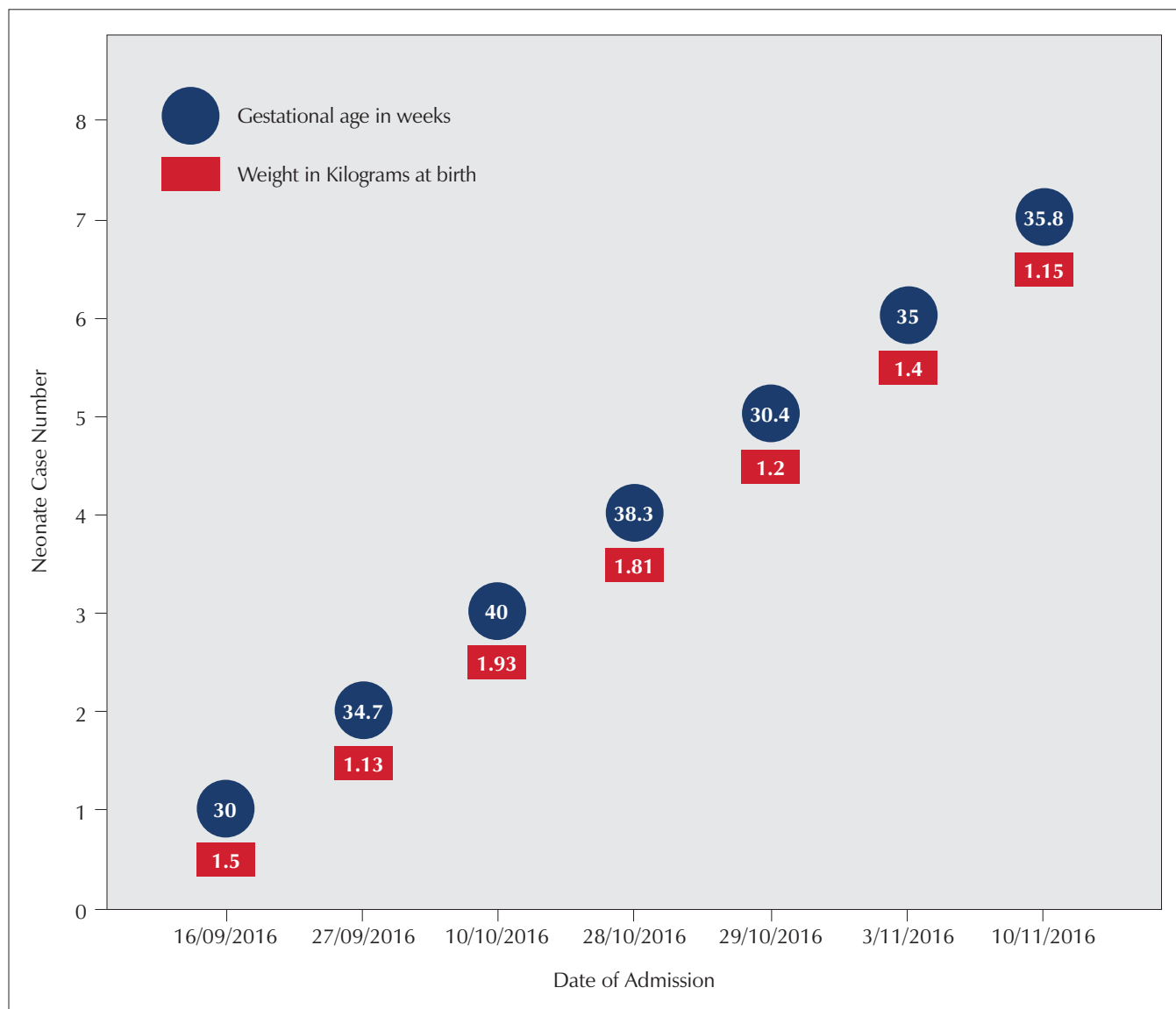
prolonged neonatal jaundice and was diagnosed with neonatal hemochromatosis. He started having feed intolerance at day 24 of life and was suspected of having late onset sepsis. A septic screen was done along with a blood culture which yielded growth of *S. marcescens*. All isolates had a similar antibiogram giving an indication of an outbreak in NICU.

In subsequent weeks, four more neonates, one with neonatal jaundice and three with prematurity were admitted for establishment of feed also developed late onset sepsis secondary to the same *S. marcescens*.

All culture positive neonates were treated with Meropenem and Amikacin as per the sensitivity. They responded well to treatment, evidenced by a falling CRP and normalization of sepsis markers.

*Bacterial identification*

All blood samples were received in automated BACTEC™ bottles. Once flagged positive by BACTEC™ system, Gram staining was carried out. Initial results were communicated to the attending



neonatologist for specific Gram negative cover. At the same time samples were sub cultured on blood and MacConkey agar and plates were incubated at  $35 \pm 2^\circ \text{C}$  for 18 hours. Next day isolate was identified by colony morphology, Gram staining and basic biochemical tests. All suspected isolates were confirmed by API 20 E (bio Mérieux, Marcy L'Etoile, France) and VITEK 2 (bio Mérieux, Marcy L'Etoile, France) Gram negative panel.

#### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by disk diffusion method and results were interpreted as per Clinical & Laboratory Standards Institute (CLSI) recommendations. Further test for minimum inhibitory concentrations (MICs) were carried out by VITEK 2 using N2O2 card.

#### Surveillance and environmental investigation

To identify the source of this outbreak, 32 environmental samples were taken from NICU including incubators, cradle, feeding trolley, suction fluid, laryngoscope, stethoscope, feeding cup, water flask, antiseptic solution, suction tube, door knobs, nursing counter. Hand swabs from 10 HCWs, including one neonatologist, one resident, one house officer, four nursing staff, two sanitary workers and one food handler were taken. The swabs were inoculated on blood agar, MacConkey agar and incubated at  $35 \pm 2^\circ \text{C}$  for 18-48 hours and all isolates were identified as per standard protocol. An isolate identified as *S. marcescens* was confirmed by API 20 E and further confirmed by VITEK 2 Gram negative panel.

#### Genotyping

PFGE was used to create a DNA fingerprint of *S. marcescens* isolates and confirm the source of the outbreak.

## RESULTS

An outbreak of *S. marcescens* was identified from 16 Sep to 29 Nov 2016 in a 20 cots tertiary care NICU. Before the start of surveillance in the affected unit, *S. marcescens* was isolated from the blood cultures of three neonates with clinical suspicion of septicemia. An infection control meeting was held between neonatologist, nursery staff and microbiologist to establish infection control measures. Environmental samples including surface swabs and hand swabs of HCW were negative for *S. marcescens*. However, samples from one environmental source, water flask yielded growth of multiple organisms including *S. aureus*, *K. pneumonia* and *S. marcescens*. *Serratia* isolates from blood cultures of seven neonates and the one isolated from water flask had similar biochemical profile and antibiogram confirming the possible source.

#### Clinical details of neonates

Neonatal septicemia caused by *S. marcescens* was diagnosed in seven neonates out of 25 who were admitted in NICU during this time period.

#### Microbiological results

*S. marcescens* was isolated from blood samples of seven neonates and one environmental source, a water flask.

Antimicrobial susceptibility pattern was substantially observed and maintained. All isolates were sensitive to amikacin, imipenem, meropenem, doxycycline and tigecycline and resistant to ampicillin, amoxicillin-clavulanate, cefepime, ceftriaxone, ciprofloxacin, cotrimoxazole, gentamycin, tazobactam-piperacillin.

By PFGE typing, seven isolates from blood samples and the isolated *S. marcescens* from water flask had the same strain (pattern A). This specific clone was responsible for the outbreak from Sept. to Nov., suggesting cross-transmission of particular isolate in the NICU.

## DISCUSSION

*S. marcescens* is an important nosocomial pathogen, responsible for hospital acquired infections in neonates (4). Gastmeier *et al.* reported 33 outbreaks in NICU caused by *Serratia spp* with the mortality rate of 7.7 % (9). Whereas in our case only one neonate, the index case, out of seven positive cases expired. Timely delivery of culture and sensitivity results, good liaison and effective communication between neonatologist and microbiologist were major factors that contributed to the successful management of the disease. In addition to this, cohorting of neonates whose blood culture were positive for *S. marcescens* and introduction of bundled interventions to improve hand hygiene in HCW, disinfection of environmental surfaces by using intermediate level disinfectants like isopropyl alcohol along with good cleaning practices for food utensils items helped in curbing the spread and preventing further transmission of the pathogen. Moreover, pathogen specific antibiotic therapy, intensive efforts by targeted post outbreak surveillance and implementation of infection control measures with special focus on horizontal infection prevention approaches played a major role in cessation of an outbreak.

*S. marcescens* is an environmental microorganism that colonizes neonates through various routes such as skin, respiratory tract, gastrointestinal and genitourinary and can cause life threatening septicemia (5). *S. marcescens* bacteremia in neonatal ICUs is typically associated with an outbreak generally linked to environmental sources (3,4,6,7,8).

This report describes a successfully contained severe neonatal septicemia outbreak caused by *S. marcescens*. Since the source was removed and strict infection control measures were followed, no new cases were reported.

Unlike reports of other outbreaks described elsewhere (3,5) in this outbreak septicemia was likely, secondary to environmental source (water flask) being used in feed preparation for the neonates.

Blood stream infections are not only the most frequent health care-associated infections in NICU outbreaks, they also represent the most frequent endemic infections in neonates (9,10). Extensive use of indwelling catheters and prolonged parenteral nutrition in sick infants are among the major causes for the high prevalence of bloodstream infections. As in present scenario, all the neonates suffering from septicemia had prolonged stay because of co-morbid conditions and acquired the infection during their stay in the hospital.



*S. marcescens* outbreak represents a serious challenge in hospitals especially in NICUs. Therefore extensive surveillance procedures are essential in infection control, whilst implementation of standard measures, such as maintaining hand hygiene, cleaning of hospital environment and multidisciplinary effort plays a crucial role in successfully controlling an outbreak.

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## EMERGING TECHNOLOGIES

# A new method to sterilize multichannel flexible colonoscopes

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## ABSTRACT

**Background:** Flexible gastrointestinal (GI) endoscopes have been associated with patient-to-patient transfer of multidrug-resistant bacteria that are not inactivated by high-level disinfection. This has resulted in calls to reprocess GI endoscopes by sterilization. However, traditional low-temperature sterilization methods are not cleared by the United States FDA to terminally sterilize complex multichannel endoscopes.

**Aim:** Demonstrate that the STERIZONE® VP4 Sterilizer (VP4 Sterilizer) can sterilize a multichannel colonoscope using a new gravity-based inoculation method.

**Methods:** In accordance with US, EU and Canadian requirements, a direct-inoculation method was developed to demonstrate that the VP4 Sterilizer can sterilize a multichannel colonoscope under both half-cycle and simulated-use conditions.

**Findings:** Half-cycle and simulated-use testing demonstrated that the VP4 Sterilizer can sterilize a multichannel colonoscope with a sterility assurance level of SAL-6. Validation of the inoculation method using surrogate lumens, confirmed that the center of each lumen contained  $>10^6$  test organisms. Furthermore, both high and low-level recovery was achieved for each lumen within a multichannel colonoscope.

**Conclusion:** Flexible colonoscopes can be terminally sterilized using the VP4 Sterilizer. It is the first vapor-based sterilization technology that is FDA cleared to sterilize a four-channel flexible colonoscope.

## KEY WORDS

colonoscope, sterilization

## INTRODUCTION

In accordance with the Spaulding Classification scheme, flexible GI endoscopes including colonoscopes and gastroscopes have been traditionally classified as semi-critical devices, meaning that they should be sterilized before use, or if this is not possible, reprocessed using high-level disinfection (HLD) (1). Because flexible endoscopes are temperature sensitive, HLD has been the preferred reprocessing method, reflecting the inadequacy of available low-temperature sterilization technologies. Recently however, both regulatory agencies and the medical community have recognized that GI endoscopes should be reclassified from semi-critical to critical devices, which requires reprocessing by sterilization and not HLD (2).

The desire to sterilize GI endoscopes is in large part caused by recent publicity involving patient-to-patient transfer of multidrug-resistant organisms (MDROs) attributed to endoscopes, particularly duodenoscopes (3). Although some infectious outbreaks have been caused by breaches of reprocessing (4), others have occurred even when endoscopes have been reprocessed according to manufacturer's instructions-for-use (IFU) (3). In particular, Ofstead *et al*, found that viable microbes were identified on GI endoscopes reprocessed using cleaning and disinfection methods provided by the device manufacturer (5).

To address this problem, some device manufacturers have begun to validate the use of ethylene oxide (EtO) as a method for sterilizing GI scopes. However, EtO requires lengthy aeration times and is associated with occupational health and environmental risks. Also, EtO sterilizers are limited in the US to sterilization of devices with a maximum of two lumens (6), which by definition excludes modern GI endoscopes. Furthermore, in studies published by Alfa *et al* involving inoculation and sterilization of flexible surrogate lumens, data shows that EtO efficacy is compromised when inoculum is mixed with inorganic contaminants (7), which are intended to reflect "simulated-use" conditions commonly found in a clinical setting.

Liquid chemical sterilization using peracetic acid is indicated for reprocessing reusable critical and semi-critical heat-sensitive medical devices including flexible endoscopes (8). As reported by McDonnell *et al* (9), half-cycle testing using a peracetic-acid system and commercial duodenoscopes, demonstrated a sterility assurance level of SAL<sup>-6</sup>. However, reprocessed scopes must be used at point-of-care, since the method does not allow for *terminal* sterilization, which facilitates sterile storage.

Additionally, the effectiveness of first-generation vaporized hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) sterilizers in sterilizing multi-lumen devices has been evaluated and found inadequate to reprocess a modern GI endoscope. Claim language varies by sterilizer

**Conflict of Interest:** This study was funded by TSO3, Inc. Sylvie Dufresne and Vanessa Molloy-Simard are employees of TSO3, Inc.

manufacturer, but at best is limited to only dual-channel flexible scopes with the longest lumen  $\geq 1$  mm in Inner Diameter (ID) and  $\leq 1000$  mm in length, which is well short of the requirements for a modern colonoscope (10).

The process for validating sterilization claims for new device designs is dictated by both international standards (11) and regulatory guidance, such as provided by FDA (12). Specifically, ISO 14937 requires that a sterilizer manufacturer demonstrate that test devices, inoculated with at least  $10^6$  CFU of a highly resistant organism, can be sterilized under half-cycle conditions. Furthermore, the inoculation must provide the greatest challenge to sterilant penetration, which for vapor-based processes, is in the middle of a lumen.

In addition, FDA requires that test devices must pass simulated-use testing, wherein the microbe suspension is mixed with organic and inorganic soils and inoculated onto devices. For a successful simulated-use validation, testing is to be performed in triplicate with no growth observed following sterilization.

Because of the urgent need for a viable method to terminally sterilize complex GI endoscopes, the effectiveness of a new low-temperature dual-sterilant method was evaluated for reprocessing a flexible video colonoscope. This in turn was completed by use of a new validated test method for direct inoculation of long-lumen multichannel flexible endoscopes.

## METHODS

### *Sterilizer*

The STERIZONE® VP4 Sterilizer (VP4 Sterilizer) (TSO3, Inc., Quebec Canada) was used in this study. A detailed description of the device has been previously published (13). The device uses dual sterilants (vaporized  $H_2O_2$  and ozone), in a multiphase process. The device is intended for use in terminal sterilization of cleaned, rinsed, and dried metal and non-metal reusable medical devices. The VP4 Sterilizer uses only a single sterilization cycle irrespective of load configuration, with a maximum load limit of 34 kg (75 pounds).

### *Test organism*

The most resistant microorganism to either hydrogen peroxide or ozone sterilants is *Geobacillus stearothermophilus* spores (14).

Spore suspensions of *G. stearothermophilus* ATCC 7953 (Lot AR-469; population  $2.2 \times 10^8$  colony forming unit (CFU)/mL) were purchased from iuvo BioScience (Rush, NY). The spore suspension populations were verified and adjusted to achieve a final concentration of  $1.0\text{--}2.5 \times 10^6$  CFU/10  $\mu$ L, which was used for validation of high-level recovery, as well as half-cycle and simulated-use testing (the latter in combination with 400 ppm AOAC hard water and 5% fetal bovine serum).

The spore suspension was further diluted to 10-100 CFU/10  $\mu$ L for validation of low-level recovery.

### *Lumen devices or surrogates*

For the purpose of validating expanded sterilization claims, a Pentax Video Colonoscope Model EC-3890Li (Pentax Medical, Tokyo, Japan) was used. The manufacturer identifies seven discrete lumens, consisting of four “channels” (Instrument, Air,

Water, and Forward Water Jet, extending from the distal end of the device to the handle) and three “tubes” or umbilical lumens (Suction, Air Feeding, and Water Feeding, extending from the handle to the suction source, air pump, and water bottle, respectively; see Figure 1). Channel dimensions, which are the basis for FDA labeling claims for the VP4 Sterilizer, are  $\geq 1,45$  mm ID and  $\leq 3\,500$  mm in length, and/or  $\geq 1,2$  mm ID and  $\leq 1\,955$  mm in length. Tube dimensions, are all  $\geq 2,4$  mm ID and  $\leq 1\,580$  mm in length. Validation studies were completed on all channels and tubes (seven in total) as defined by Dufresne (15), since all lumens can become contaminated, although the device is commonly referred to as a “four-channel” endoscope (consisting of air, water, suction, and instrument channels).

Development and validation of the inoculation method as well as high-level recovery method was completed by use of surrogate fluoropolymers tubing such as polytetrafluoroethylene (PTFE) or perfluoroalkoxy alkanes (PFA) tubing, which are part of the same group of fluoropolymers tubing used for commercial flexible endoscopes. Tubing diameter and length was selected to correspond to the dimensions found in the Pentax colonoscope. Thus, surrogate PTFE tubing, ranging between 1 mm ID  $\times$  3 500 mm length, and 4 mm ID  $\times$  1 840 mm length, were selected based on worst-case lumen dimensions (smallest ID and longest length).

### *Inoculation and recovery method using surrogate lumens*

PTFE lumens (three samples per dimension) were used to develop the inoculation method for each lumen found in the colonoscope, as well as to validate that a minimum of  $10^6$  spores were deposited in the center of the lumen, as required by FDA.

Each lumen was temporarily placed on a vertical wall such that the middle of the lumen was at the lowest height. A minimum volume of sterile diluent solution (between 40-400  $\mu$ L, depending on the lumen dimension) was added to 10  $\mu$ L of inoculum (with and without hard water and serum) in order that the collective volume would flow to the middle of the test lumen. A micropipette with a low retention tip was used to introduce the diluted inoculum into the lumen orifice. Minimal visible droplets were observed on the sides of the tube confirming that the inoculum was deposited in the middle of the lumen. The objective was to use the smallest diluent necessary in order to minimize drying time and to ensure that inoculum was visibly collected in the center of the test lumen. The inoculated tubes were left to dry.

After overnight drying of surrogate lumens, verification of the spore count deposited in the middle of the tube was performed by cutting the middle part of the PTFE tube (about 10 % of its total length) and separating it from the remainder of the tubing. This portion of the tubing underwent recovery with a 100 mL buffer solution. A pour plate method using Trypticase Soy Agar (TSA) was performed to evaluate the population. The plates were incubated at 55-60°C for a minimum of 48 hours. The acceptance criteria for a successful high-level validation required recovery of  $> 10^6$  spores.

### Inoculation of the Pentax Colonoscope for half-cycle test and simulated use test

The channels and tubes of each colonoscope were inoculated with  $1.0\text{-}2.5 \times 10^6$  CFU/10  $\mu\text{L}$  using a direct inoculation method based on gravity. A volume of 10  $\mu\text{L}$  of inoculum was diluted with 40-400  $\mu\text{L}$  of sterile diluent solution, which was introduced into each lumen orifice separately using a gel loading micropipette. For simulated-use, the inoculum was mixed with hard water and serum as described previously (*Test Organism Section*).

The endoscope was inoculated in two groups: Group 1 included only the Forward Water Jet Channel and Group 2 included all other channels and tubes (six lumens in Group 2). The Forward Water Jet Channel had to be inoculated separately due to its considerable length, extending from the distal end of the scope to the umbilical (Figure 1).

### Sterilization

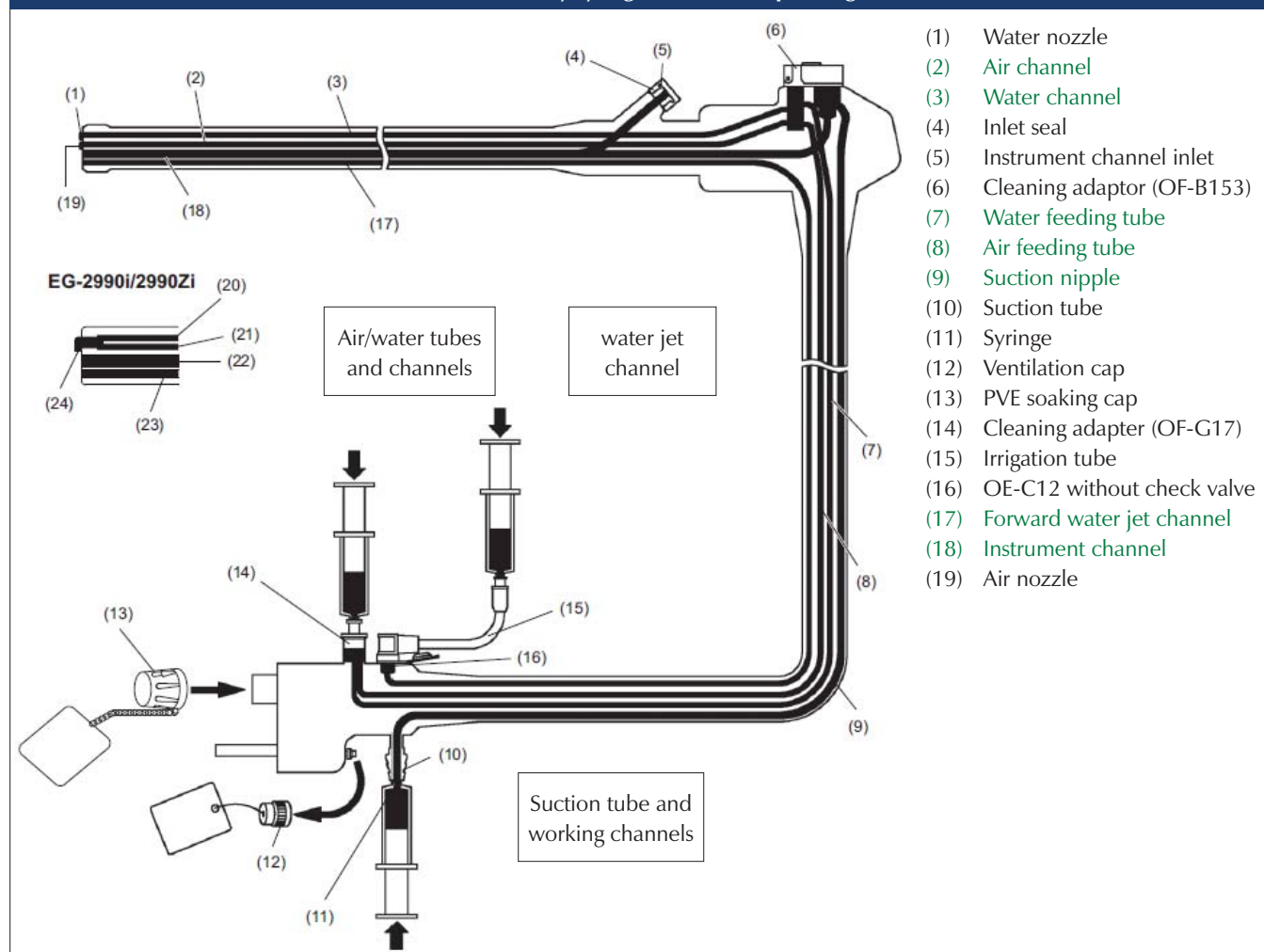
The endoscope was placed in a stainless steel basket and packaged in a full length SteriTite® Container (Case Medical Inc, South Hackensack, NJ). The container was placed on the lower shelf of the sterilizer loading rack.

The load conditions used for the half-cycle and simulated-use validation testing were selected to represent the worst case conditions for sterile efficacy testing. The recommended load temperature to be processed in the STERIZONE® VP4 Sterilizer is 20°C to 26°C. Thus the validation loads were pre-conditioned at 26°C prior to being processed in the sterilizer. The pre-conditioning temperature of 26°C was chosen, due to the fact that this load condition requires the shortest sterilant exposure time and results in the lowest mass of sterilant, and therefore represent the most challenging condition for achieving sterilization efficacy.

For the half-cycle test, the load was exposed to the first phase of the process only. For simulated-use, the load was as exposed to the complete Cycle (two sterilization pulses and full aeration).

Tests were performed in triplicate for each inoculation group under worst-case conditions. Prior to each test, the colonoscope was reprocessed in accordance with the manufacturer's instructions before initiation of the next test, which included cleaning, drying, and storage.

**FIGURE 1: Component legend for PENTAX Colonoscope. Inoculated channels and tubes are identified in green (Numbers 2, 3, 7, 8, 9, 17, & 18 – seven lumens in total). Three recovery syringes, with corresponding channels and tubes, are also identified.**





### Recovery

Recovery of viable spores was achieved by using a 60 mL syringe and the cleaning connector provided by Pentax, following the cleaning method described in the scope-reprocessing manual.

Three luer-lock connectors are available on the colonoscope, with two of the three connectors associated with more than one channel, and the Forward Water Jet having its own connector (Figure 1). Thus, recovery buffer was passed through more than one channel/tube (with the exception of the Forward Water Jet) using syringes filled with recovery buffer.

The amount of recovery buffer used per channel/tube or group of lumens was 100x the combined internal volume for each lumen or group of lumens. Recovered buffer solution was filtered using a 0,45 µm filter and placed on a TSA plate. Plates were incubated at 55°-60°C for a minimum of 48 hours.

### Controls: High level recovery

For high level recovery, each lumen of the Pentax endoscope was tested individually. Each channel was inoculated as described for

the half-cycle and simulated use tests. After drying overnight, recovery was performed. A pour plate method using TSA was performed to evaluate the population after heat shock (95-100°C for 15 min) (16). The plates were incubated at 55-60°C for a minimum of 48 hours. A successful high-level validation required recovery of > 10<sup>6</sup> spores.

### Controls: Low level recovery

In order to confirm low-level recovery, the standard spore suspension was diluted to 10-100 CFU/10 µL. Each channel was inoculated as described for the half-cycle test, but using 10-100 CFU/10 µL spore suspension. After drying overnight, recovery was performed. Recovered buffer solution was filtered using a 0,45 µm filter and placed on a TSA plate. Plates were incubated at 55°-60°C for a minimum of 48 hours. The recovery percentage was calculated using the count of the inoculating spore suspension as 100%. A successful low-level validation required recovery of a minimum of 25% spores.

**TABLE 1: Half-cycle and simulated-use validation results**

Channel description	Half-cycle Results (# positive lumens/# lumens tested)	Simulated-use Results (# positive lumens/# lumens tested)
Instrument Channel	0/3	0/3
Suction Tube	0/3	0/3
Air Channel	0/3	0/3
Air Feeding Tube	0/3	0/3
Water Channel	0/3	0/3
Water Feeding Tube	0/3	0/3
Forward Water Jet Channel	0/3	0/3

**TABLE 2: Recovered population from the middle of the test surrogate (PTFE) lumens**

Tubes description	Middle section length	Spore alone		Spore mixed with 5% serum and 400 ppm hard water	
		Recovered population	Percentage	Recovered Population	Percentage
1 mm × 3 500 mm	35 cm	1,27 × 10 <sup>6</sup>	79 ± 7%	1,09 × 10 <sup>6</sup>	79 ± 7%
		1,29 × 10 <sup>6</sup>		1,24 × 10 <sup>6</sup>	
		1,47 × 10 <sup>6</sup>		0,83 × 10 <sup>6</sup>	
2 mm × 1 580 mm	16 cm	1,27 × 10 <sup>6</sup>	78 ± 3%	1,12 × 10 <sup>6</sup>	79 ± 8%
		1,34 × 10 <sup>6</sup>		1,02 × 10 <sup>6</sup>	
		1,38 × 10 <sup>6</sup>		1,21 × 10 <sup>6</sup>	
3 mm × 1 580 mm	16 cm	1,39 × 10 <sup>6</sup>	90 ± 8%	1,27 × 10 <sup>6</sup>	80 ± 6%
		1,64 × 10 <sup>6</sup>		1,34 × 10 <sup>6</sup>	
		1,38 × 10 <sup>6</sup>		1,38 × 10 <sup>6</sup>	
4 mm × 1 840 mm	18 cm	1,52 × 10 <sup>6</sup>	92 ± 5%	1,15 × 10 <sup>6</sup>	79 ± 8%
		1,66 × 10 <sup>6</sup>		1,01 × 10 <sup>6</sup>	
		1,53 × 10 <sup>6</sup>		1,03 × 10 <sup>6</sup>	

## RESULTS

### Half-cycle and simulated-use testing of video colonoscope

No viable microorganisms were recovered from any of the inoculated challenges subsequent to exposure to either half-cycle or simulated-use testing conditions (Table 1), despite the fact that six inoculated lumens (within Group 2) were sterilized simultaneously.

### Controls – verification of inoculum in the center of test lumens

All lumens were inoculated with a spore suspension of  $1,71 \times 10^6$  CFU/10 $\mu$ L (spore alone) or between  $1,02$  and  $1,53 \times 10^6$  CFU/10 $\mu$ L when spores were mixed with 5% serum and 400 ppm hard water. High-level recovery using PTFE lumens confirmed that a population of at least  $10^6$  spores was recovered from the middle of all test lumens, irrespective of ID or length. This was true if the suspension was used either alone (78-92% recovery) or if combined with serum and hard water (74-80% recovery – See Table 2).

### Controls: High level recovery

The population of the spore suspension used for high level recovery was  $1,71 \times 10^6$  CFU/10 $\mu$ L (spores alone) or between  $1,02$  and  $1,53 \times 10^6$  CFU/10 $\mu$ L for spores mixed with 5% serum and 400 ppm hard water. High-level recovery for

each inoculated channel and tube within the colonoscope also confirmed a population of at least  $10^6$  spores. This was confirmed when the suspension was used alone (77-95% recovery by lumen) or with serum and hard water (91-105% recovery by lumen – See Table 3).

### Controls: Low level recovery

The population of the spore suspension used for low level recovery was determined to be between 69-90 CFU/10 $\mu$ L; low-level recovery was not done with spores mixed with serum and hard water. Low-level recovery was lower than with high-level recovery, but was judged to be satisfactory, particularly considering the long lengths and complicated access found with the test endoscope (range 29-67 % recovery by lumen – See Table 4).

## DISCUSSION

In 2015, the STERIZONE® VP4 Sterilizer was approved by Health Canada and the EU to include sterilization of multichannel flexible GI endoscopes including colonoscopes and gastroscopes. It was subsequently cleared by FDA in June 2016 to include sterilization of flexible endoscopes with lumens  $\geq 1,45$  mm ID and  $\leq 3$  500 mm in length (and/or  $\geq 1,2$  mm ID

**TABLE 3: High level recovery for each inoculated channel and tube found in the Pentax video colonoscope**

Channel description	Spores alone		Spores mixed with 5% serum and 400 ppm hard water	
	Population recovered (CFU)	Recovery percentage (%) $\pm$ SD	Population recovered (CFU)	Recovery percentage (%) $\pm$ SD
Instrument Channel	$1,27 \times 10^6$	$87 \pm 12$	$1,02 \times 10^6$	$96 \pm 8$
	$1,55 \times 10^6$		$1,11 \times 10^6$	
	$1,66 \times 10^6$		$1,02 \times 10^6$	
Suction Tube	$1,39 \times 10^6$	$90 \pm 9$	$1,05 \times 10^6$	$103 \pm 4$
	$1,54 \times 10^6$		$1,42 \times 10^6$	
	$1,71 \times 10^6$		$1,11 \times 10^6$	
Air Channel	$1,43 \times 10^6$	$84 \pm 7$	$1,11 \times 10^6$	$99 \pm 3$
	$1,32 \times 10^6$		$1,05 \times 10^6$	
	$1,56 \times 10^6$		$1,14 \times 10^6$	
Air Feeding Tube	$1,50 \times 10^6$	$95 \pm 6$	$1,02 \times 10^6$	$91 \pm 3$
	$1,70 \times 10^6$		$1,00 \times 10^6$	
	$1,65 \times 10^6$		$1,01 \times 10^6$	
Water Channel	$1,57 \times 10^6$	$94 \pm 2$	$1,02 \times 10^6$	$99 \pm 3$
	$1,63 \times 10^6$		$1,27 \times 10^6$	
	$1,63 \times 10^6$		$1,13 \times 10^6$	
Water Feeding Tube	$1,61 \times 10^6$	$84 \pm 10$	$1,03 \times 10^6$	$105 \pm 9$
	$1,42 \times 10^6$		$1,18 \times 10^6$	
	$1,27 \times 10^6$		$1,20 \times 10^6$	
Forward Water Jet Channel	$1,29 \times 10^6$	$77 \pm 2$	$1,15 \times 10^6$	$95 \pm 17$
	$1,30 \times 10^6$		$1,14 \times 10^6$	
	$1,35 \times 10^6$		$1,04 \times 10^6$	

and  $\leq 1\,955$  mm in length). To date, the VP4 Sterilizer is the only vapor-based sterilizer to receive FDA clearance to sterilize a four-channel flexible GI endoscope.

Numerous methods have been published on how to inoculate and recover test organisms from lumens for use in sterilization validation studies. However, in general the methods have been validated for only simple lumen devices, and do not reflect multiple, long lumens as found in a GI endoscope. Furthermore, many of the methods require use of a surrogate lumen and not actual endoscopes, as mandated by FDA.

For example, Okpara-Hofmann *et al* described the use of either stainless steel squares or wire carriers, inoculated with  $10^6$  bacterial spores, and placed in the middle of an endoscope biopsy channel (17). The longest endoscope evaluated had a biopsy channel of 2,8 mm ID and was only 1 160 mm long. The author's counseled against direct inoculation of the endoscope due to low colony counts in recovery, caused by the spore suspension being lost in "niches and lumens."

Diab-Elschahawi *et al* also described use of an inoculated wire carrier placed in the midpoint of a surrogate stainless steel lumen measuring  $0,7\text{ mm} \times 500\text{ mm}^{18}$ . Although the carrier was significantly longer than used by Okpara-Hofmann, the carrier was not qualified for use in long flexible lumens.

Dufresne *et al* tested surrogate lumens made of stainless steel tubing with diameters ranging between 0,5-4,0 mm, and lengths ranging between 450-700 mm (19). For the smallest diameter lumens, tubing was directly inoculated with spore suspension. For all other tubing, the microbial challenge was created by placing an inoculated wire inside the channel, which was longer than the lumen to be sterilized.

Finally, McDonnell *et al* reported the direct inoculation of a four-channel duodenoscope by flushing 0,5 mL spore suspension (with a titer of  $10^8$  CFU/mL) through the port and through each channel of the device (9). Satisfactory high and low-level validation was reported. Nonetheless, the method would not satisfy FDA requirements for validation of a vapor-based sterilization process, which requires confirmation that the inoculum is deposited into the middle of each channel.

Due to the complexity of modern GI endoscopes, and FDA's specific requirements for the location of inoculum and validation of spore recovery, neither carriers nor conventional direct inoculation methods are satisfactory. In particular, carriers are difficult to insert into endoscope lumens due to valves and other restrictions, which are not found in surrogate lumens. In addition, spores may be lost due to the interaction of the carrier with lumen walls during insertion. Therefore, a new validated test method was required for direct inoculation of long-lumen multichannel flexible endoscopes. The gravity-based inoculation method described herein satisfied FDA requirements for targeted inoculation and recovery efficacy.

Application of the direct inoculation method confirmed that the VP4 Sterilizer achieves a six log spore reduction in each of seven colonoscope lumens under half-cycle and simulated-use conditions. This represents the first sterilization validation of a modern multichannel GI endoscope using a vapor-based sterilant.

The development of sterilization methods for long-lumen devices is an important advancement. It is reported that more than 10 million GI endoscopic procedures are performed every year in the US, which equates to a significant risk of patient-to-patient transfer of MDROs (2). However, sterilization does not necessarily compensate for inadequate or timely cleaning of the endoscope immediately following a procedure. Thus, successful reprocessing of a complex endoscope must be viewed in the context of thorough bedside cleaning, manual cleaning, automated endoscope reprocessing, and terminal sterilization.

## CONCLUSIONS

A new gravity based inoculation method using sterile diluent demonstrated that spores were consistently deposited in the center of each test lumen as required by FDA for sterilization validation studies. Furthermore, both high and low-level recovery confirmed that spores could be recovered from inoculated lumens. Application of the method to half-cycle and simulated-use testing with a multichannel colonoscope was confirmed, verifying that complex GI scopes can be terminally sterilized using the STERIZONE® VP4 Sterilizer. The FDA's clearance of this device for the terminal sterilization of multichannel video colonoscopes is a milestone in reducing risk for patients using these critical medical devices.

**TABLE 4: Low level recovery for each inoculated channel and tube found in the Pentax video colonoscope**

Channel description	Inoculum (CFU) population	Recovery (CFU) percentage	Recovery percentage (%)
Instrument Channel	76	45	67 ± 14
	78	45	
	78	65	
Suction Tube	69	42	63 ± 13
	69	35	
	69	53	
Air Channel	76	44	52 ± 13
	78	48	
	78	29	
Air Feeding Tube	90	42	47 ± 6
	90	48	
	80	33	
Water Channel	69	20	54 ± 28
	69	34	
	69	58	
Water Feeding Tube	88	19	29 ± 7
	88	30	
	88	28	
Forward Water Jet Channel	84	42	39 ± 10
	84	26	
	76	28	

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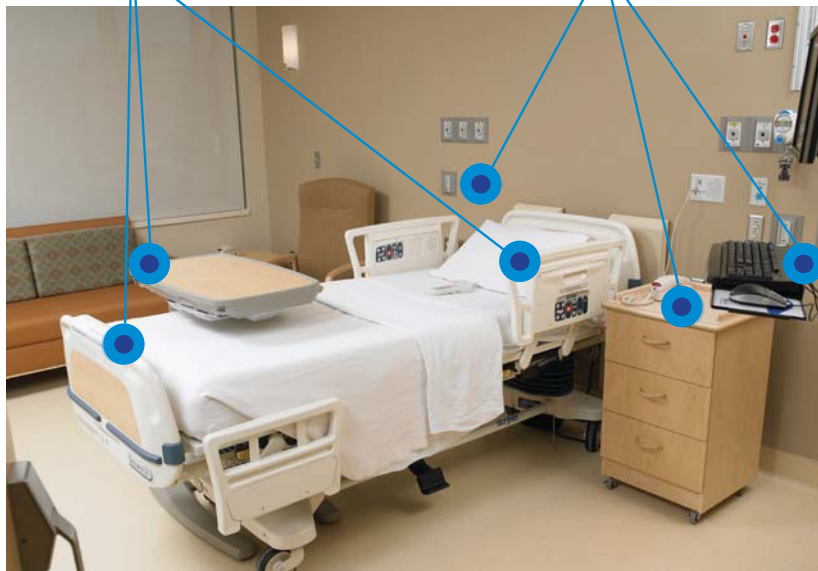


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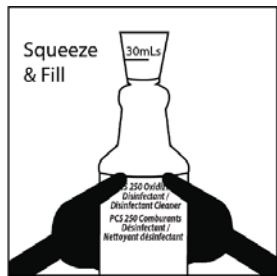
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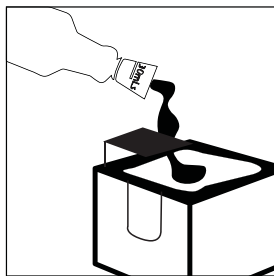


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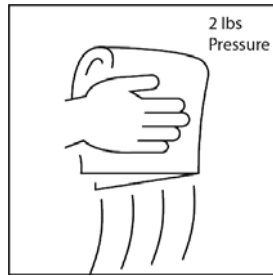
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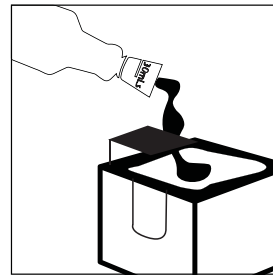
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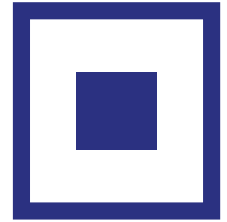


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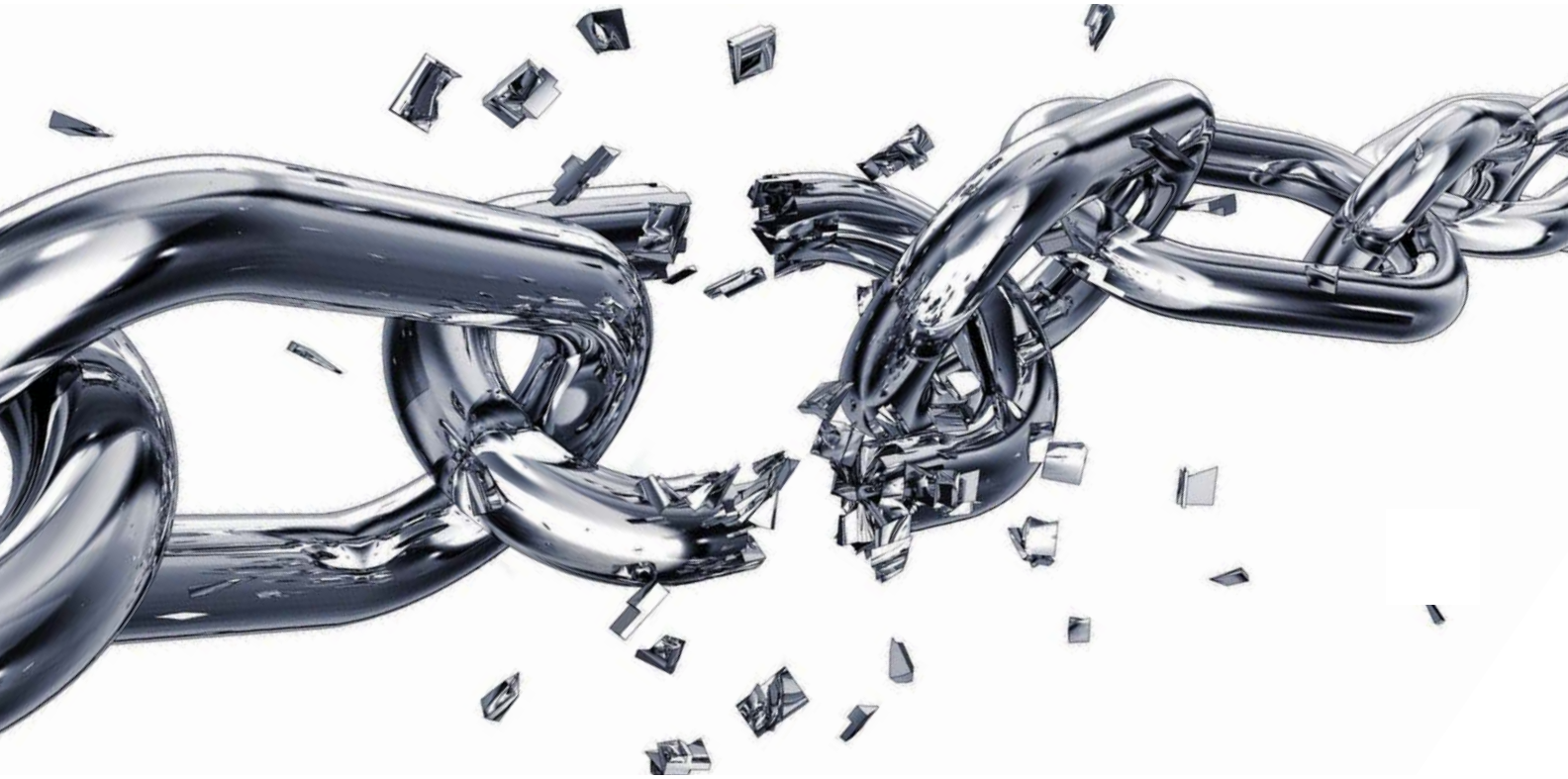




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<sup>1</sup> Health Canada Guidance Document: Human-Use Antiseptic Drugs, Ottawa, 2009/11/27.  
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