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Healthcare Acquired Infection Risk and Toothbrush Contamination in the ICU.

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Healthcare Acquired Infection Risk and Toothbrush Contamination in the ICU.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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List of Tables, Figures and Abbreviations

Abbreviations:

American Association of Critical Care Nurses ..... AACN

Analysis of variance ..... ANOVA

Centers for Disease Control and Prevention..... CDC

Colony forming units ..... CFU

Decayed missing filled surfaces..... DMF

Healthcare acquired infections..... HAI

Intensive care unit..... ICU

Methicillin-resistant Staphylococcus aureus ..... MRSA

Multi-locus sequence typing..... MLST

National Institute of Nursing Research..... NINR

Polymerase chain reaction..... PCR

Potential pathogenic microorganisms ..... PPM

Randomized control trials ..... RCT

Time in environment ..... TIE

Toothbrush..... TB

United States ..... US

Vancomycin resistant *Enterococcus spp.* ..... VRE

Virginia Commonwealth University ..... VCU

Ventilator-associated pneumonia..... VAP

World Health Organization..... WHO

ABSTRACT

HEALTHCARE ACQUIRED INFECTION RISK AND TOOTHBRUSH CONTAMINATION IN THE ICU.

By Michelle R. Frazelle, RN, MSN, CCRN

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Hospital acquired infections (HAIs) are a complex and multi-factorial problem associated with high morbidity, mortality, and cost. Toothbrushes (TBs) may be at risk for contamination with potential pathogenic microorganisms (PPMs) from the patient care environment or autoinoculation from the patient. We focused on three PPMs: methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus spp.* (VRE), and A*cinetobacter spp*. Specific aims were to (1) describe environmental factors associated with TB

contamination in the ICU and (2) describe the relationship between TB contamination and oral colonization in critically ill adults. A cross-sectional design was used to examine the physical environment in which TBs were found as well as microbial flora in 100 paired samples (subjects and their TBs) over a 72 hour period (at 24, 48 and 72 hours). Concordance among microbial cultures was determined by genetic typing. Data were analyzed by linear and logistic regression, chi-square analysis, Fisher's exact test and ANOVA.

Fourteen TBs were found to be contaminated; 1 TB had more than one PPM species. Contamination occurred at all three time points. All but one of the contaminated TBs was located on the nursing cart; TBs in cart drawers had the highest recovery rates for all PPMs. Toothbrush contamination increased as the distance to the bathroom increased. Toothbrush contamination increased as the distance to the sink decreased. Ten of the contaminated TBs were in contact with some type of patient care article. There was a significant association between the presence of TB contamination and the use of a storage container. The toothbrush weight (moisture and debris) was associated with TB contamination. Baseline oral colonization for PPMs was 19% while repeat was 20%.

We found that TBs in the ICU became contaminated with all 3 PPMs; TBs might act as fomites and increase the risk of infection in the critically ill. Additional research linking contamination to patient outcomes is critical in understanding the level of risk. Nurses should carefully consider handling and storage of TBs. A closed drawer or storage with other care items is not ideal.

CHAPTER 1: Introduction to the Study

Healthcare-acquired infections (HAIs) cause approximately 270 deaths per day or 99,000 deaths per year in the United States (US)[46]. In addition to significant morbidity and mortality[46], medical costs resulting from HAIs range from 35.7 billion to 45 billion dollars a year in the US alone[63]. Approximately 1 in 10 hospitalized patients acquire an infection after admission[35] with the highest infection rates found in the intensive care unit (ICU)[46]. Research to identify risk factors for HAIs could reduce their occurrence. The problem of HAIs is complex and multi-factorial, and some areas such as the importance of hand washing have been the subject of intense research[10, 20, 48]. However, one potential risk factor is environmental contamination with potentially pathogenic microorganisms (PPMs). ICU patients are cared for in an environment, including surfaces and equipment that are widely contaminated with PPMs creating a reservoir for infection[10, 65]. Contaminated objects used in direct patient care may become fomites, transmitting PPMs and resulting in increased risk of HAIs. Toothbrushes are advocated for nurse-administered oral care in critically ill patients. However, toothbrushes may be at risk for contamination because they are stored in the patient care environment (environmental contamination) and use repeatedly without decontamination (leading to repeated autoinoculation of a patient harboring PPMs in the oral cavity). These factors increase the risk of ongoing

contamination of the toothbrush. Several studies have shown that the toothbrushes of healthy adults quickly become contaminated with PPMs found in the environment and the oral cavity[10, 11, 25, 32]. Biofilms develop on toothbrushes after use and may harbor PPMs obtained from both the patient and the environment. Biofilms are communities of bacteria that accumulate on a surface[71]. Areas where toothbrushes are commonly stored may also be contaminated with PPMs[12, 29, 42] thus increasing risk of toothbrush contamination. There are no studies that examine toothbrush contamination in the ICU despite multiple studies supporting toothbrush contamination and the relationship between contamination and disease transmission. Examining the toothbrush as a potential source of PPMs in the ICU is important for assessing potential risks and benefits of oral care and informing nursing practice for critically ill patients.

A conceptual model of the relationships of interest is shown in Figure 1.0. The model includes five major concepts: toothbrush, ICU environment, pathogens, critically ill adults, and oral care. In the model, the environment is central to the constant interaction between PPMs, the toothbrush, and the critically ill patient. Vulnerable, critically ill patients are at increased risk for HAIs from contact with contaminated objects in their environment[4, 10, 37]. The same patients may further introduce PPMs into the environment, creating a reservoir of PPMs and continuing the cyclic relationship. Inanimate objects, such as the toothbrush, may become fomites for PPMs increasing the risk for HAIs in critically ill patients[10, 50]. Oral care practices in the ICU may further contribute to the contamination of toothbrushes through ineffective plaque control, toothbrush

storage choices, infrequency of toothbrush replacement and toothbrush handling and placement during use[8, 34, 52]. The relationships among environmental factors, toothbrush contamination and patient oral colonization will inform development of oral care guidelines for critically ill adults that minimize risks related to toothbrush contamination. Such evidence-based guidelines for practice could reduce risk of HAIs.

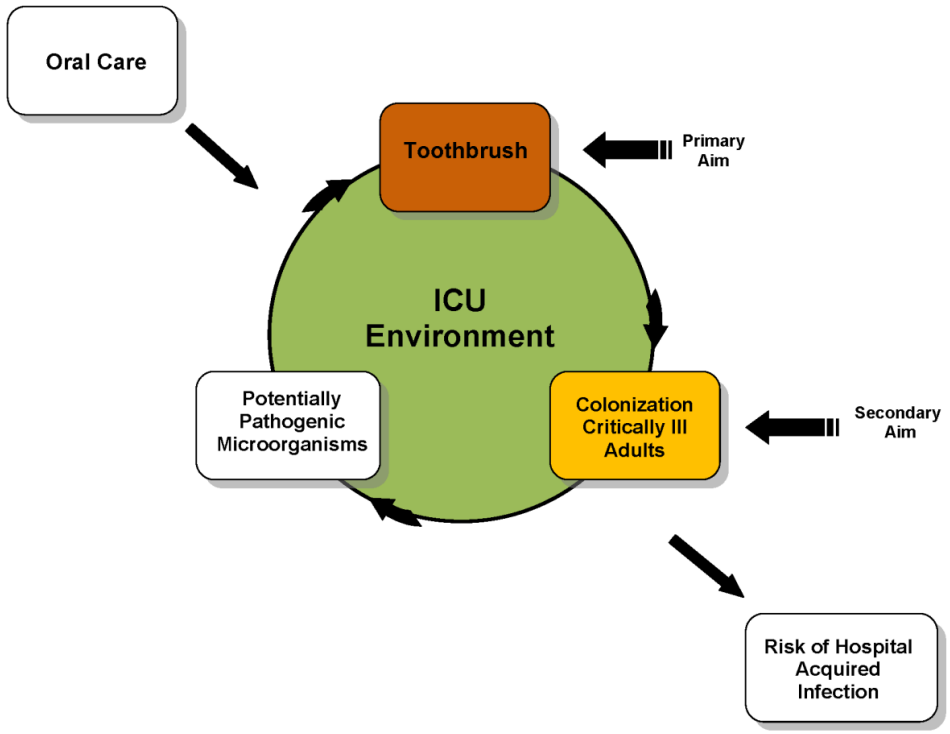

Figure 1.0 Conceptual Model

A *toothbrush* is an instrument used for cleaning teeth and is commonly used by nurses for oral care of critically ill patients. A comprehensive review of

the literature to analyze the evidence related to toothbrush contamination is presented in Chapter 2.

Pathogens are living microorganisms capable of causing disease. The Centers for Disease Control and Prevention (CDC) defines a HAI as an infection that patients acquire during the course of receiving treatment for other conditions within a healthcare setting and are caused by PPMs in the hospital environment[14]. HAIs are one of the ten leading causes of death in the United States[13]. HAIs may be caused by pathogens from endogenous or internal body sites normally inhabited by microorganisms or exogenous or external sites (environment or other individuals)[14]. Three major pathogens representative of ICU HAIs are: vancomycin-resistant *Enterococcus spp.* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter*. Rates of VRE continue to increase in the hospital setting and are more prevalent in critical care units[21]. VRE bacteremia is associated with mortality (37%) in the ICU setting[21]. Known in the media as “the superbug”, MRSA is a major healthcare-acquired pathogen around the world and is most common in the ICU setting. Eight percent of HAIs in the ICU are due to MRSA[40]. MRSA can colonize dental plaque in ICU patients[53]. *Acinetobacter* has been increasing in frequency as a cause of HAIs in the ICU setting and is resistant to many antibiotics[5, 68, 70]

The *ICU environment* includes the surfaces and equipment in close or direct contact with the ICU patient and plays a significant role in the transmission of HAIs. The environment of patients may be heavily contaminated with PPMs implicated in HAI[10, 65]. Hardy *et al.* examined MRSA in the ICU environment and

its relationship to patient acquisition of MRSA. Environmental and subject samples were obtained and subjected to pulse-field gel electrophoresis for concordance. The study found that 26 patients acquired MRSA during their ICU stay with 3 acquiring it as a direct result of environmental contamination[37]. Bonten *et al.* found that environmental contamination occurred in rooms of patients not previously colonized with VRE, 23% of whom later acquired VRE[9]. Surfaces in close contact with the patient such as bed-frames, countertops, sinks, bedside tables, linens and mattresses may act as fomites[50]. PPMs such as *Pseudomonas*, *Acinetobacter* and MRSA have been found on hospital surfaces and equipment, and *Clostridium difficile* bacteria were found on 58% of bedside surfaces in a study by Hota[41]. MRSA and VRE persist for days to weeks on environmental surfaces[10], and PPMs can survive for days to months on hospital fabrics and plastic[48, 55]. In a recent study, Johnson *et al.* found hospital bath basins to be contaminated and an environmental source for PPMs[42]. Aygun *et al* found that the ICU environment, including the patient bed, tables, and equipment, was heavily contaminated with *Acinetobacter* [70]. *Acinetobacter* has been found in both dry and moist conditions and survives for up to 6 days in the environment[6, 58, 68]. In a review of several studies, Boyce found that environmental contamination contributes to HAIs and eliminating contaminated equipment used in direct patient care, such as thermometers, reduces transmission of VRE[10]. The American Dental Association guidelines for healthy adults recommend rinsing the toothbrush after use, keeping it separate from other items that may harbor bacteria, storing it in the air in dry conditions, avoiding moist containers and

replacing it when frayed and worn or more frequently when the user is susceptible to infections or immunocompromised[4]. PPMs in the ICU environment may adhere to toothbrushes when they are placed on a contaminated surface and/or stored in conditions that encourage bacterial growth. The storage conditions of toothbrushes play an important role in bacterial survival: toothbrushes stored in aerated conditions had a lower number of bacteria than those stored in plastic bags and bacterial growth on the toothbrush increased 70% in a moist, covered environment[18, 51, 56]

Critically Ill Adults are persons over the age of 18 who are experiencing a physiologic instability or alteration requiring urgent and advanced medical care. Critically ill patients represent a vulnerable population at higher risk of colonization by PPMs due to decreased host defenses, changes in their normal oral physiology, and the use of medical therapy.

The oral cavity of healthy adults may contain at least 500 different bacterial species that are considered normal flora[7, 47]. Healthy adults have several defenses important in protection of the oral cavity against dental plaque, which is an accumulation of oral microorganisms and debris. As it matures, plaque becomes hard and porous creating areas for bacteria to attach and multiply. Eating and drinking stimulate saliva production which helps to prevent pathogenic bacteria from attaching to oral surfaces, regulates oral pH, maintains tooth integrity, washes the mouth with antimicrobials and reduces bacterial growth. Saliva washes food particles and bacteria away from the surfaces and also includes immune substances that fight infection. Oral enzymes normally

protect the mucous membranes from bacterial attachment acting as an additional host defense mechanism[57].

Critically ill patients have increased oral biofilm formation, a shift in oral flora to PPMs, and risk of micro aspiration of PPMs from the oral biofilm[23, 28, 53, 69]. Several studies found that dental plaque significantly increased during the ICU stay and that dental plaque cultures that were positive for PPMs were significantly associated with HAIs[23, 26, 29, 62] . The bacteria found in critically ill patients are more virulent compared to healthy adults resulting in an increased risk for HAIs[53]. Bacteria that are normally found in the mouth are predominantly gram-positive viridans streptococcal species, but the oral flora of critically ill patients may contain PPMs such as VRE, MRSA, and *Pseudomonas,* which are not generally found in healthy adults[53, 54, 62]. In critically ill adults, oral proteases in secretions increase, resulting in deceased glycoproteins that act as host defenses of the oral tissues[7]. Without this protection, it is easier for PPMs to attach to the cell surfaces and potentially infect the patient[7]. As bacterial levels rise in the mouth, dental plaque biofilms form on the tissues, teeth, endotracheal tubes, oral bite blocks, and orogastric tubes and may act as a reservoir and source for infection.

Medical therapy in the ICU may create additional oral complications for critically ill adults. In mechanically ventilated patients, the endotracheal tube, oral gastric tube, bite block and tape securing the devices create limited access into the oral cavity for oral care. This equipment becomes heavily contaminated with bacteria from the oral cavity[3]. PPMs accumulating in the mouth can invade and

infect the patient through openings in the oral tissues as conditions become favorable for bacterial survival and proliferation. Mechanically ventilated patient's mouths are always open, resulting in dry and often cracked mucosa. Non-mechanically ventilated patients often have drying oxygen therapy in place. In addition, many of the medications used to treat critically ill patients, such as diuretics, antibiotics, steroids, and anticholinergics cause dryness of the mucous membranes. Xerostomia is prevalent in critically ill patients[53]. Any reduction of saliva in the oral cavity reduces natural protection of the patient and allows PPM growth to occur. The combined effects of these factors lead to overwhelming risk factors for the development of HAIs.

Oral care is the process of cleaning the oral cavity to remove dental plaque and maintain moisture in the oral cavity. Healthy adults typically brush their teeth 2-3 times a day. Oral care in the ICU varies and is not standardized[7, 34, 52]. Guidelines from the American Association of Critical Care Nurses (AACN), co-authored by Dr. Munro and Dr. Grap, recommend the toothbrush as the tool of choice for oral care. A study by Kite et al. found that the toothbrush was the best tool for decreasing plaque and preventing disease[44]. Numerous studies show that oral care in the ICU is inconsistent and a low priority for nurses[17, 28, 34, 52, 59]. Evidence shows that the current standard of oral care in the ICU is insufficient to control plaque formation, leaving ICU patients at greater risk for infection through the oral cavity[26, 53]. A survey of oral care practices by ICU nurses found that more than half felt they needed further training in oral care and oral assessment[43]. Poor oral hygiene may place the patient at increased risk for infection from

aspiration of bacteria accumulated in the oral cavity or in dental plaque[72]. Several studies support the need for an oral care protocol to include more specific guidelines related to tooth brushing[34, 52]. However, two recent randomized controlled trials (RCTs) have evaluated tooth brushing in the ICU and have failed to demonstrate a reduction in ventilator-associated pneumonia (VAP) through the use of a toothbrush[54, 60]. Therefore additional knowledge related to the benefits and risks of toothbrush use in the ICU is essential.

A study based on the conceptual model above is described in Chapter 3. A cross-sectional study was initiated to examine the physical environment in which toothbrushes were found in the ICU as well as to compare microbial flora of the toothbrush and oral cavity in 100 subjects over a 72-hour period. Data were examined in three time-in-environment (TIE) groups (24, 48, or 72 hours). Toothbrush contamination and oral colonization were examined by standard microbiological methods to identify three selected PPMs (VRE, MRSA, and *Acinetobacter spp.*). Oral and toothbrush isolates were compared using molecular strain typing on any subjects that had MRSA, *Acinetobacter spp.* or VRE isolated from more than one source (both toothbrush and oral swab) to determine if the strains identified in the toothbrush and the mouth were the same. Patient characteristics (type of airway, length of stay, antibiotic therapy, oral care frequency, and history of HAIs) were also examined for possible association with toothbrush contamination.

Chapter 2: Toothbrush Contamination: a literature review.

Introduction

Toothbrushes play an essential role in oral hygiene and are commonly found in both community and hospital settings. Toothbrushes may play a significant role in disease transmission and increase the risk of infection since they can serve as a reservoir for microorganisms in healthy, oral-diseased, and medically ill adults[29]. Contamination is the retention and survival of infectious organisms that occur on animate or inanimate objects. In healthy adults, contamination of toothbrushes occurs early after initial use and increases with repeated use[9, 13]. Toothbrushes can become contaminated from the oral cavity, environment, hands, aerosol contamination, and storage containers. Bacteria which attach to, accumulate, and survive on toothbrushes may be transmitted to the individual causing disease[4, 12]. In the hospital setting, toothbrushes are commonly used for oral care by nurses. Examining the toothbrush as a possible source of potentially pathogenic microorganisms is clinically relevant for assessing the risks and benefits of oral care and informing nursing practice. This review of peer-reviewed literature was conducted to evaluate the cumulative state of knowledge related to toothbrush contamination, its possible role in

disease transmission, and in preparation for a research study related to toothbrush contamination in critically ill adults

Methods

A systematic review of the scientific literature was conducted. There were no relevant articles available in print prior to 1977. Articles published from 1977 to 2011, on human subjects and using the English language were obtained. The review included studies that evaluated toothbrush contamination in healthy and oral-diseased adults, guidelines for toothbrush and oral care in both healthy and medically ill persons, hospitalized and non-hospitalized patients, and interventions for reducing contamination of toothbrushes. Experimental and non-experimental designs were included in the review. The following databases were searched: Pub Med (clinical inquiries and MESH), CINAHL, Cochrane Library, National Guidelines Clearinghouse, Web of Science, and Google Scholar. Key search terms used in the review were: *toothbrush, tooth brushing, colonization, bacterial contamination, contamination, oral hygiene, oral health, nursing practice, microbial contamination* and *adults.* This search strategy was verified by a health sciences librarian. A total of 3 separate searches were conducted in a systematic fashion using the inclusion and exclusion criteria and search terms. The first search (search 1) identified articles in the selected databases and complete copies of articles that were considered to have met the inclusion criteria were obtained for further review (Table 1.0). Articles were excluded if they did not meet the inclusion criteria listed above, were conducted on a pediatric

population, were duplicates from other databases, or only explored antibacterial methods.

Database	Initial Number of Articles Located
Pub Med	26
CINAHL	16
Cochrane Library	10
National Guidelines Clearinghouse	None
Web of Science	22
Google Scholar	376

Table 1.0 – Results of Search 1

The second search (search 2) included articles identified through cited articles and were reviewed following the same criteria. There were a total of 23 new articles identified through the second search. A third search (search 3) was conducted 1 year after the first search in order to capture any recently published articles. There were 3 new articles identified in the third search. After a review of the abstracts for the articles obtained through the three searches, a total of 88 relevant articles were identified for further evaluation. After inclusion criteria were applied, 38 articles were selected; after exclusion criteria were applied, 10 articles were retrieved to be read in their entirety and included in this review (Figure 3.0).

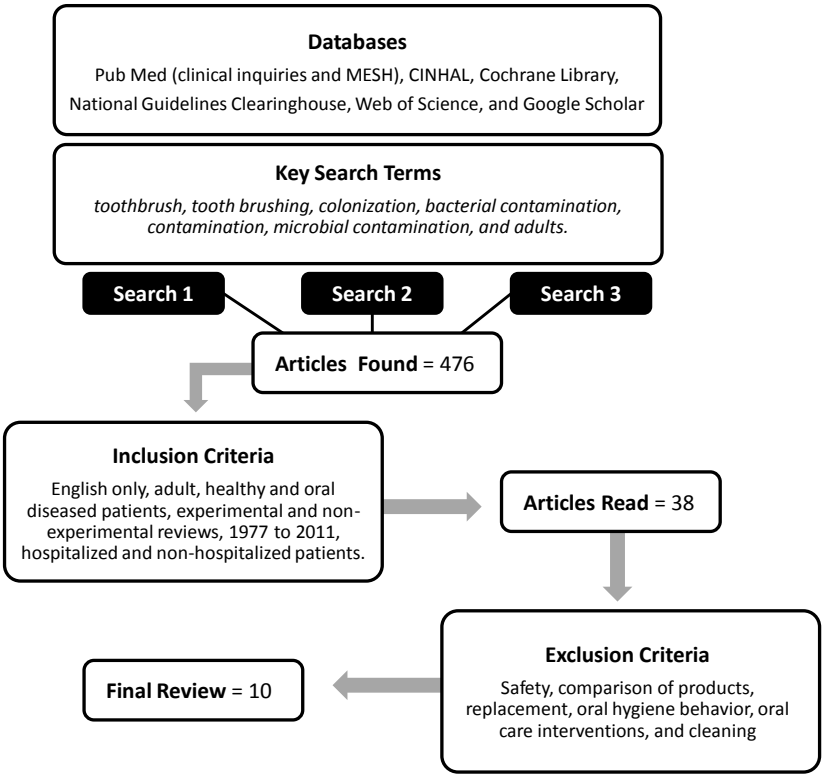

Figure 2.0 - **Literature Search Process**

Results

A comprehensive summary of the studies is listed in Table 2.0. Studies that were reviewed included: 7 experimental and 3 descriptive studies. The selected studies are grouped by setting: in vivo, in vitro, and studies that combined both types of settings. The sample sizes ranged from 3 to 103 with the majority of studies having a sample size under 30. Overall, the studies evaluated several perspectives related to toothbrush contamination to include: contamination, methods for decontamination, storage, design, and environmental factors.

STUDY	PURPOSE	DESIGN	SAMPLE	RESULTS
In Vitro Studies				
Bunetel et al. (2000)	Does retention and survival of microorganisms on toothbrushes pose a threat to patients at risk of infection?	Experimental	N = 3 toothbrush types with two series of experiments	Contamination of toothbrushes occurs early in the life of the brush and tends to increase with repeated use.
Dayoub et al. (1977)	To determine the degree of bacterial contamination of toothbrushes after contamination and storage in vented containers or in air.	Experimental	N = 103 toothbrushes	The numbers of bacteria on toothbrushes stored in room air after use decrease more quickly than on brushes in containers.
Glass & Jensen (1994)	To evaluate toothbrush design and UV sanitation on microbial growth.	Experimental	N = 72 toothbrushes	UV sanitizing kills bacteria; viruses can survive on toothbrushes for 24 hours; toothbrush design, color, opacity, and bristle arrangement is a major factor in retaining microorganisms.
In Vivo Studies				
Efstratiou et al. (2007)	To examine the contamination and the survival rate of periodontopathic and cariogenic species on new toothbrushes with antibacterial properties after a single use in periodontic patients.	Experimental	N = 10 patients; 4 toothbrushes per patient.	Immediately after brushing, the toothbrushes harbored a significant number of microorganisms with no difference between the types of toothbrushes. The antibacterial toothbrush did not limit bacterial contamination.
Mehta et al. (2007)	To determine the extent of bacterial contamination of toothbrushes after use, evaluate the efficacy of chlorhexidine and Listerine in decontamination, and effectiveness of covering the toothbrush head with a cap.	Experimental	N = 10 patients	Toothbrushes become contaminated during use; retention of moisture and the presence of organic matter may promote bacterial growth. Toothbrush contamination may lead to colonization and infection. Caps increase bacterial growth. Chlorhexidine was more effective than Listerine.
Quirynen et al. (2003)	To evaluate the effects of coated tufts and toothpaste on toothbrush contamination.	Experimental	N = 8 patients	Toothbrushes become contaminated and toothpaste reduced bacterial growth in toothbrushes.
Taji & Rogers (1998)	To investigate the microbial contamination of toothbrushes.	Descriptive	N = 10 patients	Most toothbrushes were contaminated.
Verran & Leahy-Gilmartin (1996)	To evaluate toothbrush contamination using a range of selective and non-selective media.	Descriptive	N = 28 toothbrushes	Used toothbrushes supported a wide variety of microorganisms. All media showed growth.
Combination of Both In vitro and In vivo studies				
Caudry et al. (1995)	To demonstrate, quantitatively, the presence of microorganisms adherent to toothbrush bristles.	Experimental	N = 20 toothbrushes	Toothbrushes, in normal use, are heavily contaminated by microorganisms and the bacteria are extremely adherent to the bristles.
Glass et al. (1986)	Do toothbrushes harbor pathogenic microorganisms and if there is a correlation between contaminated brushes and the presence of disease.	Descriptive	N = 30 toothbrushes	Toothbrushes can harbor pathogenic microorganisms.

Table 2.0 – Studies Selected

Contamination

All of the studies examined toothbrush contamination and found significant bacterial retention and survival on toothbrushes after use[32, 36]. Glass found that toothbrushes from both healthy patients and patients with oral disease contained potentially pathogenic bacteria and viruses such as *Staphylococcus aureus*, *E coli*, *Pseudomonas,* and herpes simplex virus[29]. Glass also found toothbrushes contaminated with herpes simplex virus 1 in numbers sufficient to cause an infection in the patient[29]. Bunetel *et al*. found that toothbrushes used by patients with existing oral disease quickly became contaminated[11]. This study also found a significant relationship between repeated use and bacterial retention on toothbrushes and that the oral cavity can be inoculated from a contaminated toothbrush. Several of the studies found that toothbrushes were contaminated before use[12, 31]. Caudry *et al*. found that toothbrushes are heavily contaminated with normal use[12]. Mehta *et al*. found that 70% of the toothbrushes in their study became heavily contaminated with pathogenic microorganisms after use[51]. Studies by both Taji *et al*. and Glass found extensive toothbrush contamination after use except in cases where an oral antiseptic, such as mouthwash, was used immediately prior to brushing[30, 64]. Verran *et al.* found that toothbrushes supported many different bacteria and the amount of growth was varied[67].

Decontamination

Several studies included in this review explored decontamination techniques for contaminated toothbrushes. Bunetel *et al*. found that toothpaste, mouthwash, and oral antiseptics all decrease microbial load on toothbrushes[11].

Caudry *et al*. examined toothbrushes in healthy adults as well as possible options for disinfection[12]. Their study found that the toothbrushes became heavily contaminated after use. Soaking the toothbrush in Listerine for 20 minutes prior to and after brushing, decreased the microbial load. The use of antimicrobial coated toothbrushes in adults with oral disease was explored by Efstratiou *et al*. as a means to prevent toothbrush contamination[22]. This study, however, found that coating the bristles with triclosan did not change bacterial growth but the use of toothpaste did. Glass *et al*. explored ultraviolet light as a means of decontamination and found this method to be effective at reducing the bacterial load on toothbrushes[31]. The use of coated tufts and toothpaste was investigated in adult patients with oral disease. Quirynen *et al*. found that coated tufts did not inhibit contamination but use of toothpaste did reduce contamination[61]. Mehta *et al*. found that an overnight immersion in chlorhexidine gluconate was highly effective in decreasing toothbrush contamination and chlorhexidine was more effective than Listerine in reducing the microbial load of bacteria[51]. Sato *et al*. found that rinsing toothbrushes with tap water resulted in continued high levels of contamination and biofilm[51]. Warren et al. found that the use of regular and triclosan-containing toothpaste resulted in lower toothbrush contamination than no toothpaste use[69].

Storage and Environment

Toothbrushes can become contaminated through contact with the environment and bacterial survival is affected by toothbrush storage containers. Dayoub *et al*. found that toothbrushes placed in closed containers and

exposure to contaminated surfaces yielded higher bacterial counts than those left open to air[18]. Mehta *et al*. found that the use of a cap for toothbrush storage increased bacteria survival[51]. Glass *et al*. found that increased humidity in the environment increased bacterial survival on toothbrushes[30]. In addition, Glass found that bacteria survived more than 24 hours when moisture is present[30].

Design

Toothbrushes are manufactured in a variety of styles. Toothbrush bristles range from soft to hard with different cluster patterns and plastic shapes while toothbrush handles included different plastic shapes and decorative moldings. Different toothbrush design elements were examined by some of the studies. Bunetel *et al*. found that bacteria become trapped inside the bristles of the toothbrush and bacterial survival is dependent upon the bacteria (aerobic versus anaerobic) and toothbrush design[11]. In addition, the researchers found that solid handles had less bacteria retention and that as the surface area increased, so did the microbial load. Efstratiou *et al*. found that filament type affected bacterial retention[22]. Toothbrushes with bristles that are frayed and arranged closely together trapped and retained more bacteria[33]. This finding was also echoed in a study by Glass *et al*.[29] in a study that explored the level of bacterial retention based on toothbrush brand, color and bristle pattern. Contamination was the lowest in soft and round, clear, two bristle row toothbrushes. Glass also found that pathogenic bacteria adhere to plastic after short exposure times[29]. Caudry *et al*. found that bacteria strongly adhere to the bristles[12]. Mehta *et al*. found that the retention of moisture and oral debris in the bristles increased bacterial survival[51].

Conclusions

Due to the limited number of publications specifically related to toothbrush contamination, it was necessary to conduct a preliminary evaluation of the majority of identified articles for this review. For example, several of the articles combined an in vivo examination of bacterial survival on actual patient's toothbrushes, and then conducted an in vitro auto inoculation experiment to examine decontamination methods on sterile toothbrushes in the laboratory. This made database searching and identification of articles for the review more challenging. The selected studies all found that toothbrushes of healthy and oral diseased adults become contaminated with potentially pathogenic bacteria from the dental plaque, design, environment or a combination of factors. The trend identified in the literature is to evaluate methods to reduce toothbrush contamination or toothbrush design rather than evaluating the process related to how the toothbrush initially becomes contaminated, is stored, or is disinfected.

In a vulnerable population such as critically ill adults, pathogenic contamination may increase the risk of infection and mortality. Although some interventions such as chlorhexidine, toothpaste, mouthwash, and ultraviolet sanitizers reduce bacterial survival, oral hygiene practices in the hospital setting by nurses vary. Currently, there are no nursing guidelines related to toothbrush frequency of use, storage, and decontamination. In the hospital setting, the environment as a source of pathogenic bacteria is now a hot topic and the focus of many current infectious disease research studies. Surfaces in close contact with the patient such as bed-frames, countertops, sinks, bedside tables, linens and mattresses may act as fomites. Toothbrushes may

come into contact with these surfaces prior to or after use thus increasing risk. While there is significant literature available on environmental contamination and risk for infection, no studies have specifically examined the toothbrush on more vulnerable hospital populations such as critically ill adults.

Toothbrush storage is inconsistent in both community and hospital environments and may increase exposure to pathogenic organisms. The storage conditions of toothbrushes play an important role in bacterial survival: toothbrushes stored in aerated conditions had a lower number of bacteria than those stored in plastic and bacterial growth on the toothbrush increased 70% in a moist, covered environment[51]. In clinical practice, the author has observed that there is no standardized nursing protocol for the storage or replacement of toothbrushes and that some commonly observed nursing practices include: storing the toothbrush in the bath basin with other bathing/personal supplies and linens, in a paper towel, in a plastic wrapper, on the bedside table, next to the sink and in an oral rinse cup at the bedside. These practices may impact the contamination of toothbrushes.

In this review, the majority of studies identified had small sample sizes. Studies with larger sample sizes would be beneficial in future studies. Importantly, despite multiple studies supporting toothbrush contamination and the likely relationship between contamination and disease transmission, there are no studies that specifically examine toothbrush contamination and the role of environmental factors, toothbrush contamination and vulnerable populations in the hospital setting (e.g. critically ill adults), and toothbrush use in nursing clinical practice. Additional descriptive studies to evaluate these relationships would be beneficial and informative for future research. The

relationship between environmental factors, toothbrush contamination and patient oral colonization would inform development of nursing oral care guidelines for adults that minimize risks related to toothbrush contamination.

CHAPTER 3: Healthcare Acquired Infection Risk and Toothbrush Contamination in the ICU.

Introduction

Healthcare-acquired infections (HAIs) cause approximately 270 deaths per day or 99,000 deaths per year in the United States (U.S.)[46]. In addition to significant morbidity and mortality[46], medical costs resulting from HAIs range from 35.7 billion to 45 billion dollars a year in the U.S. alone[63]. Approximately 1 in 10 hospitalized patients acquire an infection after admission[35] with the highest infection rates found in the intensive care unit (ICU)[46]. Research to identify risk factors for HAIs could reduce their occurrence. The problem of HAIs is complex and multi-factorial, and some areas such as the importance of hand washing have been the subject of intense research[10, 19, 48, 49]. One potential risk factor is environmental contamination with potentially pathogenic microorganisms (PPMs). ICU patients are cared for in a complex environment which includes surfaces and equipment that are widely contaminated with PPMs and may serve as a reservoir for infection[10, 65]. Contaminated objects used in direct patient care may become fomites, transmitting PPMs and resulting in increased risk of HAIs. In a recent study, Johnson *et al.* found hospital bath basins to be contaminated and an environmental source for PPMs[42]. Toothbrushes are a commonly used item for nurse-administered oral care in critically ill patients. However, toothbrushes may be at risk for

contamination because they are stored in the patient care environment (environmental contamination) and used repeatedly without decontamination (leading to repeated autoinoculation of a patient harboring PPMs in the oral cavity). These factors increase the risk of ongoing contamination of the toothbrush. Several studies have shown that the toothbrushes of healthy adults quickly become contaminated with PPMs found in the environment and the oral cavity[10, 11, 25, 32]. Biofilms (communities of bacteria that accumulate on a surface)[71] develop on toothbrushes after use and may harbor PPMs obtained from both the patient and the environment. Areas where toothbrushes are commonly stored may also be contaminated with PPMs[12, 29, 42] thus increasing risk of toothbrush contamination. There are no studies that examine toothbrush contamination in the ICU despite multiple studies demonstrating toothbrush contamination in other settings or if there is a relationship between contamination and disease transmission[32,36]. Examining the toothbrush as a potential source of PPMs in the ICU is important for assessing potential risks and benefits of oral care and informing nursing practice for critically ill patients.

Specific Aims

In this study, we focused on contamination by three PPMs: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus spp.* (VRE), and A*cinetobacter spp*. These PPMs were selected for their prevalence in oral cultures of ICU patients and their importance as HAIs[54, 62]. The specific aims of this study were (1) to describe environmental factors associated with toothbrush contamination in the ICU and (2) to describe the relationship between toothbrush contamination and oral colonization in critically ill adults. In addition, we examined the influence of patient

factors (such as antibacterial therapy) on toothbrush contamination and oral colonization.

Materials and Methods

Study Design

This study was a cross-sectional design. Hospital-type toothbrushes were provided to each subject at enrollment into the study. Subject participation ended when the toothbrush was removed from the environment at a defined randomized time point (either 24, 48, or 72 hours after enrollment). The ICU environment relative to the toothbrush was assessed, and oral cultures (obtained at enrollment and the end of participation) and cultures of the toothbrush were compared.

Setting and Sample

This study was conducted in a 933-bed tertiary care, university teaching hospital in the Southeast. Subjects were recruited from the medical-respiratory, neuroscience, and surgical trauma ICUs as shown in Figure 3.0. All ICU rooms were private. All subjects admitted to the three ICUs were considered for enrollment, including mechanically ventilated subjects, non-mechanically ventilated subjects and subjects with tracheotomies. Children under the age of 18 were excluded because their oral flora and dentition differ from adults[66].

The study was reviewed and approved by the university's institutional review board. All subjects who met the inclusion criteria were assessed for competence and the ability to provide informed consent. If subjects were not able to provide informed consent, consent was obtained from the legally authorized representative.

Procedures

All of the laboratory procedures, data collection, and analysis were completed by the same researcher.

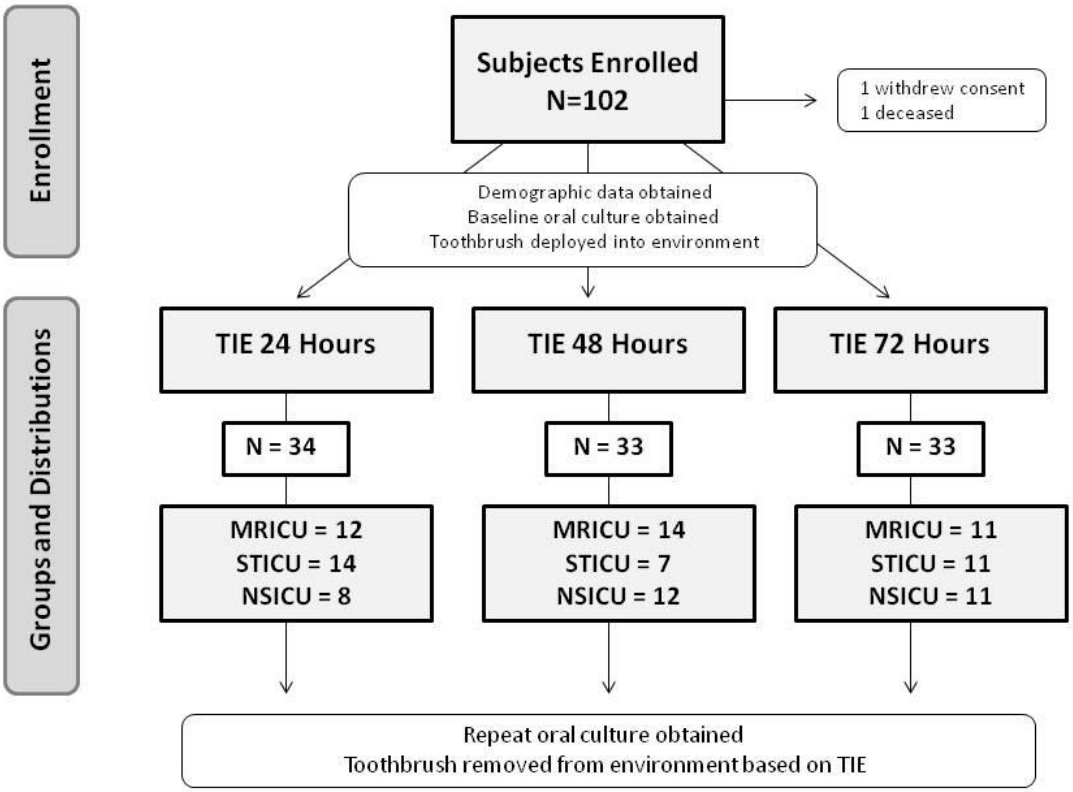

Figure 3.0 – Consort Diagram

Toothbrush Placement One new toothbrush per subject was placed in the ICU room. The introduction of the toothbrush into the environment was standardized. Specifically a labeled hospital-type toothbrush was given directly to the primary nurse caring for the patient for use in oral care. A sign was placed at the bedside indicating that the toothbrush would be collected at a later time and was not to be discarded after routine use. Nurses were told to use and store the toothbrush based on their normal practice. Each subject was

randomized into one of three “time in environment” (TIE) groups (a 24 hour group, a 48 hour group, or a 72 hour group) in order to examine the effects of TIE on contamination.

Toothbrush Weight (Moisture and Debris)

Toothbrush weight was measured in grams using a laboratory balance. Before deployment to the subject’s ICU bedside, the toothbrush was weighed and marked with an identifier. The sterile container used to collect that particular toothbrush was also weighed and marked with the same identifier. When the toothbrushes were returned to the laboratory and prior to culturing, the sterile container containing the toothbrush was weighed. The difference between pre and post deployment weights (transformed log 10 grams) reflected the weight of any fluid, moisture, and debris retained on the toothbrush after use.

Toothbrush Environment

There were three measurements used to describe the toothbrush environment: toothbrush location, contact with other articles and storage container. All three measurements were collected using direct observation prior to collection of the toothbrush. Toothbrush location was categorized into 4 groups: nursing cart; nursing drawer, bedside table, and sink area. Contact with other articles was categorized into three categories: bathing and wound care products, oral care products, and no other articles. Storage container was categorized into 4 categories: basin, paper towel, plastic bag, and none. Environmental distances (from toothbrush to bathroom and sink) were measured in inches with a Craftsman™ ACCUTRAC laser measuring tool.

Toothbrush Contamination and Oral Colonization

Toothbrush contamination and oral colonization were measured using quantitative culture methods for selected representative PPMs. The toothbrush was collected using aseptic technique at the randomized TIE for each subject. The subject's oral cavity was swabbed with a sterile cotton swab using an aseptic technique at the time the toothbrush was initially placed in the environment (baseline oral culture) and again when the toothbrush was collected from the subject (24, 48, or 72 hours).Toothbrushes and oral cultures were transported to the research laboratory in sterile containers at room temperature within 2 hours of collection. Upon arrival to the lab, the toothbrush heads were aseptically removed from the handles using a sterile wire cutter. Toothbrush heads and oral cultures were processed in the same manner. Each was placed in 20 ml of sterile saline and vortexed for 20 seconds to release organisms. The resulting suspension was centrifuged to isolate a pellet. The pellet was resuspended in 1ml of sterile saline and was then serially diluted and plated onto three types of selective media to isolate PPMs: CHROMagar™ (MRSA detection)[38], Enterococcosel™ agar supplemented with 6 mcg/ml of vancomycin (VRE detection)[9], and CHROMagar™ *Acinetobacter* medium (*Acinetobacter spp.* detection). The plates were incubated aerobically for 72 hours at 37 ºC prior to counting colonies for each species.

Oral Contamination, Clinical Information, and Oral Health Status

Demographic data were collected on each subject from the medical record. This data included age (in years), gender, race, ICU admitting diagnosis, history of existing PPMs. The ICU length of stay (in days) was calculated from the admission and discharge data. Airway status and ICU type was observed by the researcher. The

frequency of oral care over the 24 hours prior to enrollment was determined from the ICU nursing record to evaluate usual oral practices. The oral health status of each subject was measured using the World Health Organization (WHO) Decayed Missing Filled Surfaces/Teeth (DMF) index[45]. This is a count of the number of decayed, missing and filled teeth, has been validated, is well established as a measurement of global health in dental epidemiology and has been used in a variety of critical care research settings[16, 26, 27, 47, 54]. The DMF score was obtained at the time of the baseline oral culture.

Genetic Concordance of Oral, Toothbrush, and Clinical Isolates

The genetic relationship of PPM isolates obtained from the paired samples (toothbrush and oral cultures) was investigated. Polymerase chain reaction (PCR) amplification was first attempted for internal fragments of seven housekeeping genes specific for each of the three PPMs of interest. For samples for which limited or no PCR products were obtained, an additional PCR amplification was conducted using 16S rRNA primers. PCR products were column-purified and submitted for capillary DNA sequencing at the VCU Nucleic Acids Research Facility. After examining the sequences for quality and accuracy, each sequence was searched against GenBank using BlastN analysis and the species of the best matching sequences were noted. SeqMan™ software (DNASTAR, Inc.) and the online European Molecular Biology Laboratory's ClustalW2 multiple sequence alignment program[15] were then used to compare sequences of the same gene obtained from different samples in order to assess similarities in bacterial strains. Samples that yielded sequences for all seven

housekeeping genes were also submitted for Multi-Locus Sequence Typing (MLST)[24, 39]. For paired samples that yielded limited or no PCR products using the housekeeping gene primers, 16S rRNA amplification and sequence comparison were used to determine the species of the isolates.

Data Analysis

Data analysis was completed using JMP™ statistical software (SAS Institute, Inc., Cary, NC). Subject characteristics were summarized using descriptive statistics including means, SD, medians, interquartile ranges (IQR), counts and percents. For both the primary and secondary aims the outcome variables were the presence of toothbrush contamination (yes/no) and the amount of toothbrush contamination (CFU/ml) for those toothbrushes that were contaminated. The predictor, or explanatory variables, included environmental characteristics (location, distance to sink, distance to bathroom, storage container, and contact with other articles), weight (in grams), oral colonization variables (any colonization (yes/no) and the amount of colonization (CFU/ml) for those that harbored PPMs), and the oral health status as measured by the DMF score. In addition, other predictor variables, including type of airway, type of PPM, and antibiotic use were examined. Table 3.0 summarizes the various statistical methods that were used to assess the relationships between the two outcome variables and each of the predictor variables. Initially all analyses were done regardless of TIE or type of species; however when sample size permitted, analyses were done by TIE, species, and by both TIE and species. Fisher's exact tests were used in place of chi-square tests when the sample size assumption was not valid.

		Toothbrush Contamination (Y)		
		Nominal Outcome (Yes/No) **Overall and by TIE***		**Continuous Outcome (Log 10 Scale CFU/ml) by TIE***
	Sample Size	100		100
	Predictors Variables	**Statistical Methods**		**Statistical Methods**
Primary Aims	**Environmental**			
	Location (4 categories)	Fisher's		-
	Distance to Sink (inches)	Logistic		Correlation
	Distance to Bathroom (inches)	Logistic		Correlation
	Storage container (Yes/No)	Chi-square		*t*-test
	Contact with Other Articles (Yes/No)	Chi-square/Fisher's		*t*-test
	Weight (Moisture and Debris)			
	Weight (grams; log 10 scale)	Logistic		Correlation
Secondary Aims	**Oral Colonization**			
	Nominal (Yes/No)	Chi-square/Fisher's		*t*-test
	Continuous (CFU/ml)	Logistic		Correlation
	Oral Health Status			
	DMF score (positive integers)	Logistic		Correlation
Other Aims	**Other Variables**			
	Type of Airway (3 categories)	Chi-square/Fisher's		ANOVA
	Antibiotic Use (Yes/No)	Chi-square/Fisher's		*t*-test

*When sample size permits

Table 3.0 Data Analysis

Results

Sample Characteristics

One hundred subjects were enrolled from the medical-respiratory, neuroscience, and surgical trauma ICUs. The subjects were representative in ethnicity, gender, and race for the population at the university medical center where the study was conducted (see Table 4.0).

Variable	Enrolled Sample (N = 100)
Age (years), mean (SD)	53.58 (17.62)
Gender, # Male Female	 61 39
Race, # White Black/African American Asian Other	 63 36 1 0
Intensive Care Unit, # Medical Respiratory Surgical Trauma Neuroscience	 37 32 31
ICU Length of Stay (LOS), median (IQR)	9 (5 to 18.75)
Number of decayed, missing, and filled teeth (DMF Score), mean (SD)	10.92 (7.83)
Airway Status, # Non-ventilated Ventilated Tracheotomy	 59 36 5
Oral Care Frequency, mean (SD)	1.94 (1.87)
History of PPMs, # No Yes	 81 19
PPMs (oral and toothbrush) susceptible to current antibacterial therapy, # PPMs susceptible PPMs not susceptible No current antibiotic therapy	 36 33 31
ICU Admitting Diagnosis, # Neurological Condition Trauma Pulmonary Condition Cardiovascular Condition Other Oncological Condition Infectious Disease Post Surgical Condition	 23 19 17 11 9 8 7 6

Table 4.0 Subject Demographics

Toothbrush Contamination

A total of 14 toothbrushes (14%) were found to be contaminated (see Table 5.0). There was not a significant relationship between TIE and the presence of TB contamination, regardless of species (Fisher *p*-value = 0.77), or for any of the individual species: VRE (Fisher *p*-value = 0.84), MRSA (Fisher *p*-value = 0.42), *Acinetobacter* (Fisher *p*-value > 0.99), or VRE+MRSA (Fisher *p*-value = 0.66).

Presence of TB Contamination				
		TIE Group		
	Overall	**24h**	**48h**	**72h**
Contamination, Count (percent)	**14 (14%)**	**4 (12%)**	**6 (18%)**	**4 (12%)**
VRE only	3	1	1	1
MRSA only	9	2	4	3
VRE + MRSA	1	0	1	0
Acinetobacter spp. only	1	1	0	0
No Contamination	**86**	**30**	**27**	**29**

Table 5.0 Presence of Toothbrush Contamination

The means (CFU/ml) and SD for the 14 toothbrushes that grew PPMs are summarized in Table 6.0 regardless of TIE and by TIE, for each species. There was not a significant difference among the TIE groups in the amount of TB contamination (transformed Log 10 CFU/ml) for MRSA, VRE or *Acinetobacter*.

Bacteria Species	**Amount of TB Contamination (CFU/ml) (Log10 Scale)** Mean (SD)			
		TIE Groups		
	Overall	**24h**	**48h**	**72h**
VRE	2.02 (2.09)	0.52 (-)	2.73 (3.13)	2.07 (-)
MRSA	2.84 (1.97)	1.44 (1.73)	3.75 (2.22)	2.27 (1.25)
Acinetobacter spp.	2.58 (-)	2.58 (-)	-	-

Table 6.0 Amount of Toothbrush Contamination

The primary aim of the study was to describe environmental factors (location, distance to sink, distance to bathroom, storage container, and contact with other articles) associated with toothbrush contamination in the ICU.

Location

The toothbrushes were recovered from the bedside table (14%), the RN cart (46%), the RN drawer (36%), or the sink area (4%). There was a marginally significant association between location of TB and the presence of TB contamination (Fisher *p*-value = 0.05), regardless of TIE. The trend was such that TBs recovered from RN drawers or the sink area were more likely to be contaminated than those found in the bedside table or the RN cart (see Table 7.0).

TB Location (when collected)				
	BS Table	**RN Cart**	**RN Drawer**	**Sink Area**
Total Number Recovered	14	46	36	4
Total Number (%) Contaminated	0 (0%)	4 (9%)	9 (25%)	1 (25%)
24 hours	0 (6%)	0 (13%)	4 (15%)	0 (0%)
VRE	0	0	1	0
MRSA	0	0	2	0
Acinetobacter spp.	0	0	1	0
VRE + MRSA	0	0	0	0
48 hours	0 (6%)	3 (17%)	3 (8%)	0 (2%)
VRE	0	0	1	0
MRSA	0	2	2	0
Acinetobacter spp.	0	0	0	0
VRE + MRSA	0	1	0	0
72 hours	0 (2%)	1 (16%)	2 (13%)	1 (2%)
VRE	0	0	1	0
MRSA	0	1	1	1
Acinetobacter spp.	0	0	0	0
VRE + MRSA	0	0	0	0

Table 7.0 Toothbrush Location

There was a marginally significant relationship between location of TB and the presence of TB contamination (Fisher *p*-value = 0.08) at 24 hours, but the association was not significant at 48 hours (Fisher *p*-value = 0.42) or 72 hours (Fisher *p*-value = 0.33).

Distance to Sink

On average, TBs were recovered 100.3 inches from the sink (SD = 48.9, range = 1 inch to 190 inches). There was not a significant relationship between the distance to the sink and the presence of TB contamination, regardless of TIE (*p*-value = 0.8757), nor at 24 (*p*-value = 0.5610), 48 (*p*-value = 0.852), or 72 hours (*p*-value = 0.8529) in the environment.

Toothbrush Distance to Sink Mean (SD)				
Bacteria Species	**Overall**	**TIE Groups**		
		24 hours	**48 hours**	**72 hours**
VRE	120.7 (35.6)	150.0 (-)	81.0 (-)	131.0 (-)
MRSA	98.2 (57.0)	73.0 (26.9)	108.3 (44.9)	101.7 (93.6)
VRE+MRSA	124.0 (-)	-	124.0 (-)	-
Acinetobacter spp.	61.0 (-)	61.0 (-)	-	-
None	100.0 (48.1)	104.1 (49.6)	91.5 (46.7)	103.8 (51.2)
Overall	**100.3 (48.9)**	**102.3 (48.6)**	**94.2 (45.0)**	**104.4 (53.5)**

Table 8.0 Distance to the Sink when Toothbrush Recovered

There was not a significant relationship between the amount of TB contamination (transformed Log 10 CFU/ml) and the distance to the sink regardless of TIE.

Distance to Bathroom

On average, TBs were recovered 132.1 inches from the bathroom (SD = 58.9, range = 26 inch to 269 inches) (see Table 9.0). There was a trend for the presence of toothbrush contamination to increase as the distance to the bathroom decreased. There was a significant negative relationship between the distance to the bathroom and the

presence of TB contamination, regardless of TIE (*p*-value = 0.045). For every 12 inches closer to the bathroom the TB was recovered, the odds of TB contamination multiplied by 1.13 (95% CI = 1.00, 1.31). There were marginally significant negative relationships between distance to bathroom and the presence of TB contamination at 24 hours (p-value = 0.09) and 48 hours (p-value = 0.09), but no significant relationship at 72 hours (*p*-value = 0.99) in the environment. There were no significant relationships between distance to the bathroom and the amount of TB contamination (transformed Log 10 CFU/ml) regardless of time for the VRE (*r* = 0.51, *p*-value = 0.29) or MRSA (*r* = 0.04, *p*-value = 0.57) bacteria; sample sizes were too small for comparisons of *Acinetobacter.*

Toothbrush Distance to Bathroom Mean (SD)				
	Overall	**24 hours**	**48 hours**	**72 hours**
VRE	105.5 (38.3)	57.0 (-)	132.0 (-)	140.0 (-)
MRSA	104.4 (45.3)	128.5 (48.8)	83.3 (45.6)	116.7 (47.7)
VRE+MRSA	93.0 (-)	-	93.0 (-)	-
Acinetobacter spp.	91.0 (-)	91.0 (-)	-	-
None	136.7 (60.3)	141.7 (50.4)	147.0 (76.3)	122.0 (51.6)
Overall	**132.1 (58.9)**	**137.0 (50.9)**	**137.2 (73.7)**	**122.1 (49.8)**

Table 9.0 Distance to the Bathroom when Toothbrush Recovered

Storage Container

Ninety percent of toothbrushes were recovered from a storage container, either a basin (n = 27), a paper towel (n = 41), or a plastic bag (n = 22). TBs kept in storage containers had TB contamination rates of 11% while TBs not kept in storage containers had contamination rates of 40% (see Table 10.0). There was a significant association between the presence of TB contamination and the use of a storage container (*p*-value = 0.01), regardless of TIE. TBs not kept in storage containers had odds of TB contamination that were 5.33 times greater than those TBs kept in storage containers

(95% CI = 1.20, 22.20). This relationship was not significant at 24 hours (*p*-value = 0.38), but was marginally significant at 48 hours (*p*-value = 0.08) and 72 hours (*p*-value = 0.09).

TB Storage Container				
	Basin	**Paper Towel**	**Plastic Bag**	**None**
Total Number Recovered	27	41	22	10
Total Number Contaminated	**4 (15%)**	**4 (10%)**	**2 (9%)**	**4 (40%)**
24 hours Number (%) Contaminated	2 (15%)	0 (9%)	1 (6%)	1 (4%)
VRE	0	0	0	1
MRSA	2	0	0	0
Acinetobacter spp.	0	0	1	0
VRE + MRSA	0	0	0	0
48 hours Number (%) Contaminated	2 (6%)	2 (16%)	0 (7%)	2 (4%)
VRE	1	0	0	0
MRSA	1	2	0	1
Acinetobacter spp.	0	0	0	0
VRE + MRSA	0	0	0	1
72 hours Number (%) Contaminated	0 (6%)	2 (16%)	1 (9%)	1 (2%)
VRE	0	1	0	0
MRSA	0	1	1	1
Acinetobacter spp.	0	0	0	0
VRE + MRSA	0	0	0	0

Table 10.0 Toothbrush Storage Container

For the 14 contaminated toothbrushes, the amount of contamination was examined for a relationship with the use of a storage container by species, regardless of TIE. There was not a significant relationship between the use of a storage container and the amount of contamination (transformed Log 10 CFU/ml) for VRE (*p*-value = 0.17), or MRSA (*p*-value = 0.49). Sample sizes were not large enough to examine the

relationship between the use of a storage container and the amount of contamination for each species by TIE (see Table 11.0)

Amount of Toothbrush Contamination (Log 10 Scale)			
		Storage Container	
		Yes	No
	Total Number Recovered	90	10
24 hours	**Bacteria Species**	**Mean CFU/ml (SD)**	
	VRE	-	0.5 (-)
	MRSA	1.5 (1.7)	-
	Acinetobacter spp.	2.6 (-)	-
48 hours			
	VRE	4.9 (-)	0.5 (-)
	MRSA	3.7 (0.8)	3.9 (4.3)
	Acinetobacter spp.	-	-
72 hours			
	VRE	2.0 (-)	-
	MRSA	2.0 (1.6)	2.9 (-)
	Acinetobacter spp.	-	-
Overall			
	VRE	3.5 (2.0)	0.5 (0.0)
	MRSA	2.5 (1.5)	3.5 (3.0)
	Acinetobacter spp.	2.6 (-)	-

Table 11.0 Amount of Toothbrush Contamination

Contact with Other Articles

Ninety-one percent of the contaminated toothbrushes were in contact with some type of patient care article. There were 3 categories identifying the toothbrushes' contact with other articles: bathing and wound care products, oral care products, and no other articles. Toothbrush contact with specific categories of articles was not related to the presence of toothbrush contamination, regardless of TIE (*p*-value = 0.93), nor at 24 hours (Fisher *p*-value = 0.36), 48 hours (Fisher *p*-value = 0.44), or 72 hours (Fisher *p*-value = 0.22) in the environment (see Table 12.0).

TB Contact with Other Articles			
	Bathing and Wound Care	**Oral Care**	**None**
Total Number Recovered	38	53	9
Total Number Contaminated (%)	**5 (13%)**	**8 (15%)**	**1 (11%)**
24 hours	12 (0%)	21 (40%)	1 (11%)
VRE	0	1	0
MRSA	0	2	0
Acinetobacter spp.	0	1	0
VRE + MRSA	0	0	0
48 hours	11 (29%)	16 (30%)	6 (67%)
VRE	1	0	0
MRSA	2	2	0
Acinetobacter spp.	0	0	0
VRE + MRSA	0	1	0
72 hours	15 (39%)	16 (30%)	2 (22%)
VRE	1	0	0
MRSA	1	1	1
Acinetobacter spp.	0	0	0
VRE + MRSA	0	0	0

Table 12.0 Toothbrush Contact with Other Articles

The amount of TB contamination for the 14 contaminated TBs is summarized in Table 13.0 by type of article in contact with the toothbrush. There was not a significant relationship between contact with articles and the amount of contamination (transformed Log 10 CFU/ml) for VRE (*p*-value = 0.17) or MRSA (*p*-value = 0.97). Sample sizes were not large enough to examine the relationship between contact with articles and the amount of contamination for *Acinetobacter* or for each species by TIE (see Table 13.0).

TB Contact with Other Articles (Log 10 Scale)				
		Bathing and Wound Care	**Oral Care**	**None**
	Total Number in Contact	**38**	**53**	**9**
	Total # Contaminated (%)	**5 (13%)**	**9 (17%)**	**1 (11%)**
24 hours	**Bacteria Species**	**Mean CFU/ml (SD)**		
	VRE	-	0.52 (-)	-
	MRSA	-	1.45 (1.73)	
	Acinetobacter spp.	-	2.58 (-)	-
48 hours				
	VRE	4.95 (-)	0.52 (-)	-
	MRSA	3.47 (1.01)	3.93 (3.04)	-
	Acinetobacter spp.	-	-	-
72 hours				
	VRE	2.07 (-)	-	-
	MRSA	0.82 (-)	3.08 (-)	2.90 (-)
	Acinetobacter spp.	-	-	-
Overall				
	VRE	**3.51 (2.04)**	**0.52 (0.0)**	
	MRSA	**2.59 (1.69)**	**2.96 (2.40)**	**2.90 (-)**
	Acinetobacter spp.		**2.58 (-)**	

Table 13.0: Amount of Toothbrush Contamination Contact with Other Articles

Weight (Moisture and Debris)

Ninety percent of the toothbrushes had measurable weight (grams). The amount of weight is summarized by species and overall in Table 14.0. For these 90 TBs, the amount of weight (transformed Log 10 grams) was positively associated with the presence of TB contamination at a marginal level of significance, regardless of TIE (*p*-value = 0.09), and at 24 hours (*p*-value = 0.09) and 48 hours (*p* = 0.07) in the environment; however there was no significant relationship at 72 hours (*p* = 0.60). That is, increased levels of moisture and debris were marginally associated with increases in the odds of TB contamination at 24 and 48 hours in the environment. Regardless of TIE, there were not significant relationships between the presence of TB contamination and the amount of moisture and debris for VRE (*p* = 0.88); however there was a marginally

positive relationship for MRSA (p = 0.09). There were not significant associations between the amount of TB contamination (transformed Log 10 CFU/ml) and the amount of moisture and debris for VRE (r = 0.08, p = 0.72) or MRSA (r = 0.03, p = 0.63).

		Overall	Time in Environment		
Bacteria Species	**Number of Toothbrushes ***	**Weight (grams)** *Mean* (SD)	**24 hr**	**48 hr**	**72 hr**
VRE	4	0.15 (0.13)	0.16 (-)	0.21 (0.16)	0.01 (-)
MRSA	9	0.27 (0.23)	0.33 (0.37)	0.26 (0.20)	0.24 (0.28)
Acinetobacter spp.	1	0.49 (-)	0.49 (-)	-	-
No PPM of interest	77	0.17 (0.23)	0.15 (0.17)	0.15 (0.22)	0.22 (0.29)
Total	90	0.18 (0.23)	0.17 (0.19)	0.18 (0.22)	0.21 (0.28)

* One grew more than 1 species (MRSA +VRE)

Table 14.0 Toothbrush Weight

The secondary aim of this study was to describe the relationship between toothbrush contamination and oral colonization in critically ill adults.

Oral Colonization

The baseline oral cultures were positive for PPMs in 20% of subjects (see Table 15.0). Two subjects grew more than 1 species at baseline (*Acinetobacter*+ MRSA+VRE and MRSA + VRE*)*. The presence of PPMs on repeat oral culture, completed when the toothbrush was collected, was 19%. One subject grew more than 1 species with the repeat culture (*Acinetobacter*+ MRSA+VRE*)*. Two subjects had PPM growth on their toothbrush but had negative baseline and repeat cultures (VRE and MRSA). Two subjects had negative baseline cultures followed by PPM growth on their toothbrush and repeat oral cultures (MRSA and *Acinetobacter*) (see Table 15.0).

Bacteria Species	Baseline Oral Culture (Log 10 Scale) (When TB deployed)		Repeat Oral Culture (Log 10 Scale) (When TB collected)	
	Colonized (Yes/No)	**Amount of Colonization (CFU/ml)** *Mean (SD)*	**Colonized** (Yes/No)	**Amount of Colonization (CFU/ml)** *Mean (SD)*
VRE	9	2.59 (1.64)	6	2.39 (2.28)
MRSA	10	3.90 (2.57)	11	3.29 (2.00)
Acinetobacter spp.	4	1.78 (1.31)	4	1.74 (1.43)

* Two grew more than 1 species (MRSA +VRE and MRSA + *Acinetobacter*)

Table 15.0 – Oral Colonization: All PPM Positive Baseline and Repeat Cultures

Oral Health

The trend was such that increases in DMF scores tended to decrease the probability of TB contamination. However, there was no significant association between the DMF score and the presence of toothbrush contamination, regardless of TIE (*p*-value = 0.35), nor at 24 hours (*p*-value = 0.52), 48 hours (*p*-value = 0.92), or 72 hours (*p*-value = 0.33) in the environment. The association between DMF scores and amount of toothbrush contamination were not significant for either VRE ($r = 0.66$, $p = 0.19$) or MRSA ($r = 0.01$, *p*-value = 0.80), and could not be tested for *Acinetobacter*.

Antibacterial Therapy

Of the 100 enrolled subjects, 69% of the subjects were on antibiotic therapy and 31% were not. There were 13 toothbrushes that were positive for one or more of the 3 PPMs of interest. Three of these were from subjects who were not on any antibiotic therapy at the time the culture was obtained; 6 were being treated with antibiotics to which MRSA, VRE, and *Acinetobacter* show susceptibility; 4 were being treated with

antibiotics to which MRSA, VRE, and *Acinetobacter* were not susceptible (see Table 16.0).

There were 20 baseline oral cultures that were positive for one or more of the 3 PPMs of interest. Six of these were from subjects who were not on any antibiotic therapy at the time the culture was obtained; 9 were being treated with antibiotics to which MRSA, VRE, and *Acinetobacter* showed susceptibility; 5 were being treated with antibiotics to which MRSA, VRE, and *Acinetobacter* were not susceptible.

There were 16 repeat oral cultures that were positive for one or more of the 3 PPMs of interest. Four of those were not on any antibiotic therapy at the time the culture was obtained; 7 were being treated with antibiotics to which MRSA, VRE, and *Acinetobacter* showed susceptibility; 5 were being treated with antibiotics to which MRSA, VRE, and *Acinetobacter* were not susceptible.

Bacteria Species	Toothbrush Contamination	Antibacterial Therapy	No Antibacterial Therapy
VRE	4	4	0
MRSA	10	7	3
Acinetobacter spp.	1	0	1

* One grew more than 1 species (MRSA +VRE)

Table 16.0 – Antibacterial Therapy

Clinical Sample Concordance

A total of 12 paired samples (toothbrush and oral cultures) were subjected to PCR amplification and sequencing. No paired samples yielded sequences for both strands for all seven specific housekeeping genes, which is a requirement

for MLST analysis, so MLST could not be completed.

The online nucleotide BlastN analysis confirmed that four of the paired samples were *S. aureus*. These paired samples produced at least one amplicon each using MLST housekeeping gene primers specific for *S. aureus*. In all cases, all gene sequences obtained from paired samples were determined to be identical to one another, suggesting that the paired isolates originated from the same source. Interestingly, the paired isolates from two patients (V002 and V007) were also indistinguishable, suggesting they may have had a common source (see Table 17.0).

Subject and Sample Source	**ICU**	***S. aureus* Specific Housekeeping Genes**						
		ARC	**AROE**	**GLPF**	**GMK**	**PTA**	**TPI**	**YQIL**
P009 OC1	MRICU	1	-	1	-	1	1	1
P009 TB		1	-	1	1	1	1	1
P009 OC2		1	-	-	-	1	1	-
V002 OC1	NSICU	1	1	1	-	-	-	-
V002 TB		1	1	1	1	1	1	-
V002 OC2		1	-	-	-	1	1	2
V007 OC1	MRICU	1	1	1	1	1	-	2
V007 TB		1	1	1	-	1	1	-
V007 OC2		1	1	1	1	1	1	2
V029 OC1	MRICU	-	-	-	-	-	-	-
V029 TB		2	2	1	2	1	2	3
V029 OC2		-	-	1	-	-	-	-

OC1 = Baseline oral culture OC2 = Repeat oral culture
1 = allele type one 2 = allele type two 3 = allele type three

Table 17.0 *S. aureus* Housekeeping Gene Comparison

The BlastN analysis confirmed that one of the paired samples (V033) was *E. faecalis* (see Table 18.0). All gene sequences obtained from paired samples were

identical to one another, suggesting that the paired isolates originated from the same source.

Subject and Sample Source	ICU	*E. faecalis* Specific Housekeeping Genes						
		GDH	GYD	PST	GKI	AROE	XPT	YIQL
V033 OC1	MRICU	1	1	1	1	1	1	1
V033 TB		1	1	1	1	1	1	1

OC1 = Baseline oral culture OC2 = Repeat oral culture 1 = allele type one

Table 18.0 *E. faecalis* Housekeeping Gene Comparison

BlastN analysis revealed that one of the samples (V009 baseline oral culture), which was identified by selective media as VRE, was actually *E. faecium* even though the primers were not intended for this species. No amplicons were obtained from this subject's second oral or TB samples and could not be compared.

Six of the paired samples yielded either few or no PCR products using the housekeeping gene primers, making comparison between TB and oral cultures impossible. In an attempt to determine the cause for this, these samples were subjected to 16S rRNA gene amplification and sequencing. This revealed that there were two subjects (with V035 yielding two types of PPM) who had paired samples which were not correctly identified by the selective media (see Table 19.0). Because the primers used for the housekeeping genes were intended for use with only the species of interest[24, 39], [2] this misidentification likely explains the failure of the housekeeping gene analysis for these strains.

Subject	Selective Media Identification	16S rRNA Identification		
		Baseline Oral Culture	Toothbrush	Repeat Oral Culture
V035	*Acinetobacter*	*Neisseria flavescens*	*Pseudomonas aeruginosa*	*Neisseria flavescens*
	MRSA			*Neisseria flavescens*
V087	VRE	*Lactobacillus rhamnosus*		*Lactobacillus rhamnosus*

Table 19.0 Bacteria Identification Based on Selective Media and 16S rRNA Sequence Analysis

Discussion

We found that toothbrushes in the ICU became contaminated with MRSA, VRE, and *Acinetobacter*. PPMs were cultured from toothbrushes at 24, 48 and 72 hours after deployment, which is consistent with previous studies in other environments that found bacterial survival and retention on toothbrushes after use[10, 11, 25, 32, 36]. As previously reported in the literature, contaminated toothbrushes had varied bacterial loads, with some retaining more than one species at the same time[67]. Since bacteria are able to accumulate and survive on toothbrushes, toothbrushes might act as fomites and increase risk of infection in the critically ill. Additional studies linking contamination to patient outcomes are critical in understanding the level of risk.

This study explored multiple environmental factors possibly related to toothbrush contamination: location, distance to the bathroom and sink, storage containers, contact with other articles, and moisture. In the ICU environments included in this study, each patient room has a large rolling cart with five large drawers used for storing nursing supplies and patient care equipment. The majority of the toothbrushes (82%) were located on top of the nursing cart or in the drawer of the nursing cart and constituted all but one of the contaminated toothbrushes in this study. In addition, all but 1 of the

contaminated toothbrushes in this study were in contact with other patient care articles. Of the 9% of toothbrushes not in contact with other articles, only one toothbrush was contaminated (MRSA) and the patient had a known history of MRSA. The drawers of the cart had the highest survival of all 3 PPMs. The drawers are a closed environment with decreased air flow and place the toothbrush in closer contact with other articles potentially increasing the likelihood for contamination. It seems that location of the toothbrush is an important factor, and one that nurses generally decide based on convenience or tradition rather than potential for contamination. Based on our data, a closed drawer or storage with multiple other care items is not ideal; the bedside table, which tends to have less use in procedural care, may be preferred. Alternately, more attention could be paid to reducing cross contamination related to the nursing cart. There is no current policy for the routine decontamination of the cart or drawers during the patient's ICU stay. Further research is needed to explore contamination of nursing carts in the ICU.

We anticipated that a shorter distance to the bathroom or sink would be associated with more contamination. We found it surprising that there was no significant relationship between toothbrush contamination and distance to the sink. For the distance to the bathroom, there was a trend for the presence of TB contamination to increase as the distance to the bathroom increased. The small number of contaminated toothbrushes and the relative lack of variability in room arrangement may have affected our ability to detect an effect of distance if one exists.

The use of a storage container was associated with the presence toothbrush contamination which is consistent with published literature[18]. Contamination also occurred with toothbrushes not found in a storage container which may be a result of contact with contaminated hands, surfaces or aerosol contamination. The American Dental Association (ADA) recommends keeping toothbrushes separate from items that may harbor bacteria[4]. There was significant variation in nursing practice related to toothbrush storage and included storing the toothbrush in contact with items that are known to harbor bacteria[12, 29, 42].

Ninety percent of the used toothbrushes had measurable additional weight (retained moisture and debris). Previous studies found that increased humidity and moisture supported bacteria survival on toothbrushes[30] which may have contributed to the contamination of the toothbrushes in this study. Some toothbrushes were visibly moist, while others were not. The mean weight for toothbrushes contaminated with *Acinetobacter* was higher (0.525 grams) than the other 2 PPMs which is consistent with *Acinetobacter*'s affinity for moist environments[6]. There was a positive trend for MRSA; however, the finding was limited by low power. Another limitation was the inability of our moisture measurement (weight) to differentiate between moisture and debris retained on the toothbrushes. We did not examine the effect of toothpaste, mouthwash, or chlorhexidine use on toothbrush contamination in this study. Further research to examine the effect of specific oral care products on toothbrush contamination in the ICU would be useful.

We examined the relationship between oral health, oral contamination and toothbrush contamination. Two of the subjects had negative baseline oral cultures,

positive toothbrush contamination, and a subsequent positive oral culture that matched the toothbrush species. In addition, we found one toothbrush was contaminated with a different species of bacteria than the species found in the oral cultures. The mean DMF score of this sample was 10.92 which indicate the presence of caries and disease in approximately 40% of the teeth. For VRE, as the patient's oral health decreased, the risk of toothbrush contamination increased. Decreased oral health in combination with potentially contaminated oral equipment, and altered oral physiology in ICU patients create a favorable environment for bacterial survival and proliferation.

We examined the genetic relationship of PPM isolates obtained from paired samples (toothbrush and oral cultures). We found that the selective media did not correctly identify the PPM of interest in six of the individual samples. Genetic evaluation was only conducted on the samples in which there was a match between the TB and one or both of the oral cultures. In future studies, we would recommend the use of 16S rRNA sequencing to determine species prior to sequencing of housekeeping genes to evaluate bacterial strains for all positive samples. The results of the allele comparison for MRSA suggested that there was one strain shared by two subjects. It is possible that there is a dominant strain of MRSA in this hospital environment. Future studies examining particular strains in the three ICUs would be useful.

There was significant disparity in nursing practice related to toothbrush use and oral care which was echoed in previous studies[17, 28, 34, 43, 52, 59]. Toothbrush use and practice was varied between nurses. There was variation in the number of times oral care was documented in a 24 hour period. This documentation did not specify tooth brushing versus swabbing. It is recommended that healthy adults brush their teeth 2-3

times a day[4]. There were twelve toothbrushes that appeared not to have been used, indicating that some nurses may not brush the patient's teeth at all. Many nurses verbalized a preference for oral swabs to toothbrushes, especially in intubated and facial trauma patients where tooth brushing often leads to increased agitation and pain. AACN guidelines recommend the toothbrush as the tool of choice for oral care and that toothbrushes are the best tool for reducing plaque and preventing disease[1,44].

There is a need for standardized nursing guidelines to prevent toothbrush contamination, which may increase risk of infection from PPMs. Toothbrushes will remain in the ICU environment, since tooth brushing is an important part of maintaining oral hygiene and other products such as foam swabs are not acceptable alternatives. Based on our study and what is known from studies of contamination in other settings, we think it is reasonable for nurses to carefully consider their handling and storage of this personal care item. While guidelines for toothbrush decontamination, storage, and reuse and oral care education have not been tested in the ICU, several actions are reasonable based on available data. Contamination is less likely if the toothbrush is rinsed well after use, stored in a dry, well ventilated space and kept apart from other patient care items (particularly bathing, continence, and wound care items).

References

1. AACN. Oral care for patients at risk for ventilator-associated pneumonia. In: AACN, ed; 2010.
2. Aanensen D. Multi Locus Sequence Typing. Available at: http://www.mlst.net/, 2011.
3. Abidia RF. Oral care in the intensive care unit: a review. *J Contemp Dent Pract.* Jan 1 2007;8(1):76-82.
4. ADA. ADA statement on toothbrush care: Cleaing, storage and replacement. Available at: http://www.ada.org/1887.aspx. Accessed 1/27, 2009.
5. Aygun G, Demirkiran O, Utku T, et al. Environmental contamination during a carbapenem-resistant Acinetobacter baumannii outbreak in an intensive care unit. *J Hosp Infect.* Dec 2002;52(4):259-262.
6. Bergogne-Berezin ET, K. . Acinetobacter spp. As nosocomial pathogens: microbiological, clinical, and epidemiological features. . *Clinical Microbiology Reviews.* 1996;9(2):148-165.
7. Berry A, & Davidson, P. . Beyond comfort: Oral hygiene as a critical nursing activity in the intensive care unit. . *Intensive & Critical Care Nursing.* 2006;22(6):318-328.

8. Berry AM, Davidson PM, Masters J, Rolls K. Systematic literature review of oral hygiene practices for intensive care patients receiving mechanical ventilation. *Am J Crit Care.* Nov 2007;16(6):552-562; quiz 563.

9. Bonten M, Hayden, M, Nathan, C., van Voorhis, J., Matushek, M., Slaughter, S., Rice, T. & Weinstein, R. . Epidemiology of colonization of patients and environment with vancomycin-resistant enterococci. *The Lancet.* 1996;348:1615-1619.

10. Boyce JM. Environmental contamination makes an important contribution to hospital infection. *J Hosp Infect.* Jun 2007;65 Suppl 2(S2):50-54.

11. Bunetel L, Tricot-Doleux S, Agnani G, Bonnaure-Mallet M. In vitro evaluation of the retention of three species of pathogenic microorganisms by three different types of toothbrush. *Oral Microbiol Immunol.* Oct 2000;15(5):313-316.

12. Caudry S, Klitorinos, A., & Chan, E. . Contaminated toothbrushes and their disinfection. . *Journal Canadian Dental Association.* 1995;61(6):511-516.

13. CDC. In CDC's National Center for Infectious Diseases. Available at: http://www.cdc.gov/ncidod/dhqp/hai.html, 2009.

14. CDC. Healthcare-Associated Infections: Recovery Act. Available at: http://www.cdc.gov/nhsn/ra/index.html, 2009.

15. Chenna R, Sugawara, H., Koike, T., Lopez, R., Gibson, TJ., Higgins, DG., Thompson, JD. Multiple sequence alignment with the Clustal series of programs. . *Nucleic Acids Res.* 2003;31(13):3497-3500.

16. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. *Clin Infect Dis.* Jan 1 2003;36(1):53-59.

17. Cutler C, & Davis, N. . Improving oral care in patients receiving mechanical ventilation. . *American Journal of Critical Care.* 2005;14(5):389-394.

18. Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. *J Dent Res.* Jun 1977;56(6):706.

19. Edmond MB, Ober JF, Dawson JD, Weinbaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. *Clin Infect Dis.* Dec 1996;23(6):1234-1239.

20. Edmond MB, Wenzel RP, Pasculle AW. Vancomycin-resistant Staphylococcus aureus: perspectives on measures needed for control. *Ann Intern Med.* Feb 1 1996;124(3):329-334.

21. Edmond MB. Nosocomial infections in the United States hospitals: a three year analysis. *Clinical Infectious Diseases.* 1999;29(2):239-244.

22. Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos IA, Panis V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. *J Dent.* Apr 2007;35(4):331-337.

23. El-Solh AA, Pietrantoni C, Bhat A, et al. Colonization of dental plaques: a reservoir of respiratory pathogens for hospital-acquired pneumonia in institutionalized elders. *Chest.* Nov 2004;126(5):1575-1582.

24. Enright M. DN, Davies C., Peacock S. & Spratt B. . Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. . *Journal of Clinical Microbiology.* 2000;38(3):1008-1015.

25. Fitch JA, Munro CL, Glass CA, Pellegrini JM. Oral care in the adult intensive care unit. *Am J Crit Care.* Sep 1999;8(5):314-318.

26. Fourrier F, Duvivier B, Boutigny H, Roussel-Delvallez M, Chopin C. Colonization of dental plaque: a source of nosocomial infections in intensive care unit patients. *Crit Care Med.* Feb 1998;26(2):301-308.

27. Fourrier F, Cau-Pottier E, Boutigny H, Roussel-Delvallez M, Jourdain M, Chopin C. Effects of dental plaque antiseptic decontamination on bacterial colonization and nosocomial infections in critically ill patients. *Intensive Care Med.* Sep 2000;26(9):1239-1247.

28. Furr L, Binkley, C., McCurren,C., & Carrico, R. . Factors affecting quality of oral care in intensive care units. . *Journal of Advanced Nursing.* 2004;48(5):454-462.

29. Glass RT. The infected toothbrush, the infected denture, and transmission of disease: a review. *Compendium.* Jul 1992;13(7):592, 594, 596-598.

30. Glass RT. Toothbrush types and retention of microorganisms: How to choose a biologically sound toothbrush. . *Journal - Oklahoma Dental Association.* 1992;82(3):26-28.

31. Glass RT, & Jensen, H. G. . The effectiveness of a u-v toothbrush sanitizing device in reducing the number of bacteria, yeasts and viruses on toothbrushes. . *Journal - Oklahoma Dental Association.* 1994;84(4):24-28.

32. Glass RT, & Lare, M. M. . Toothbrush contamination: A potential health risk? . *Quintessence International.* 1986;17(1):39-42.

33. Goldschmidt MC, Warren, D. P., Keene, H. J., Tate, W. H., & Gowda, C. Effects of an antimicrobial additive to toothbrushes on residual periodontal pathogens. . *The Journal of Clinical Dentistry.* 2004;15(3):66-70.

34. Grap MJ, Munro, C. L., Ashtiani, B., & Bryant, S. . Oral care interventions in critical care: Frequency and documentation. . *American Journal of Critical Care.* 2003;12(2):113-118.

35. Graves N. Economics and preventing hospital-acquired infection. . *Emerging Infectious Diseases.* 2004;10(4):561-566.

36. Grewal N, & Swaranjit, K. . A study of toothbrush contamination at different time intervals and comparative effectiveness of various disinfecting solutions in reducing toothbrush contamination. . *Journal of the Indian Society of Pedodontics and Preventive Dentistry.* 1996;14(1):10-13.

37. Hardy K, Oppenheim, B., Gossain, S., Gao, F. & Hawkey, P. . A study of the relationship between environmental contamination with Methicillin-Resistant Staphylococcus Aureus (MRSA) and patient's acquisition of MRSA. *Infection Control and Hospital Epidemiology.* 2006;27(2):127-132.

38. Hedin G, Fang H. Evaluation of two new chromogenic media, CHROMagar MRSA and S. aureus ID, for identifying Staphylococcus aureus and screening methicillin-resistant S. aureus. *J Clin Microbiol.* Aug 2005;43(8):4242-4244.

39. Heo S, Haase, E., Lesse, A., Gill, S. & Scannapieco, F. . Genetic Relationships between Respiratory Pathogens Isolated from Dental Plaque and Bronchoalveolar Lavage Fluid from Patients in the Intensive Care Unit Undergoing Mechanical Ventilation. . *Clinical Infectious Diseases.* 2008;47:1562–1570.

40. Hidron A, Edwards, J., Patel, J., Horan, T., Sievert, D., Pollock, D. & Fridkin, S. . Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Annual Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. . *Infection Control and Hospital Epidemiology.* 2008;29(11):996-1011.

41. Hota B. Contamination, disinfection and cross-contamination: Are hospital surfaces reservoirs for nosocomial infection? . *Clinical Infectious Diseases.* 2004;39:1182-1189.

42. Johnson D, Lineweaver, L. & Maze, L. . Patient's Bath Basins as Potential Sources of Infection: A Multicenter Sampling Study. . *American Journal of Critical Care.* 2009;18(1):31-40.

43. Jones H, Newton, J., & Bower, E. . A survey of the oral care practices of intensive care nurses. . *Intensive & Critical Care Nursing.* 2004;20(2):69-76.

44. Kite K, & Pearson, L. . A rationale for mouth care: The integration of theory with practice. . *Intensive & Critical Care Nursing.* 1995;11(2):71-76.

45. Klein HP, C.E. Studies on Dental Caries: X. a procedure for the recording andstatistical processing of dental examination findings. *Journal of Dental Research.* 1940;19.

46. Klevens R, Edwards, J., Richards, C., Horan, T., Gaynes, R., Pollock, D. & Cardo, D. . Estimating health care-associated infections and deaths in U.S. hospitals, 2002. . *Public Health Reports.* 2007;122:160-166.

47. Kolenbrander P, Andersen, R., Blehert, D., Egland, P., Foster, J. & Palmer, R. . Communication among oral bacteria. . *Microbiology and Molecular Biology Reviews.* 2002;66(3):486-505.

48. Kramer A, Schwebke, I. & Kampf, G. . How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. . *BMC Infectious Diseases.* 2006;6(130):1-8.

49. Larson Eea. Assessment of two hand hygiene regimens for intensive care unit personnel. *Critical Care Medicine.* 2001;29(5):944-951.

50. Levin P, Shatz, O., Sviri, S., Moriah, D., Or-Barbash, A., Sprung, C., Moses, A. & Block, C. . Contamination of portable radiographic equipment with resistant bacteria in the ICU. . *Chest.* 2009;136(2):426-432.

51. Mehta A, Sequeira, P. S., & Bhat, G. . Bacterial contamination and decontamination of toothbrushes after use. . *The New York State Dental Journal.* 2007;73(3):20-22.

52. Munro C, & Grap, M. . Oral health and care in the intensive care unit: State of the science. . *American Journal of Critical Care.* 2004;13(1):25-33.

53. Munro C, Grap, M., Elswick, R., McKinney, J., Sessler, C. & Hummel, R. . Effect of oral health status on the development of ventilator associated pneumonia: A descriptive study. . *American Journal of Critical Care.* 2006;15:453-460.

54. Munro CL, Grap MJ, Jones DJ, McClish DK, Sessler CN. Chlorhexidine, toothbrushing, and preventing ventilator-associated pneumonia in critically ill adults. *Am J Crit Care.* Sep 2009;18(5):428-437; quiz 438.

55. Neely AM, M. . Survival of enterococci and staphylococci on hospital fabrics and plastic. . *Journal of Clinical Microbiology.* 2000;38(2):724-726.

56. Nelson P, Macari, S., Faria, G., Assed, S., & Ito, I. . Microbial contamination of toothbrushes and their decontamination. . *Pediatric Dentistry.* 2000;22(5):381-384.

57. O'Reilly M. Oral care of the critically ill: A review of the literature and guidelines for practice. . *Australian Critical Care.* 2003;16(3):101-110.

58. Paterson DL. The epidemiological profile of infections with multidrug-resistant Pseudomonas aeruginosa and Acinetobacter species. *Clin Infect Dis.* Sep 1 2006;43 Suppl 2(S2):S43-48.

59. Pellegrini JM, Fitch, J., Munro, C., & Glass, C. . Oral hygiene in the intensive care unit: An interdisciplinary approach to oral health. . *Journal of Practical Hygiene.* 1997;6:15-17.

60. Pobo A, Lisboa T, Rodriguez A, et al. A randomized trial of dental brushing for preventing ventilator-associated pneumonia. *Chest.* Aug 2009;136(2):433-439.

61. Quirynen M, De Soete, M., Pauwels, M., Gizani, S., Van Meerbeek, B., & van Steenberghe, D. . Can toothpaste or a toothbrush with antibacterial tufts prevent toothbrush contamination? . *Journal of Periodontology.* 2003;74(3):312-322.

62. Scannapieco F, Stewart, E., & Mylotte, J. . Colonization of dental plaque by respiratory pathogens in medical intensive care patients. . *Critical Care Medicine.* 1992;20(6):740-745.

63. Scott D. The direct medical costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention. . 2009.

64. Taji SR, A. . ADRF trebitsch scholarship. the microbial contamination of toothbrushes. A pilot study. . *Australian Dental Journal.* 1998;43(2):128-130.

65. Talon D. The role of the hospital environment in the epidemiology of multi-resistant bacteria. . *Journal of Hospital Infection.* 1999;43(13-17).

66. Todar K. The Normal Bacterial Flora of Humans. Available at: http://www.textbookofbacteriology.net/normalflora.html.

67. Verran J, & Leahy-Gilmartin, A. . Investigations into the microbial contamination of toothbrushes. . *Microbios.* 1996;85(345):231-238.

68. Villegas MV, Hartstein AI. Acinetobacter outbreaks, 1977-2000. *Infect Control Hosp Epidemiol.* Apr 2003;24(4):284-295.

69. Warren D, Goldschmidt, M., Thompson, M., Adler-Storthz, K., & Keene, H. . The effects of toothpastes on the residual microbial contamination of toothbrushes. . *Journal of the American Dental Association.* 2001;132(9):1241-1245.

70. Wilks M, Wilson, A., Warwick, S., Price, E., Kennedy, D., Ely, A. & Millar, M. Control of an outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus colonization and infection in an intensive care unit (ICU) without closing the ICU or placing patients in isolation. . *Infection Control Hospital Epidemiology.* 2006;27:654-658.

71. Wilson M. Bacterial biofilms and human disease. *Sci Prog.* 2001;84(Pt 3):235-254.

72. Yoon M, & Steele, C. . The oral care imperative: The link between oral hygiene and aspiration pneumonia. . *Topics in Geriatric Rehabilitation.* 2007;23(3):280-288.

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