TITLE

Pulsed UV Xenon Disinfection to Prevent Resistant Healthcare Associated Infection

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Pulsed UV Xenon Disinfection to Prevent Resistant Healthcare Associated Infection

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SPECIFIC AIMS: In 2011, 721,800 healthcare-associated infections (HAIs) were reported in the United States. The hospital environment has been increasingly recognized as an important determinant of HAI acquisition. Terminal cleaning of a patient room is recognized as a critically important process to help prevent HAI and involves extensive disinfection of the room after a patient has been discharged from and before the subsequent patient has been admitted to the room. Pulsed xenon ultraviolet light (PX-UV) has been shown to be effective in killing a variety of pathogens including endospores of *Clostridium difficil*e, vegetative bacteria and viruses that are responsible for HAIs in the patient care environment. In recent observational studies, hospital-wide implementation of PX-UV was associated with a decrease in healthcare associated *C. difficile* infections and resultant mortality and morbidity. To date, however, the clinical impact of adding PX-UV to terminal cleaning has not been demonstrated in clinical trials with sham controls or in a crossover design. Determining the efficacy of PX-UV light in reducing HAIs is of critical importance, as HAIs have been recognized as a major patient safety threat. Our **long-term goal** is to establish the efficacy of terminal cleaning plus PX-UV in reducing rates of hospital-acquired environmentally-implicated HAI (eiHAI). In this proposal, eiHAI will be defined as acquisition of one or more of the following pathogens by a patient while on study unit: *C. difficile*, vancomycin-resistant enterococci (VRE), *Klebsiella pneumonia* and *Escherichiae coli*-producing extended-spectrum beta-lactamases (ESBLs), methicillin-resistant *Staphylococcus aureus* (MRSA) or *Acinetobacter baumannii.*

OBJECTIVE: To conduct a prospective, sham controlled, double-blinded, interventional crossover trial to compare standard terminal cleaning plus sham PX-UV (control) with standard terminal cleaning plus PX-UV (intervention) with crossover at 12 months, following a 6-month washout period. Outcome measures include the rates of eiHAIs, as well as the recurrence of genetically identical strains of eiHAIs in patients on study units. The study will be conducted in 2 hospitals and 16 hospital units consisting of a total of 379 beds. A total of three PX-UV devices and three sham devices will be used as part of terminal cleaning following each discharge on study units.
Our central hypothesis is that the addition of PX-UV to standard terminal cleaning will be associated with superior infection prevention, resulting in a reduction in the rate of eiHAIs, as well as a reduction in the recovery of genetically identical strains of drug-resistant organisms.
At the conclusion of the proposed project, novel data will be generated from a rigorously controlled study regarding the effectiveness of PX-UV in reducing eiHAIs in a representative healthcare setting.

Aim 1: To evaluate the impact of PX-UV disinfection on rates of eiHAIs on study units by:

1.1. Comparing rates of eiHAIs on a) study units where PX-UV is added to standard terminal cleaning practices to b) units where a sham UV disinfection system is added to standard terminal cleaning.

1.2. Comparing Rates of eiHAIs on the same medical ward during each of two 12-month phases of a crossover study (one phase when a PX-UV device is added and one when a sham device is added to standard terminal cleaning).

Method 1: PX-UV Devices will be used following each discharge on study units, with intervention devices emitting germicidal UV on 50% of the units and sham devices not emitting germicidal UV on 50% of the units, with device users and the study team blinded to the device's sham/intervention status. Rates of eiHAIs will be the primary outcome. Other key variables to be evaluated will include: types and quantities of antibiotics administered, hand hygiene compliance, room cleanliness quality monitoring through fluorescent marking, colonization pressure on study units; and co-morbid conditions and demographics of enrolled patients.

Aim 2: To evaluate the pattern of repetition of genetically identical strains of eiHAIs in patients identified as infected or colonized with those organisms on the study units throughout the study period.
Method 2: Samples from patients with clinical cultures positive for VRE, MRSA, ESBL and *A. baumannii* strains will be typed using REP-PCR with overlay and validated as genetically identical using next-gen whole genome sequencing. These identical strains will be analyzed with regards to timing and location of recovery, and clinical characteristics.

Expected Outcomes
In this project, we aim to quantify the reduction in eiHAIs associated with the additional use of PX-UV disinfection to standard terminal cleaning.

RESEARCH STRATEGY

SIGNIFICANCE

Role of the Environment in Infection
Environmental hygiene has been identified as an area of importance by the Agency for Healthcare Quality (AHRQ) and plays a critical role in the spread of hospital pathogens in hospital settings.[1] Manual cleaning is an important component of hospital environmental hygiene, but often, there are missed opportunities for optimal environmental cleaning.[2-4] It has been demonstrated that the environment can contaminate the hands of patients and healthcare workers directly, causing an increase in the risk of pathogenic transmission.[5] Pathogenic organisms such as *C. difficile*, *Enterococcus* spp, *Acinetobacter* spp. and *E. coli* have the ability to persist in the environment at least five months.[6] This persistence creates an ongoing risk of transmission of these pathogens in the hosptial.[7].

Inadequacy of Current Manual Disinfection Approaches
Current interventions to reduce microbial contamination in the environment rely on the manual disinfection of surfaces by environmental service workers.

Multiple studies have shown that the manual disinfection process is often inadequate, as up to 50% or more of high-touch surfaces may be routinely missed during cleaning. Improper use of chemical cleaning can result in inadequate disinfection and cross-contamination and time pressure can result in truncated dwell times for chemicals.[3,9,10] These inadequacies increase the risk for nosocomial transmission of pathogens in healthcare settings. Even in situations in which the manual cleaning process has been considerably improved, some environmental surfaces remain inadequately cleaned.[11] Therefore, it is plausible that supplements to manual cleaning might be needed to optimize risk reduction for pathogenic transmission in the patient care environment.[11]

Furthermore, it has been documented that healthcare workers' hands, gloves and gowns can become contaminated with VRE and MRSA from contact exclusively with the environment of the patient room in a similar rate at which hands, gloves and gowns become contaminated from direct contact with the patient. For example, contact with the environment in a VRE-positive patient room resulted in hand/glove VRE contamination 52.3% of the time while contact directly with the patient resulted in contamination 69.5% of the time ($p=0.1$).[12] Similarly, contact with the environment in MRSA-positive rooms by healthcare workers resulted in glove contamination 42% of the time, compared with contamination from direct patient contact, which occurred 58% of the time.[13] Finally, environmental contamination was independently associated with

contamination of healthcare worker gloves or gown with multiple drug-resistant organisms (MDROs) including *A. baumannii*, VRE and MRSA (OR 4.15 for recovery of one or more of these MDROs).[14] These frequent contaminations of healthcare worker's hands, gloves and/or gown contribute directly to acquisition of eiHAIs and adversely affect patient outcomes.

The Potential for Automated Disinfection Technologies to Prevent Pathogen Transmission in Hospitals

Commercially available automated disinfection systems can be used to greatly reduce or eliminate risks associated with sub-optimal environmental cleaning via surface and air disinfection through the application of technology using hydrogen peroxide vapor, ozone gas and ultraviolet light. The ultraviolet (UV) systems consist of UV light created using mercury vapor gas (Hg-UV) or pulsing a xenon flashlamp (PX-UV). Both types of technologies are currently being used in over 500 U.S. hospitals.[15-21] The mercury-based systems, according to the published literature, require more than 45 minutes for disinfection and thus, are not practical for routine use as a supplement to terminal cleaning following patient discharge from the hosptial.[18] Hydrogen peroxide vapor systems are even less practical for routine use in the hospital, because the cycle time to treat one room is reported at over 3 hours.[5]

PX-UV produces germicidal UV in a range of 200-320 nm using an intense pulse,that provides PX-UV with unique advantages compared to other UV systems. These advantages include production of a higher maximum intensity UV output and production of high energy UV which damages micro-organisms by a variety of methods. This PX-UV is produced in a flashlamp using xenon, an inert gas, and the device uses a motion sensor to make certain that the room is unoccupied before UV is emitted. PX-UV is currently used in over 250 hospitals in the U.S. for the routine disinfection of patient rooms, operating rooms and other areas. The device is designed for movement from room-to-room by a single staff for disinfection and has the capacity of disinfecting over 30 rooms per day on a routine basis. According to Ghantoji *et al*, a PX-UV system reduces environmental contamination by *C. difficile* in less than 15 minutes of operating time.[22] PX-UV systems have demonstrated a 50% reduction (or greater) in the rate of hospital acquired *C. difficile* infections[23-25], a 57% reduction in hospital-acquired MRSA[26] and reductions of more than 40% in surgical site infections.[27] However, these studies are limited by their retrospective designed and do not constitute strong evidence of the impact of PX-UV on HAI rates. In addition, there was no sham or control arm in these studies to account for potential biases introduced by health-care providers or managerial staff. Furthermore, the quality of cleaning by the environmental services staff was not monitored during the study period in these studies. In addition, in these studies, cleaning personnel were aware that a research project was ongoing, potentially creating a bias by drawing attention to environmental hygiene which could have resulted in improved processes during the study period (i.e., the Hawthorne Effect). Finally, a recently presented CDC-funded trial demonstrated a reduction in MDROs associated with mercury UV light, but several questions remain unanswered (see B5 below).

While the literature on automated UV-based disinfection shows promise, there is a lack of high-quality data demonstrating the impact of germicidal UV on eiHAIs. This study plans to address these gaps by doing the following: 1) using sham control PX-UV devices so that all units will have identical devices and cleaning processes during both intervention phases of the study period; 2) using PX-UV disinfection (or sham disinfection) following terminal cleaning on all rooms (as opposed to selected rooms) on study units; 3) using eiHAI definitions that mirror those used by CDC and Centers for Medicare & Medicaid Services;[28] and 4) using advanced molecular epidemiological techniques to demonstrate persistence of specific pathogen strains during the study period.

In summary, hundreds of hospitals in the U.S. and around the world are using automated disinfection technologies, such as PX-UV. Such technologies show promise in reducing HAIs by decreasing environmental contamination. However, well-controlled studies evaluating routine use of automated systems using rigorous designs have not been conducted. Given the potential for PX-UV technology to reduce the large burden of HAI in the U.S., pivotal clinical trial data are now needed to establish efficacy of PX-UV in preventing spread of eiHAIs and to determine if PX-UV should be routinely included as part of terminal cleaning in hospitals.

INNOVATION

Increasing the level of proof for infection control interventions

While the literature pertaining to PX-UV shows promise, all but one of the published studies used a quasi-experimental design, using historical comparison data to reach conclusions about the impact of PX-UV on HAI rates[23-25,27,29,30]. In addition, previous PX-UV studies have not effectively controlled for many factors contributing to the spread of eiHAIs in hospitals (e.g., thoroughness of manual cleaning, hand hygiene and pathogen colonization pressure). We propose to conduct a prospective, double blinded, placebo-control crossover study to more effectively address the limitations of studies in the published literature.

Targeting all discharges

Prior research with automated disinfection systems focused on the terminal disinfection of isolation rooms only. This is a problem, as patients might be infected or colonized with an eiHAI but the eiHAI might not be detected during the hospital stay. This type of approach probably will likely "miss" certain rooms where a patient is a silent carrier of an eiHAI. This type of approach also results in a low daily use of such technologies and limits the global effect of UV disinfection on a hospital unit's environmental hygiene. In the proposed project, the automated disinfection system (PX-UV) will be used following every discharge on participating units. This approach will significantly increase the frequency, breadth and impact of the intervention on study units in a way that has not been previously studied.

Use of sham device and double-blind status

The proposed study is also novel in its use of sham automated devices to provide a "placebo" intervention and facilitate control for many potential confounders associated with the implementation of automated disinfection devices on the study units, such as changes in general infection control awareness and practices and environmental hygiene. Additionally, all healthcare personnel (including device operators and environmental services staff), will be blinded to the intervention/sham status of the devices. In addition, the study personnel including the PI and individuals performing data analysis, will be blinded to the intervention/sham status of the devices. The device status will be written on a piece of paper by the manufacturer with the serial numbers of each device indicated, sealed in an envelope and kept in a secure location by the study statistician (Dr. George Divine) for later retrieval when trial data analysis has been completed.

Use of crossover design

The crossover study design represents an improvement in study design quality compared with studies in the current literature and allows for the control of additional measured and unmeasured confounders. A crossover design, with each unit observed with both the active and sham intervention, means each unit serves is its own control, which will improve the precision of the comparison, reducing the number of units and total expense required for the study.

Notable differences from recent CDC-sponsored trial

Study NCT01579370 (Anderson, PI: *A Four-arm Prospective, Multicenter Study to Assess the Efficacy, Effectiveness, and Feasibility of Enhanced Terminal Room Disinfection with Chlorine and UV Light Using Clinical and Microbiologic Outcomes*) compared the impact of a mercury UV system with two cleaning protocols for the disinfection of isolation rooms only. The study abstract has been published at the IDWeek website, but the results have not yet been fully published or presented at the time of submission. The study abstract reported the following results, with group A being terminal cleaning with quaternary ammonia only; group B quaternary ammonia plus a germicidal UV device; group C terminal cleaning with bleach and group D, terminal cleaning with bleach plus a germicidal UV device: The clinical incidence of all target MDROs was 37% lower in Group B (p=0.03) and 32% lower in Group D (p=0.01) compared to Group A. While this study suggests benefits related to the addition of germicidal UV disinfection to manual room cleaning, there remain many unanswered questions. For outcomes measurements, this study included MDROs occurring as far out as 90 days following discharge from the study unit and included *C. difficile* cases occurring as far out as 28 days following discharge. Such a long follow-up period introduces many competing risks and statistical "noise" that complicate interpretation of study results. The primary outcome measures that will be used in the proposed study (recovery of eiHAIs while on study units or shortly after discharge from the units) will be a more direct, precise and relevant measure of the impact of PX-UV on infection prevention. Another limitation of the CDC-funded study was the fact that no sham control devices were used on control units. It is possible that on units where UV disinfection devices were used, adding UV disinfection devices to routine terminal disinfection processes might have positively influenced the thoroughness of manual room cleaning and infection control practices. Another limitation was that UV disinfection was only used in isolation rooms as opposed on all rooms following patient discharge. This limitation was necessary because of the cycle time of more than 45 minutes of the mercury UV device chosen for the study. Use of PX-UV disinfection, as proposed in the current study, has a much shorter cycle time (15 minutes) allowing for incorporation of UV disinfection following terminal cleaning for ALL rooms on study units. This broader, more routine use of PX-UV disinfection will likely have a greater impact on environmental hygiene on study units and on infection reduction. Thus, the proposed study would offer additional information and real-world experience with PX-UV that will not only supplement the findings of the findings from the CDC-sponsored trial but will provide innovative approaches and experience that can be used to optimize the use of UV disinfection in healthcare settings.

APPROACH

PRELIMINARY STUDIES

Impact of PX-UV on environmental contamination

Multiple studies have evaluated the impact of PX-UV on levels of microbial contamination after terminal cleaning, the level of interdependence of manual cleaning/disinfection and PX-UV, and the change in nosocomial infection rate for *C. difficile* and other organisms. In one study, Jinadatha (Co-Investigator) showed that colony counts in 20 rooms (10 per arm) prior to cleaning varied by cleaning protocol: for HPC, manual (mean = 255, median = 278, interquartile range [IQR] 132-304) *vs* PPX-UV (mean = 449, median = 365, IQR 332-530), and for MRSA, manual (mean = 127; median = 28.5; IQR 8-143) vs PPX-UV (mean = 108; median = 123; IQR 14-183). PPX-UV was superior to manual cleaning for MRSA (adjusted incident rate ratio [IRR] = 7; 95% CI <1-41) and for HPC (IRR = 13; 95% CI 4-48). [31] In another study, Stibich (Co-

Investigator) obtained two hundred thirty-nine samples from 21 surfaces from 12 rooms from which patients with VRE colonization and/or infection were discharged. The mean HPC for before cleaning, after cleaning, and after UV treatment was 33.0, 27.4, and 1.2 CFU/cm2, and the number of VRE-positive surfaces was 17 (23.3%), 4 (8.2%), and 0 (0%), respectively.[15] These studies demonstrate a consistent and significant reduction in environmental pathogens with use of PX-UV.

Impact of PX-UV on hospital acquired *C. difficil*e infection (CDI) rates
Investigators examined the impact of PX-UV on nosocomial *C. difficile* and VRE infection rates. PX-UV disinfection was evaluated in three patient units (intervention arm), while three units with similar baseline CDI rates and patient populations served as controls. The intervention and control arms each included two Hematology/Oncology units and one Medical-Surgical unit. Patient rooms on the intervention units received PX-UV in three 5-minute positions after standard terminal cleaning. Infection control measures (i.e. bleach use, hand hygiene and isolation compliance rates) were similar in the two study arms. CDIs, diagnosed by polymerase chain reaction (PCR), were classified as HAIs and attributed to the unit if CDI was diagnosed > three days after admission or within four weeks of dismissal from that unit. Incidence rates expressed as the number of HAI CDIs per 10,000 patient-days were analyzed using a negative binomial regression model. Within units of the intervention arm, 85% of rooms were disinfected with PX-UV. Baseline HAI CDI rates in the intervention and control arms were not significantly different (18.3 and 20.1 per 10,000 patient-days, respectively [p=0.28]). During the 6 month period of PX-UV disinfection, the HAI CDI rate in the intervention units decreased to 11.2 per 10,000 patient-days compared to an increase to 28.7 per 10,000 patient-days in control units (p=0.03). The rates of HA-VRE for the control units were 1.91 at baseline and 1.96 at completion of the study (p=0.363). The rates of HA-VRE for the intervention units were 3.25 at baseline and 1.23 at completion of the study (p=0.016).[32] Thus, in the PX-UV arm, there was a 38.8% decrease in *C. difficile* rates, while rates increased by 42.8% in the control arm.

Recovery of Genetically Identical VRE Strains
Co-investigators Jinadatha and Stibich have collaborated on a research project that examined occurrence and frequency of recovery of genetically identical VRE strains at the MD Anderson Cancer Center. Both time between isolation of an identical strain and location of isolation of the identical strain were determined. Of the 82 VRE transmissions, 32.9% were repetitions of genetically identical strains. The average time between the reoccurrence of a strain was 49 days [median: 36, range: 0-183 days]. One strain reoccurrence was in the same room as the previous occurrence of the strain, 6 were in the same pod, 14 were on the same floor and 6 were on a different floor. Twelve of the reoccurrences were from patients on the leukemia floors, 10 were on the hematopoietic stem cell transplant unit and 5 were between these services.[33]

Epidemiology and Prevention of eiHAIs at DMC The PI (Kaye) on this proposal is the Medical Director of Infection Prevention and Hospital Epidemiology at DMC and oversees all infection control activities at study hospitals. The PI has published extensively at DMC on hospital-acquired infections, including VRE,[34] MRSA,[35] ESBLs,[36] *A. baumannii*[37] and *C. difficile*[38]. Clearly these eiHAI pathogens are a persistent problem at the study sites. The PI's research and infection control teams have notable experience in controlling the spread of these pathogens in the hospital. Because of the strong infection control infrastructure already in place at the study sites, it is highly likely that the PX-UV disinfection and sham device interventions will proceed successfully on study units.

RESEARCH DESIGN AND METHODS

Study overview
We will conduct a prospective, double-blind, two-armed crossover clinical trial with an initial intervention period of 12 months, followed by a 6-month washout period and a second intervention period of 12 months. The 6-month washout period will be necessary because of the long-term survivability of eiHAIs, such as *C. difficile*, on environmental surfaces.

Sample size estimation
For the 16 units to be studied, over 8 months from December 2014 through July 2015, the average rate of *C. difficile* infections on day 4+ of a patient's hospital stay was 1.3 per 1000 days. Rates of ESBL+VRE+ MRSA + *A. baumannii* are 1.5 times that for *C. difficile*, so the total rate of all four infection types would be 3.2 (=1.3x2.5). The total average daily census on the 16 units was 291. Assuming the average patient stay is 5.2 days, 42.3% of patient days will be day 4+, so the expected number of daily eligible patient days would be 123. For 24 months of intervention the total number of eligible days would be 123x730 = 89,790, or 44,895 per arm.

If the power for the Poisson model is approximated by that for a binomial test of proportions, and with a final alpha level of 0.049 to take into account one interim analysis, a 31% reduction in eiHAI incidence (from 3.2 to 2.208 per 1000 eligible days) would be detectable with 81% power.

Hospital Unit Selection
Patient care units from two healthcare facilities have been identified for this study based on a relatively high average recovery of eiHAIs. These units will be randomized to receive either the intervention or sham PX-UV devices in Phase 1 of the study, and the intervention and sham PX-UV devices will switch in Phase 2 of the study. The protocol for use of the devices is outlined below.

Clinical Sites
Two DMC acute-care hospitals are being proposed for this project, Sinai-Grace Hospital (SGH) and Detroit Receiving Hospital (DRH). SGH is a 383-bed hospital, and DRH is a 248-bed hospital. The study will be conducted in eight hospital units in SGH and eight hospital units in DRH, including two medical intensive care units (ICUs), two surgical ICUs and 12 non-ICU medical-surgical wards. The 16 study units include a total of 379 beds. During the 24-month study intervention period, we anticipate that 40,852 total patients will be cared for on study units accounting for 89,790 eligible patient days out of a total of 212,430 patient days. If past experience continues unchanged, during the 24-months of intervention, 287 eiHAIs attributable to these units would be observed.

Devices will be deployed on these study units with the sham and intervention devices split evenly between SGH and DRH. Within each study site, the types of devices (PX-UV vs sham) will be assigned randomly to the units in Phase 1 and then switched in Phase 2.

Patient Selection

Inclusion Criteria: All patients admitted to the study units will be eligible. Only patients who remain in the hospital for four calendar days or longer will be eligible for evaluation of eiHAI outcomes (the day of admission counts as calendar day one). Patients who do not develop an eiHAI at the time of unit discharge will be eligible for eiHAI outcomes for up to calendar 3 days following unit discharge.

Data Collection and Treatment Outcomes

Variables that are attributes of patients:

- *C. difficile* laboratory test results: hospital-acquired *C. difficile* will be defined as a laboratory-positive PCR test on calendar day 4 or beyond on the study unit; or within 3 days after discharge from unit.[28]
- Clinical cultures positive for VRE, MRSA, ESBLs or *A. baumannii* on day 4 or beyond on the study unit; or within 3 days after discharge from unit.[28]
- ICD-9 and ICD-10: primary and secondary *ICD*-9 and *ICD*-10 diagnoses.
- Patient demographics: age, gender, insurance status and zip code.
- Admission and transfer information: admission source and admission and discharge dates for both the study units and the entire episode of care, including room numbers.
- Antibiotic use: the dose, duration and type of antibiotic prescribed to the patient on the study units
- Infections and colonizations due to eiHAIs as well as other pathogens, defined according to National Healthcare Safety Network (NHSN) criteria.[39]
- PX-UV disinfection status: sham/intervention disinfection occurring before patient is admitted to the room.

Variables that are attributes of the unit: For unit-level variables that are measured monthly, the value used for each patient will consist of the average of the immediately previous and immediately following values for the variable, each weighted by the number of days from the mid-point of the patient stay on the unit.

- Hand Hygiene compliance: monthly measures of hand hygiene compliance according to standard infection control guidelines and also according to “secret observers” who are members of the study team. Approximately 30 observations will be performed each month on study units.
- Environmental cleaning quality: monthly measures of cleaning quality using EnCompass™ Environmental Monitoring Program will be conducted by environmental services at study hospitals with a minimum of 2 room inspections marking 10-15 high-touch surfaces in a patient room with an invisible marker per unit per month (for a total of 16 inspections per month).[40] In addition, every other month, a minimum of 1 room per unit will be visually inspected but a study team member using the EnCompass™ Environmental Monitoring Program.
- Staffing levels: Average number of nurses per patient on study units.
- Frequency of eiHAI pathogens recovered within the first 72 hours of unit admission to determine eiHAI colonization pressure.
- PX-UV compliance: number of rooms receiving sham/intervention PX-UV divided by the number of discharges
- PX-UV Sham/Intervention status: this variable will be blinded to both the healthcare workers and patients on the units and to the research team.

Description of Intervention Protocol

The PX-UV devices will be placed in three positions in a standard patient room for a UV cycle time of five minutes each. An operator will move the PX-UV devices between positions. While the device is operating in the bathroom, workers can clean in the main room to save time. Before PX-UV disinfection, the room will be cleaned according to the standard terminal cleaning protocol for that room, which may include the use of sodium hypochlorite in the case of *C. difficile* isolation rooms. Between positions 2 and 3, the operator will make certain that objects, such as telephones, TV remote controls and call buttons are inverted to allow for UV exposure on all sides.

Description of sham device

For the sham units, a non-UV producing bulb has been designed that will produce a flash that will be visually and audibly indistinguishable from the flash produced by the device that creates germicidal UV. The cart and other features of both the intervention and sham devices will be indistinguishable. The study team and room cleaning staff (environmental services personnel) will be blinded to the sham/intervention status of all devices.

APPROACHES TO ADDRESS EACH SPECIFIC AIM

Aim 1: To evaluate the impact of PX-UV disinfection on rates of eiHAIs on study units by:

1.1. Comparing rates of eiHAIs on study units where a) PX-UV is added to standard terminal cleaning practices to b) units where a sham UV disinfection system is added to standard terminal cleaning.

1.2. Comparing Rates of eiHAIs on the same medical ward during each of two 12-month phases of a crossover study (one phase when a PX-UV device is added and one when a sham device is added to standard terminal cleaning).

Data collection: With the exception of the environmental cleaning quality, the hand hygiene compliance measures and compliance with the use of the PX-UV device, all data collected will be compiled from infection control databases, the admissions and transfer database and the electronic medical record. Data will be collected on all subjects who stay on study units during the study period via abstraction from hospital databases and medical records. Variables to be collected include demographics; admission and discharge dates to study units, other units and the hospital; co-morbid conditions; antimicrobial exposures; clinical culture positivity with eiHAI pathogens; occurrence of CDC-defined HAIs including bloodstream infection, urinary tract infection, skin and skin structure infection, pneumonia and *C. difficile* infection (CDI).[39] Data will be collected and stored securely in an Access database.

PX-UV device monitoring: The PX-UV device utilization data are recorded automatically and uploaded in real time to a cloud-based portal. Alerts in the portal can be set to determine if a PX-UV device is not used the predicted number of times per day or is used in a manner inconsistent with the protocol.

Specific Aim 2: To evaluate the pattern of repetition of genetically identical strains of drug resistant bacteria strains in patients identified as infected or colonized with those organisms on the units selected above using REP-PCR with overlay and next-gen whole genome sequencing for validation throughout the course of the study period.

Sample Selection

For the molecular pathogen analysis, all clinical microbiologic patient isolations of eiHAIs (excluding *C. difficile*) will be preserved by the clinical laboratory. Because *C. difficile* is detected at the study sites by a PCR test and not by clinical culture, *C. difficile* will not be included in Aim 2. eiHAIs (excluding *C. difficile*) will be shipped by the clinical microbiology laboratory per standard procedures to the laboratory at VISN-17 where REP-PCR and next-generation whole genome sequencing will be performed on randomly selected strains. To detect genetically identical strains of MDROs, a two-step process will be used. In the first step, all samples will be tested using Rep-PCR with overlay. Samples that appear identical after undergoing that process will be then tested using next generation whole genome sequencing. All laboratory testing will be done at the Olin E. Teague Veterans' Medical Center under the supervision of co-investigator, Dr. Jinadatha.

REP-PCR: Rep Sequencing Strain Typing System Process and Analysis

In previous studies, REP sequence strain typing has been used to detect genetically different strains.[41-43] To REP sequence strain type, using UltraClean® Microbial DNA Isolation Kit (MO-BIO Laboratories, Inc., Solana Beach, CA), genomic DNA will be extracted from 1 µL of organism. A working concentration of 25-50 nanograms/mL of DNA will be verified using a NanoDropTM ND-1000 spectrophotometer. Using the BioMerieux Diversilab® kits (Marcy L'Etoile, France), Rep-PCR will be performed to produce the repetitive elements of the genome for each isolate. The fingerprints of the DNA will be automatically downloaded onto the Diversilab® website, and using Kullback-Leibler distance comparison software, the isolates will be analyzed. The software uses an unweighted pair-group method to create dendrograms, scatter plots, and electropherograms for data interpretation. Each individual electropherogram will be examined by an expert microbiologist (Dr. Thomas Huber, Central Texas Veterans Healthcare System) by comparing its overlay pattern to the Diversilab® VRE library (DL) and a unique facility-specific library created for Diversilab® unmatched strains.

Validation of similarity scores given from the Diversilab® analysis is necessary because a high percentage agreement (95% similarity or greater) between isolates does not guarantee that strains are identical. Further, a few small peaks of difference will not impact the percent agreement score enough to substantially reduce the similarity score, but these differences may indicate that the strains are not identical. Hence, each pair of isolates with more than 90% similarity will be overlaid. The tracings of the overlay will be examined closely to see if peaks were in the same position and height. If two peaks are different, isolates will be classified as "not identical" and if the isolates are indistinguishable, they will be subjected to next generation whole genome sequencing for validation.

Whole Genome Sequencing

Whole genome sequencing provides the most comprehensive collection of an organisms' genetic variations. With the falling costs of sequencing technology, whole genome sequencing has become a useful tool for genetic sequencing of isolates for comparing strain mapping. The reference-based assembly approach involves mapping each read to a reference genome sequence, which will help in identifying genetic variation (single nucleotide polymorphisms, small indels, and copy number variants). Whole genome sequencing is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. This entails sequencing all of an organism's chromosomal DNA as well as DNA contained in the mitochondria.[44,45]

Whole genome sequencing for this project will be performed on a table top sequencer called MiSeq (Illumina, San Diego, CA). The following process will be used to verify isolates determined to be "genetically identical" by the REP-PCR process described above. We estimate 5% of total samples will need to undergo this whole genome sequencing process for validation.

The sequencer operates on a patent technology that uses sequencing by synthesis (SBS). It integrates the functions of cluster generation, SBS, and data analysis in a single instrument and can go from sample to answer (analyzed data) within a single day (as few as 8 hours). The sequencer can perform single 36 base pair reads (120MB output) up to 2 × 150 paired end reads (1–1.5 GB output). The entire process involves three basic steps. 1. Preparation of the isolate. 2. Sequencing of the isolate 3. Analyzing the isolate to the library.[46]

Preparation of the isolate: The first step of the prep is to Denature and Dilute libraries. 1 ml of freshly diluted 0.2 N Sodium hydroxide (NaOH) is used for denaturing libraries for cluster generation. The tube containing thawed Hybridization Buffer (HT1) is used to dilute denatured libraries before loading libraries onto the reagent cartridge for sequencing. Next denaturation and dilution of the deoxyribonucleic acid (DNA) is performed by combining a 4nM sample of DNA (5 µl) with 0.2 N NaOH (5 µl) in a microcentrifuge tube. The solution is vortexed briefly to mix the sample solution, and then centrifuged 280 × g for 1 minute. It is incubated for 5 minutes at room temperature to denature the DNA into single strands. Further Pre-chilled HT1 (990 µl) is added to the tube containing (10 µl) denatured DNA. The result is a 20 pM denatured library in 1 mM NaOH. The denatured DNA is placed on ice until final dilution. This is achieved by diluting the 20 pM DNA further to give 600 µl of the desired input concentration. A PhiX Control is prepared using similar denaturation and dilution method as described above.

Sequencing of the isolate: The pre-filled reagent cartridge is prepared for use by thawing completely. The library mix is loaded onto the reagent cartridge in the designated reservoir making sure not to pierce any other reagent positions. The other reagent positions will be pierced automatically during the second phase (sequencing). After this step, 600 µl of prepared libraries is pipetted into the "Load Samples" reservoir

After appropriately loading all libraries, MiSeq Control Software (MCS) is set up for use. From the software interface, Sequence to start the run setup steps is selected. Next, the flow cell is loaded. After closing the flow cell latch, the wash buffer/PR2 bottle is loaded. And finally, the reagent cartridge is loaded inside the reagent chiller door. After making sure of run parameters and pre-run check results, the sequencer is run and the quality of run is monitored using the MCS interface or from another computer using Sequencing Analysis Viewer (SAV).

Analyzing the isolate to library: The isolate sequencing will be interpreted by an experienced microbiologist who will be masked to all clinical, epidemiological and microbiological study information. A reference isolate will be defined initially to compare subsequent isolates to the reference isolates for mapping the clonal spread of an isolate. The genome data will be analyzed to identify single-nucleotide polymorphisms (SNPs) and insertions or deletions. Then reference isolates will be compared to subsequent isolates for mapping clonality of the isolates to define the strain relatedness, and to define any repetition of a particular strain over a given period of time. Further, we can also define if an intervention such as pulsed-xenon disrupts the repetition pattern of strains and compare it other intervention such as manual disinfection.

Statistical Analysis

Aim 1. The primary analysis will use a Poisson regression model to compare PX-UV disinfection with sham disinfection. Analysis will begin with testing for a carryover effect, but the long 6-month washout is expected to make carryover unlikely. An interim analysis for efficacy will be made after the first 12-month intervention period using an O'Brien-Flemming boundary, the trial may be halted for efficacy if a chi square statistic of 8.78 or greater is observed for the interim test ($p \leq 0.003$). With the interim analysis, the p-value for the test at end of the study will need to be 0.049 or lower to be significant. The primary regression model will include patient age, sex, race and indicator variables for co-morbid conditions. Secondary analysis will summarize and compare study arms (and periods) on: administered antibiotic doses and types, hand hygiene compliance, room cleanliness quality (monitoring through florescent marking) and eiHAI colonization pressure.

Aim 2 The frequencies of genetically identical strains of eiHAIs in the selected units will be summarized descriptively with regards to timing, location and clinical strain characteristics.

Study Timeline

ACTIVITY TO ADDRESS STUDY AIMS	**Year 1**				**Year 2**				**Year 3**				**Year 4**				**Year 5**	
Quarter	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**
IRB, training of environmental services staff	X	X	X	X		X				X				X				X
Phase 1of crossover				X	X	X	X											
Wash-out period								X	X									
Phase 2 of crossover										X	X	X	X					
Laboratory analysis							X	X	X				X	X	X			
Data analysis and manuscript preparation										X	X	X		X	X	X	X	X

Potential Problems, Alternative Strategies, and Benchmarks for Success

While not anticipated, delays in the intervention could occur due to staffing and other logistical issues. Based on the experience of current customers of the PX-UV system, we do not anticipate a disruption to the workflow and patient through-put on the study units, but such a disruption could impact compliance with the use of the PX-UV devices. We have safeguarded against these potential issues, by garnering the full support of Detroit Medical Center, Xenex and the environmental services company, Sodexo. We have budgeted for the time and effort of Sodexo employees to carry out the PX-UV intervention. We do not anticipate any problems with laboratory aspects of the project because of the use of standardized methods in an experienced laboratory.

The benchmarks for success are: (1) the successful operationalization of the intervention (both PX-UV devices and sham controls) without disruption of patient flow; (2) maintaining routine, objective measurements of hand hygiene and room disinfection throughout the study period; (3) the reduction of both the rate of eiHAIs and the recovery of genetically identical strains of drug resistant organisms on study units; and (4) development of an automatic, replicable and scalable intervention to reduce the risk of *C. difficile* and other eiHAIs.

Protection of Human Subjects

1. Risks to the Subjects

a. Human Subjects Involvement and Characteristics

The human subjects involved in this study will be cared for on units where either PX-UV or sham unit disinfection occurs following each patient discharge. The room disinfection will only occur when the room is unoccupied, so there is no direct risk to the patient from PX-UV disinfection treatments. The medical treatment that patients receive will not be impacted in any way by this study and the patients will not undergo any additional testing as a result of participating in the study. We anticipate that 40,852 human subjects will participate in the study. The anticipated age range is 18-100 years. All patients will be hospitalized on study units.

The criteria for inclusion in the study is admission to the study units. Only patients who remain in the hospital for four calendar days or longer will be eligible for evaluation of eiHAI outcomes (the day of admission counts as calendar day one). Patients who do not develop an eiHAI at the time of unit discharge will be eligible for eiHAI outcomes for up to calendar 3 days following unit discharge.

Rationale for the involvement of special classes of subjects: Fetuses, neonates and children will not be included because they are not cared for on the types of units where the study is being performed.

Prisoners, institutionalized individuals and others who may be reasonably believed to be vulnerable populations will be enrolled in the study because the study presents no more than minimal risk and no more than inconvenience to the participants. In addition, it would not be practical to exclude these populations without adversely effecting the study's impact and objectives. These subjects are not the primary focus of this research study.

Pregnant women will be enrolled in the study if they meet the inclusion criteria specified above and also in the Research Strategy. These subjects are not the primary focus of this research study.

Collaborating sites where human subjects research will be performed are listed below. Investigators at these sites will be responsible for enrolling patients and collecting the appropriate samples and data. Collaborating sites
are:
- Detroit Receiving Hospital, Detroit, MI, USA
- Sinai Grace Hospital, Detroit, MI, USA
-

b. Sources of Materials

No research material will be obtained from living human subjects. Bacterial isolates derived from cultures taken from subjects as part of their routine clinical care will be

collected from the participating clinical microbiology laboratories.

The data recorded on human subjects involved in the project will include demographic details (age, gender, race, etc.), underlying illnesses, antibiotic use, occurrence of infections and admission and discharge dates to and from units and the hospital. The data will be collected by review of the subject's medical records. Subject identities will be known only by the study personnel at the participating centers. Study personnel include the co-investigators and study coordinator. Subject identities will not be revealed to the statistician (Dr. Divine) or to other participating centers. Subjects will be identified by a code comprising the hospital location followed by a triple digit number. This code will be linked to subject identities on a secure, password-protected computer of the Project Coordinator at Wayne State. The list linking codes to subject identity will be kept on a password-protected computer or as a paper copy in a locked filing cabinet in a locked room of the study coordinator at Wayne State.

c. Potential Risks
Risks associated with confidentiality:

We will preserve subject confidentiality by assigning a special research code number to medical record information stored. No personal identifying information will be entered in the Research Registry. Information linking the research code number to name and other personal identifiers will be stored in a separate secure location of the Project Coordinator. Access to any information contained within the Research Registry will be limited to investigators associated with the study and their research staff.

2. Adequacy of Protection against Risks
a. Recruitment and Informed Consent
All subjects admitted to study units during the study period will be enrolled. The study will request a waiver of consent.

b. Protection against Risk
Protection against loss of confidentiality of personal medical information will be by ensuring that only the clinical investigators and relevant study personnel will have access to any personal data obtained. Personal information will be kept in a locked filing cabinet or a password-protected computer within a locked room of the Project Coordinator at Wayne State. No medications or surgical interventions will be performed for the purpose of the study.

3. Potential Benefits of the Proposed Research to the Subjects and Others
Individual subjects in the study may benefit from their involvement in the study. If the PX-UV intervention is effective their risk for acquiring HAI might be reduced. The potential benefits in this study outweigh the minimal potential risks

4. Importance of the Knowledge to be Gained
HAIs are a top national safety priority. The objectives of this study are to analyze a novel and potentially improved method for hospital room disinfection that if successful, will decrease risk for HAIs among hospitalized patients and would improve patient safety, not only for study patients but for hospitalized patients across the USA.

Inclusion of Women and Minorities

Inclusion of Women

Women will be included in this study. There is no intent to restrict entry into this study on the basis of gender. We anticipate that the mixture of men and women enrolled in the study will be approximately equal.

Inclusion of Minorities

Minorities will be included in this study. There is no intent to restrict entry into this study on the basis of race, religious affiliation, political persuasion etc. Please see Targeted Planned Enrollment Table for the anticipated ethnic and racial categories of subjects. We anticipate that the ethnic and racial categories of subjects will be representative of those in the general community.

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