

Effectiveness of Ultraviolet C-Light in Eliminating Microbial Pathogens from Stethoscope used in the Neonatal Intensive Care Unit of East Avenue Medical Center*

Glendie Patricia Bakilan-Gallardo, MD¹; Ma. Theresa Policarpio, MD¹

ABSTRACT

Background: Instruments used to examine infected patients may be contaminated by disease-causing microorganisms during contact. If these instruments are not sterilized properly prior to being used on other patients, pathogen transfer may occur via this route. Stethoscopes are the most commonly used equipment by healthcare providers. Microbes and viruses may be transmitted from one patient to another and from healthcare worker to patient via stethoscope membranes.

Objective: To determine the effectiveness of ultraviolet c-light in eliminating microbial pathogens from stethoscopes used in the Neonatal Intensive Care Unit of East Avenue Medical Center.

Methodology: This is a two-arm, double blind, randomized controlled trial. The minimum sample size computed for this study was 26 stethoscopes. Thirteen (13) stethoscopes each were randomly allocated to Ultraviolet C (Group A) and standard of care (Group B) groups.

Data Analysis: Summary statistics were reported in tables as means, standard deviations, percentages and frequencies min-max for quantitative discrete outcome measures or percentages for qualitative measures.

Results: The predominant microbial pathogens colonized in the stethoscopes were different species of Coagulase Negative Staphylococcus (CoNS) namely: *Staphylococcus Heamolyticus* (34.62%), *Staphylococcus Epidermidis* (26.92%) and *Staphylococcus Hominis* (19.23%). Both UVC light and standard of care were equally effective in decreasing the CFUs on the stethoscopes. There was no significant difference in the post-test colony-forming units (CFUs) between the two groups ($t = -.594, p > .05$).

Conclusion: UVC light sterilization is comparative to the standard of care in eliminating microbial pathogens. It works faster and is more reliable, durable and cost-effective. It is recommended as an alternative method for decontaminating stethoscopes used at the EAMC-NICU due to its numerous advantages.

Keywords: *ultraviolet c light, neonatal intensive care unit, stethoscope*

Disclosures: The author has formally acknowledged and signed a disclosure affirming the absence of any financial or other relationships (including personal connections), intellectual biases, political or religious affiliations, and institutional ties that could potentially result in a conflict of interest.

¹Department of Pediatrics, East Avenue Medical Center

INTRODUCTION

Background of the Study

Special care should be exercised when handling newborn skin since it is the main port of entry for infections. Hand washing is the best way to prevent microbial pathogens from spreading. However, this may not be enough to eradicate microbes that may be spread via medical equipment or devices commonly used by physicians and other healthcare providers.

Instruments used to examine infected patients may be contaminated by disease-causing microorganisms during contact. If these instruments are not meticulously cleaned and sterilized prior to being used on other patients, these pathogens may be transmitted from one patient to another.

Stethoscopes are the most commonly used equipment by healthcare providers. Microbes and viruses may be transmitted from one patient to another and from healthcare worker to patient via stethoscope membranes.¹

Gastmeier et al in 2003 reported that strains of *Klebsiella pneumoniae* were found on the stethoscopes of incubators at a neonatal intensive care unit in Germany. Clinical isolates of this bacteria were found in the blood of two patients.²

Cleaning, disinfection and sterilization are essential in preventing the spread of infections.³ According to Gurevich et al, sterilization is defined as destruction of all microorganisms, endospores and viruses.⁴

Rutala et al concluded that ethanol and isopropyl alcohol, commonly used as disinfectants, are only effective for eradicating vegetative bacteria and viruses but not spores.⁵

Nosocomial infections often result from inadequate or superficial management of cleaning, disinfection and sterilization.⁶ Cross-infections are transmitted from one patient to another through contaminated equipment, instruments and materials and are major causes of nosocomial diseases. Healthcare-associated infections have a huge impact on the patient's condition including prolonged hospital stay, more nursing care and rehabilitation and increased health complications that may ultimately result in death.

Ultraviolet (UV) C light has already been used for stimulation of vitamin D production, for psoriasis treatment and for air, water and environmental sanitation purposes. At its typical wavelength of 200-280 nm, UVC radiation induces pyrimidine dimers in thymine and cytosine, breaking DNA molecules, inactivating germs and preventing growth and reproduction.⁷

The nursing bottle sterilizer has been reported to sterilize and eliminate 99.9% of bacteria on surfaces using UV-C light. The sterilizer measures 325mm x 340mm x 403mm and weighs 5kgs. It has a power consumption of 80W, a voltage of AC 220V/60Hz and UV lamp of 4W. Aside from its UV Sterilizing properties, it also has heater a fan which dries the items inside the portable device. It can sterilize, dry, and store items such as baby bottles, bottle nipples, pacifiers, breast pump parts, utensils, plates and teething toys. It can also sterilize mobile phones, remote controls, stuffed toys and accessories. This device is relatively safe since it is enclosed by a tinted glass and vessel, preventing the UVC light from penetrating the eyes. KCL Korea Conformity Laboratories has validated the sterilizer's ability to eliminate harmful bacteria including *Escherichia Coli*, *Staphylococcus Aureus*, *Salmonella Typhimurium* and *Pseudomonas Aeruginosa*.

In this study, the device will be used as an alternative to sterilize stethoscopes used at the Neonatal Intensive Care Unit of the East Avenue Medical Center.

Significance of the Study

Elimination of microbial pathogen spread in Neonatal Intensive Care Units is crucial in the successful management of newborns. Hence, additional means to prevent infection should be investigated.

The UVC light sterilizer may be a user-friendly and cost-effective way of disinfection that can help in preventing nosocomial infections which lead to longer hospital stays and cause greater economic burden to the patients' families.

The data gathered from this study may help supplement current literature on the use of UVC light sterilizers to eliminate pathogens on stethoscopes and other medical equipment or devices. Based on the results of the study, recommendations on the use of this sterilizer in the EAMC-NICU may also be made.

Review of Related Literature

The negative impact of nosocomial infections including damage to patient health, clinical complications, mortality and prolonged hospital stay leading to extended care and increased costs has been widely documented in literature.⁸

Health care-associated infections (HAI) are a significant public health problem worldwide,^{9,10} with significant negative consequences, including impairment of patient's health, mortality and longer hospitalization, with the need for longer treatment and the associated higher costs.^{11,12} The main vehicle for microbe transmission are the hands of health care professionals,^{11,13} but contaminated medical devices such as stethoscopes, otoscopes and thermometers may also transmit microorganisms to patients.^{9,10,14} In

particular, stethoscopes, probably the most widely used medical device, may cause cross-contamination if not disinfected properly.^{15,16}

All objects that come into contact with and are shared between personnel and patients are possible carriers of microorganisms. Lee et al in 2012 reported that the hands of health care personnel are the main vehicles of transmission of microbes and viruses.¹⁴

In addition, although the importance of careful disinfection of these tools has been repeatedly suggested,^{11,15,17,18} their disinfection is not yet common practice. The US Centres for Disease Control and Prevention (CDC) published medical equipment disinfection guidelines to minimize the risk of cross-infections. These guidelines follow the Spaulding classification, categorizing medical devices for the type of contact for which are used (critical, semi-critical, non-critical items). Stethoscopes belong to the non-critical contact category and it is recommended that these be disinfected for "each patient or once daily or once weekly".⁵

The role of the stethoscope in spreading bacteria cannot be overemphasized. Hence, cleaning of stethoscopes between patients is recommended to avoid increasing contamination.²⁰

Longtin Y et al in 2014 documented that the substantial bacterial contamination level on stethoscopes is similar to that on physicians' hands, which are a major source of nosocomial infections.²¹

Following contact with infected skin, pathogens can attach and grow on the diaphragms of stethoscopes. Without proper disinfection, these pathogens may be transmitted to other patients.²² The diaphragm and bell of stethoscopes are colonized with micro-organisms around 87.3% of the time. It has been reported that

14% of stethoscopes carry MRSA while 16.5% carry gram-negative species.²³

The World Health Organization (WHO) has defined Health-Care Associated Infections (HCAIs) as infections that patients acquire while receiving treatment for medical or surgical conditions and are the most frequent adverse events during care delivery.²⁴ Despite the difficulty in establishing a direct association between stethoscope contamination and HCAIs, microbial contamination of stethoscopes has been demonstrated and this may contribute to the spread of pathogens involved in HCAIs.²⁵

The results of the study of Parmaretal in 2004 showed that 66% ethyl alcohol was an effective disinfectant for each of the 100 stethoscopes used in their study. Four samples were taken: One before cleaning (Group A), one immediately after cleaning (Group B), one after five days without cleaning (Group C), and one five days after cleaning once a day (Group D). Ninety percent (90%) of the stethoscopes in Group A were contaminated. A significant reduction in the contamination rate of stethoscopes was noted in Group B (28%) and in Group D (25%). In Group C, the rate of contamination was 95%. No statistically significant differences were found between Groups A and C.²⁶

Datta et al in 2018 documented that Isopropyl Alcohol (IPA) was significantly effective as a disinfectant. A total of 100 stethoscope diaphragms were analyzed before and after cleaning with IPA. Fifty-six membranes were noted to be colonized before disinfection. After disinfection, there were only five colonized membranes ($p < 0.001$).²⁷

In addition, Raghubanshi et al in 2017 reported that both 90% ethanol and IPA are equally effective in decontaminating diaphragm of the stethoscopes.¹⁸

In another study by Gurevich et al in 1996, it was documented that wiping the head of a stethoscope with a 70% alcohol pledget or wiping it with the antiseptic used for hand hygiene or a hospital surface disinfectant greatly reduced disease and usually eliminated the bioburden of aerobic bacterial contamination. However, not all microbial pathogens are removed by this method. Alcohol-based hand rubs that have been used for hygienic hand disinfection in hospitals are not enough to kill microbial DNA especially microbes which are resistant to the bactericidal effect of the alcohol.²⁸

The use of an Ethanol-Based Hand Sanitizer (EBHS) (gel or foam) was also reported to be effective for disinfection.²⁹⁻³¹

In a study conducted by Grandiere-Perez et al in 2015 it was noted that prior to disinfection, 38 out of 40 stethoscopes examined were culture positive with a mean of 29.9 CFU per culture plate. After disinfection, the mean was 1.1 CFU $p < 0.001$.²⁹

Similarly, Schroeder et al in 2009 found a mean of 28.4 CFU (95% CI, 20.2–36.6), in the pre-wash sample, and a mean of 3.2 (95% CI, 1.8–4.6; $p < 0.001$) in the post-wash sample.³¹

The use of disinfectant wipes is a practical and fast method for disinfecting stethoscopes and other authors have also evaluated the effectiveness of this method.^{30,32,33}

Leprat et al in 1998 demonstrated the effectiveness of wipes with benzalkonium against *coagulase-negative staphylococci* (CNS), CNS resistant to methicillin (MR-CNS) and *methicillin-susceptible Staphylococcus aureus* (MSSA). After cleaning, all contaminated stethoscopes showed no bacterial growth. However, in developing countries, they are not easily available because of their cost.³³

Ultraviolet (UV) C radiation denatures the DNA of microorganisms which have a high absorbance of the UV spectrum at 254 nm via formation of pyrimidine dimers. The bacteria are inactivated through DNA replication blockage.³⁴

Several previous studies have demonstrated that the use of UVC on a wheeled device to disinfect hospital rooms and environments is plausible, fast, and practicable. Nerandzic et al in 2012 tested portable units emitting UVC for disinfection of the environment.³⁵

The results of the study of Leontsini et al in 2012 showed the contamination of the stethoscopes with different species of *coagulase negative Staphylococcus* (CoNS) with high antibiotic resistance and possess the potential risk of causing hospital infections.³⁶

Earpieces of stethoscopes are also frequently colonized with microorganisms either by the hands of the users or by the users' ear canals.³⁷ The use of Ultraviolet C light (UVC) emitted by Light Emitting Diodes (LEDs) has been proposed and investigated as a method for disinfecting the stethoscope membrane.³⁸

In a 2015 study by Messina et al, a prototype UVC light was configured as a simple circular cover for application to stethoscope head. The size of the cover was obtained by analyzing the classical dimensions of stethoscope membranes and the design was created using Sketchup 3D modeling software and a 3D printer. A significant reduction in CFU counts after Ultraviolet C-light treatment ($P < .01$) was found for all bacteria: *E. faecalis* (85.5%), *S. aureus* (87.5%), *E. coli* (94.3%) and *P. aeruginosa* (74.9%). The results showed that the treatment technique was effective and efficient in disinfecting the membranes.³⁸

In another study in 2016, Messina et al conducted a pre- and post-intervention study to verify the effectiveness and reliability of UVC LEDs in stethoscope membrane disinfection after prolonged use. UVC LEDs demonstrated the capacity to maintain high levels of disinfection after more than 240 hours of use and they were effective against common microorganisms that are causative agents of HCAs including *S. aureus* and *E. coli*. Statistically significant differences ($p < 0.001$) were also found relating to the reduction of specific bacteria (no CFUs observed for *S. aureus* and *E. coli*). The authors concluded that after prolonged use of the UVC light, it was still effective in reducing microbial contamination.³⁹

Several things need to be considered when comparing disinfection of stethoscopes using alcohol versus UVC light. The UVC sterilizer, though obviously more expensive, is durable and more cost effective since there is no need for other materials such as alcohol and cotton balls.

OBJECTIVES of the STUDY

General Objective

To determine the effectiveness of ultraviolet c-light in eliminating microbial pathogens from stethoscopes used in the Neonatal Intensive Care Unit of East Avenue Medical Center

Specific Objectives

1. To determine the prevalence of identified microbial pathogens colonizing the stethoscopes before exposure to UVC light and standard of care
2. To determine the quantity of growth (in Colony Forming Units/CFUs) present in the stethoscopes used at NICU-EAMC before exposure to UVC light and standard of care
3. To determine the effect of UV-C light and standard of care in reducing isolated pathogens in stethoscopes used at NICU-EAMC

4. To compare the effectiveness of UVC light and the standard of care in eliminating microbial pathogens from stethoscopes used in the NICU-EAMC

OPERATIONAL DEFINITION OF TERMS AND VARIABLES

Stethoscope– medical device that is used in the NICU section for auscultation of admitted patients

Colony-forming unit (CFU)–used to estimate the number of bacterial growth in the study

Pre-test – before exposure to the different interventions

Post-test – after exposure to the different interventions

UVC light (ECOMOM) – A UV lamp (253.7nm) incorporated in the nursing bottle sterilizer portable machine that is operated by touch icon in the operating panel. It is operated by tapping the icon in the operation panel. It features a touch type button, which is simple and convenient to use. It also releases anion which is excellent for removing contaminated substances, dirt and odors to every corner inside the stored item which is not sterilized by UV. Moreover; it has a PTC fan drying method heater that efficiently dries the remaining humidity and moisture in the item thereby combating the reproducing power of virus in humid condition. The device has a surface of high-class stainless steel inner reflection plate that can refract UV rays to reach all corners, which increases sterilization efficiency.

Propan-2-ol, Propan-1-ol, MectroniumEthylsulfate (STERILIUM) – A classic alcohol-based hand disinfection which is used in the NICU Section as standard of care for disinfecting functional stethoscopes

METHODOLOGY

Research Design

This is a two-arm, double blind, randomized controlled trial.

Sample Size Computation

The minimum sample size computed for this study is 26 stethoscopes with culture growth based on a 95% of detecting at 1% level of significance, a decrease in the average number of colony forming units from 35 in the control group to 2 in the experimental group according to the study of Mesina et al in 2015. Thirteen (13) stethoscopes each were randomly allocated to Ultraviolet C (Group A) and standard of care (Group B) groups.

The sample size was determined based on the average of the total number of functional stethoscopes for the past two years (2020 – 2021) with $n = 52$, 95% confidence interval and 90% response rate distribution among the participants in either Group A or B.

Statistical Analysis

Valid data from evaluable subjects were included in the analysis. Missing values were accounted for during the statistical analysis of outcome variables. Summary statistics were reported in tables as means, standard deviations, percentages, and frequencies min-max for quantitative discrete outcome measures (e.g. number of colony forming units) or % for qualitative measures (e.g. reduction in CFU count). Dependent and independent sample t-tests were used to compare CFU counts before and after the intervention to determine significant differences in CFU count reduction across the two intervention groups and to compare the reduction in CFU count between each active intervention group and control group. Statistical significance was based on a $p\text{-value} \leq 0.05$. Data processing and statistical

analyses were performed using the Statistical Package for Social Sciences (SPSS) version 28.

Description of the Study Procedure

Specific Methods and Techniques

Functional stethoscopes dedicated to patients at the NICU were numbered and collected individually with the use of sterile gloves to avoid contamination by the assistant investigator and were transferred to the isolated research study site. The assistant investigator then collected samples from the diaphragm of the stethoscopes in a circular motion, centrifugally, for about 10-15 minutes, using a sterile cotton tip swab. Each swab was placed inside a tube with brain heart infusion broth as culture media. The dates and times of specimen collection were recorded during the swabbing phase.

The samples were transported to the Microbiology section of the EAMC Laboratory in not more than 30 minutes after swabbing. Collected samples were incubated at 37 degrees Celsius for 24 hours after which the culture reading was done by a medical technologist who determined the presence of bacterial growth in the sample. Two samples with no growth were excluded from the study. Twenty six (26) sample cultures were positive for bacterial growth and were randomly grouped by the assistant investigator to Group A (UVC) and Group B (standard care with Propan-2-ol, Propan-1-ol, MecetroniumEthylsulfate) through drawing of lots.

GROUP A: Exposed to UVC light for 15 minutes (as per manufacturer's recommendation)

- a. The 15-minute sterilization process began with the activation of the sterilization button.
- b. The built-in timer displayed the remaining time for the sterilization process.

- c. An alarm sounded to signal the end of the sterilization process after which the device automatically turned off.

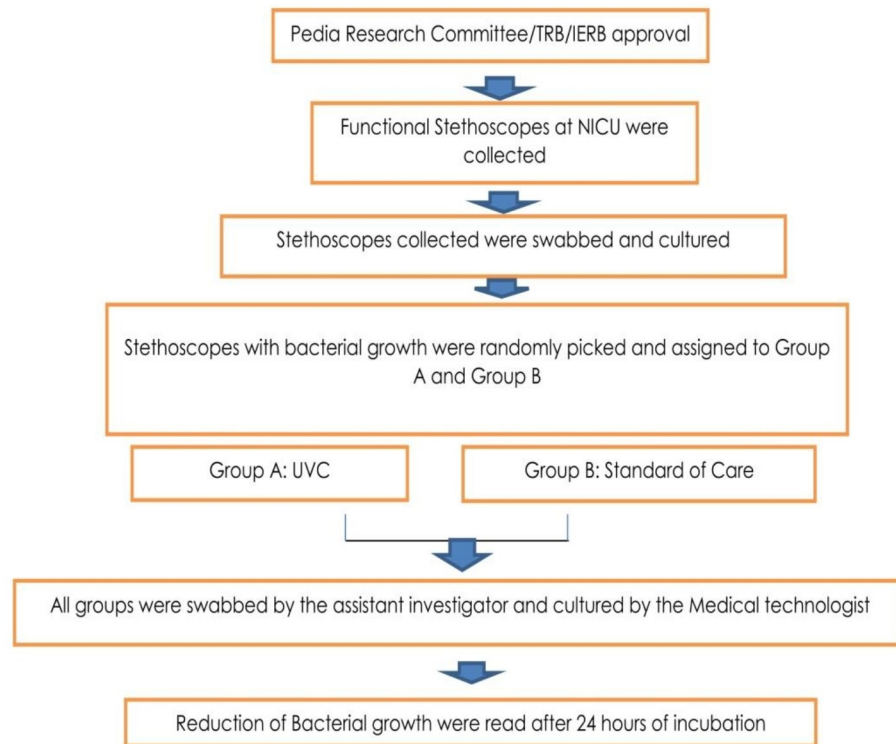
GROUP B: Disinfection with Propan-2-ol, Propan-1-ol, MecetroniumEthylsulfate

The assistant investigator disinfected each stethoscope belonging to this group for about 45 to 60 seconds using the standard of care used by the NICU staff.

After each disinfection, all stethoscopes from Group A and B were cultured by the same medical technologist in a manner similar to the process previously mentioned. The Medical Technologist was blinded with regard to the particular disinfection process used. The samples were all incubated at 37 °C and read after 24 hours. It was concluded that the specific intervention was not effective for those samples noted to have bacterial growth after the incubation period.

In order to prevent contamination of the samples, an isolated area in the NICU served as the location for sampling collection. All samples were kept at the EAMC-Laboratory Department following standard protocols.

Study Algorithm



Inclusion Criteria

Only functional stethoscopes with culture growth were included in the study.

Exclusion Criteria

Stethoscopes with no documented growth in the first culture were excluded from the study.

RESULTS

The frequency percentages of microbial pathogens were examined before exposure to UVC (Group 1) and the standard of care (Group 2).

Table 1 shows the frequency count of microbial pathogens presented in the study before exposure to the different interventions.

Table 1. Frequency Percentages of Microbial Pathogens

Microbial Pathogen	Group 1	%	Group 2	%	Total	%
<i>Pseudomonas aeruginosa</i>	0	0.00	1	7.69	1	3.85%
<i>Escherichia coli</i>	1	7.69	0	0.00	1	3.85%
<i>Klebsiella pneumoniae</i>	2	15.38	0	0.00	2	7.69%
<i>Staphylococcus epidermidis</i>	3	23.08	4	30.77	7	26.92%
<i>Staphylococcus haemolyticus</i>	5	38.46	4	30.77	9	34.62%
<i>Staphylococcus hominis</i>	2	15.38	3	23.08	5	19.23%
<i>Staphylococcus lentus</i>	0	0.00	1	7.69	1	3.85%
N	13	100.00	13	100.00	26	100.00%

In Group 1, the most frequent pathogens colonized were *Staphylococcus haemolyticus* (38.46%), *Staphylococcus epidermidis* (23.08%), *Staphylococcus hominis* and *Klebsiella Pneumoniae* (15.38%). In Group 2, the prevalent pathogens were *Staphylococcus haemolyticus* and *Staphylococcus Epidermidis* (30.77%) and

Staphylococcus hominis (23.08%). The microbial pathogens predominantly found on the stethoscopes included in the study were *Staphylococcus haemolyticus* (34.62%), *Staphylococcus epidermidis* (26.92%) and *Staphylococcus hominis* (19.23%).

Table 2. Colony-forming Units before and after the different interventions
(Group 1: UVC exposed; Group 2: Standard of care)

Groups		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Steth Pre Group 1	89230.77	13	7595.54	2106.625
	Steth Post Group 1	5384.62	13	19414.50	5384.615
Pair 2	Steth Pre Group 2	76538.46	13	21926.45	6081.303
	Steth Post Group 2	10769.23	13	26287.36	7290.804

Table 2 shows the colony-forming units before and after the interventions. For Group 1 (UVC exposed), the CFU mean scale score was 89,230.77 (SD= 7,595.54) before the exposure to UVC light. After exposure to UVC light, CFU mean scale score of Group 1 decreased from 89230.77 to 5384.62. However, the CFU mean scale score of the group after exposure to UV light intervention became heterogeneous. This means that the intervention varied greatly within the group and the decrease in CFU was arbitrary.

Meanwhile, Group 2 (standard of care) had a CFU mean scale score of 76,538.46 (SD= 21,926.45) before the intervention. After exposure to the standard of care, the CFU mean scale score decreased to 10,769.23 (SD= 26,287.36). The CFU mean scale score of the group after their exposure to the standard of care became heterogeneous. This means that the intervention varied greatly within the group and the decrease in CFU was also arbitrary. Arbitrary means that interventions rely heavily on the type of microbial pathogens and/or CFU level of the stethoscopes before intervention.

Table 3. Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	StethPreGroup 1 – StethPostGroup 1	83846.154	20631.069	5722.029	71378.923	96313.384	14.653	12	.000
Pair 2	StethPreGroup 2 – StethPostGroup 2	65769.231	33030.677	9161.062	45808.992	85729.469	7.179	12	.000

Aside from observing the decrease in CFU mean scale scores to determine the effectiveness of UVC light and standard of care interventions in decreasing the CFUs of microbial pathogens of selected stethoscopes under investigation, the CFUs of groups 1 and 2 before and after intervention were analyzed.

The results of the paired sample t-tests of groups 1 and 2 before and after the intervention are presented in **Table 3**.

Pre-test and Post-test Comparison of Group 1. Using the dependent sample t-test, the pre-test and post-test CFUs of Group 1 were compared. There was a significant difference between the pre-test and post-test CFUs of Group 1 stethoscopes ($t = 14.653$ $p < .05$). This means that UVC light was effective in decreasing the CFUs of

microbial pathogens present in the stethoscopes under investigation.

Pre-test and Post-test Comparison of Group 2. Using the dependent sample t-test, the pre- and post-test CFUs of the samples in Group 2 were compared. There was a significant difference between the pre- and post-test CFUs of Group 2 stethoscopes ($t = 7.179$, $p < .05$). This means that standard of care was effective in decreasing CFUs of microbial pathogens present in the stethoscopes under investigation.

Both the UVC light and standard care interventions were proven to be effective in addressing the microbial pathogens of stethoscopes used in the study regardless of the CFU percentages or levels.

Table 4. Group Statistics

	Exposure	N	Mean	Std. Deviation	Std. Error Mean
Steth Pretest	1.00	13	89230.77	7595.545	2106.625
	2.00	13	76538.46	21926.450	6081.303
Steth Posttest	1.00	13	5384.62	19414.507	5384.615
	2.00	13	10769.23	26287.367	7290.804

To evaluate whether UVC light and/or standard of care of intervention was more effective in decreasing the CFUs of microbial pathogens among the stethoscopes under investigation, an independent sample t-test was performed.

Table 4 shows the summary of the group statistics of the pre- and post-test results of both the UVC light and standard of care groups. Number 1 represents the UVC light exposed group while number 2 represents the standard of care group. The pre-test CFU mean scale score of the UVC light group (mean = 89,230.77, standard deviation = 7,595.54) was much higher than the standard care group (mean = 76,538.46, SD = 21,926.45). On the

other hand, the post-test of the UVC light group was much lower (mean = 5,384, SD = 19,414.50) than the standard of care group (mean = 10,769.23, SD = 26,287.36). Based on the mean CFU mean scale score alone, the UVC light was more effective in decreasing the microbial pathogens among the selected stethoscopes under investigation compared to the standard of care intervention.

Table 5. Independent Samples

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
StethPretest	Equal variances assumed	.990	.330	1.972	24	.060	12692.308	6435.846	-590.626	25975.242
	Equal variances not assumed			1.972	14.839	.068	12692.308	6435.846	-1038.339	26422.954
StethPosttest	Equal variances assumed	1.484	.235	-.594	24	.558	-5384.615	9063.658	-24091.087	13321.856
	Equal variances not assumed			-.594	22.089	.558	-5384.615	9063.658	-24177.094	13407.863

To validate the statistical differences between the pre- and post-test CFUs of the two groups, an independent sample t-test was performed.

Table 5 shows the summary of the results of the independent sample t-tests. The equal variances assumed were used to determine the significant differences since the Levene's test for equality of variances showed no significant differences between the pre- and post-test CFUs of both the UV light ($F = .990, p > .05$) and standard of care ($F = .235, p > .05$) groups.

Before Intervention (Groups 1 and 2) There was no significant difference in the pre-test CFUs of both Group 1 and Group 2 stethoscopes ($t = 1.972, p > .05$). This means that the pre-test CFU scores of both groups were comparable before their respective interventions.

After Intervention (Groups 1 and 2) There was no significant difference in the post-test CFUs of Group 1 and Group 2 stethoscopes ($t = -.594, p > .05$). This means that the changes in the post-test CFU scores and/or microbial pathogens of the stethoscopes under investigation among the two groups were comparable.

Both the UVC light and standard of care interventions were effective in preventing the proliferation of microbial pathogens present on the stethoscopes as evidenced by the decreasing the CFUs on the stethoscopes. There was no significant difference in the post-test CFUs of the stethoscopes in the UVC light or standard of care groups.

DISCUSSION

In the present study, it was shown that there was no significant difference in the pre-test CFUs of both the UVC and standard of care groups ($t = 1.972, p > .05$). For that reason, the baseline CFUs of both groups were statistically equivalent or comparable to each other before exposure to UVC light or standard of care. Specifically in both groups, the predominant microbial pathogens colonized in the stethoscopes at NICU-EAMC were

different species of Coagulase Negative Staphylococcus (CoNS) namely: *Staphylococcus Hemolyticus* (34.62%), *Staphylococcus Epidermidis* (26.92%) and *Staphylococcus Hominis* (19.23%). As documented by previous studies, stethoscopes are a major source of nosocomial infections in the NICU.²² The diaphragm and bell of stethoscopes are colonized by microorganisms 87.3% of the time, 16.5% of which are gram-negative species.²⁴

Leontsini et al reported in 2012 that the contamination of the stethoscopes with different species of *Coagulase Negative Staphylococcus* (CoNS) had high antibiotic resistance and had the potential risk of causing hospital infections.³⁷

Stethoscopes have been recognized as important modes of bacterial transmission among patients. Strategies for the sterilization of stethoscopes such as the CDC recommendation of disinfection for each patient, once daily or once weekly should be employed in order to prevent hospital infections.^{20,21}

The present study demonstrated that there was a significant difference between the pre- and post-test CFUs of the Standard of Care stethoscope group ($t = 7.179$, $p < .05$). There was also a significant difference between the pre- and post-test CFUs of the UVC stethoscope group ($t = 14.653$, $p < .05$). Thus, UV light and standard of care were shown to be both effective in decreasing the CFUs of the stethoscopes. Furthermore, a comparison of the post-test CFUs in the UVC and standard of care stethoscope groups showed that there was no significant difference ($t = -.594$, $p > .05$). Based on the results of the study, it can be concluded that UVC light is as effective as the standard of care in decreasing CFUs of stethoscopes. Hence, UVC light may be an alternative method for decontaminating stethoscopes especially since 70% alcohol or

alcohol-based products are not enough to eliminate all microbial pathogens.

Various studies have supported the fact that the UVC technique is an effective and efficient way of disinfecting stethoscope membranes via DNA denaturation of the microorganisms.³⁵

Similarly, Messina et al also reported a significant reduction in CFU counts for all bacteria (85.5% *E. faecalis*, 87.5% *S. aureus*, 94.3% *E. coli*, and 74.9% for *P.aeruginosa* after UVC light treatment ($P < .01$). The treatment technique was effective in disinfecting the membranes of stethoscopes.⁴⁰

There are several advantages of UVC over alcohol-based disinfection. UVC is plausible, fast and practicable. Nerandzic et al in their 2012 study reported that portable units could emit UVC for disinfection of the entire environment.³⁶ Likewise, Messina et al documented that UVC LED eliminated *S. aureus* and *E. coli* growths on stethoscopes and could maintain high levels of disinfection after more than 240 hours of use. UVC light was found to be effective in reducing microbial contamination even after prolonged use.⁴⁰

UVC light sterilizer may seem to be a more expensive tool compared to isopropyl alcohol disinfection. However, it may actually be more cost-effective in the long run. UVC has longer durability and requires less consumables (e.g. alcohol and cotton balls). Moreover, UVC is effective against common microorganisms that are causative agents of HCAs. Hence, UVC light is an effective and reliable method for disinfecting stethoscope membranes.

CONCLUSION

Proper disinfection of stethoscopes after each use may help in reducing cross-contamination and subsequently, decrease the likelihood of healthcare associated infections.

UVC light sterilization is comparative to the standard of care in eliminating microbial pathogens. It works faster and is more reliable, durable and cost-effective. It is recommended as an alternative method for decontaminating stethoscopes used at the EAMC-NICU due to its numerous advantages.

LIMITATIONS OF THE STUDY

The study involved only a small sample size of stethoscopes used at the Neonatal Intensive Care Unit and did not include other areas in the hospital. In addition, the culture method used was limited to bacterial pathogens. Hence, the effectiveness of UVC light against viral or fungal pathogens was not evaluated. Lastly, the microbial pathogens present in each stethoscope was heterogenous, indicating that the intervention varied greatly within each group.

RECOMMENDATIONS

A larger sample size and inclusion of stethoscopes used in other areas of the hospital as well as other medical equipment utilized are recommended in order to more accurately establish the effectiveness of UVC light in a tertiary government hospital setting and to improve infection control measures.

The use of homologous pathogens and various culture media to include detection of viral and fungal pathogens is also recommended.

A follow-up study that would compare the three methods (UVC light alone, UVC light plus standard of care and standard of care alone) may also help in guiding health care professionals in choosing the most effective method of sterilization.

Strict adherence to existing standard disinfection protocols is vital to decrease the spread of nosocomial infections. Routine surveillance, frequent supervision and regular

training of personnel with regard to infection control measures should be implemented to ensure the sterility of equipment used in the NICU.

ETHICAL CONSIDERATIONS

Approval from the EAMC-IERB was sought prior to the conduct of the study. The research was done according to the principles of good clinical practice.

The data gathered may be presented in local and international conferences and the manuscript may be submitted for future publication.

The authors declare no conflicts of interest.

No funding was received for this study. The principal investigator shouldered all study-related expenses. However, the EAMC-Laboratory, through the PETRO office granted a 50% discount on the price of the cultures requested. In addition, the UVC sterilizer was donated by the company (Ecomom) to the Department of Pediatrics during the time of the pandemic when this study was conducted. No other support was received from the donors of the UVC sterilizer.

REFERENCES

1. Africa-Purino FC, Dy ER & Coronel R. **Stethoscopes: a potential source of nosocomial infections.** Phil J Microbiol Infect Dis. 2000;12:9-13.
2. Gastmeier P, Groneberg K, Weist K & Ruden H. **A cluster of nosocomial Klebsiella pneumoniae bloodstream infections in a neonatal intensive care department: identification of transmission and intervention.** Am J Infect Control. 2003. 31: 424-430.
3. Jones JS, Hoerle D & Riekse R. **Stethoscopes: A potential vector of infection?** Ann Emerg Med. 1995. 26:296-9.
4. Gurevich I, R Dubin & BA Cunha. **Dental instrument and device sterilization and disinfection practices.** Journal of Hospital Infection. 1996. 32:295-304.
5. Rutala WA, Weber DJ & the Healthcare Infection Control Practices Advisory Committee (HICPAC). **Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008.** Update: May 2019. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, Georgia. Available from: http://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008
6. Havill NL. **Best practices in disinfection of noncritical surfaces in the health care setting: creating a bundle for success.** Am J Infect Control. 2013. 41:226-30.
7. Anderson DJ, Gergen MF, Smathers E, Sexton DJ, Chen LF, Weber DJ et al. **Decontamination of targeted pathogens from patient rooms using an automated ultraviolet C emitting device.** Infect Control Hosp Epidemiol. 2013. 34:466-71.
8. World Health Organization. **Report on the burden of endemic health care associated infection worldwide.** Available from: http://whqlibdoc.who.int/publications/2011/9789241501507_eng.pdf. Accessed June 13, 2015.
9. Thompson, RL, Landry, SM, Russell, BS, Miller, PJ, Ponce de Leon, S & Miller, GB, Jr. **Hospital-acquired infections in intensive care unit patients: An overview with emphasis on epidemics.** Infect. Control. 1983. 4:371-375.
10. Sengupta, S, Sirkar, A & Shivananda, PG. **Stethoscopes and nosocomial infection.** Ind J. Ped. 2000. 67: 197-199.
11. Messina, G, Burgassi, S, Messina, D, Montagnani, V & Cevenini, G. **A new, U.V.-LED device for automatic disinfection of stethoscope membranes.** Am. J. Infect. Control. 2015. 43, e61-e66.
12. World Health Organization. **Report on the Burden of Endemic Health Care Associated Infection Worldwide.** Available from: http://whqlibdoc.who.int/publications/2011/9789241501507_eng.pdf. Accessed February 2020.
13. Messina, G, Ceriale, E, Lenzi, Dm Burgassi, S, Azzolini, E & Manzi, P. **Environmental contaminants in hospital settings and progress in disinfecting techniques.** BioMed Res. Int. 2013, 2013, 429780.
14. Lee, YT, Chen, SC, Lee, MC, Hung, HC, Huang, HJ, Lin, HC et al. **Time-series analysis of the relationship of antimicrobial use and hand hygiene promotion with the incidence of healthcare-associated infections.** J. Antibiot. 2012. 65: 311-316.
15. Alvarez, JA, Ruiz, SR, Mosqueda, JL, Leon, X, Arreguin, V, Macias, AE et al. **Decontamination of stethoscope membranes with chlorhexidine: Should it be recommended?** Am. J. Infect. Control. 2016. 44, e205-e209.
16. West, GF, Resendiz, M, Lustik, MB & Nahid, MA. **Bacterial Contamination of Military and Civilian Uniforms in an Emergency Department.** J. Emerg. Nurs. JEN Off. Publ. Emerg. Dep. Nurses Assoc. 2018. 45, 169-177.e1.
17. Marinella, MA, Pierson, C & Chenoweth, C. **The stethoscope. A potential source of nosocomial infection?** Arch. Intern. Med. 1997. 157: 786-790.
18. Raghubanshi, BR, Sapkota, S, Adhikari, A, Dutta, A, Bhattarai, U & Bhandari, R. **Use of 90% ethanol to decontaminate stethoscopes in resource limited settings.** Antimicrob. Resist. Infect. Control. 2017. 6:68.
19. Napolitani M, Bezzini D, Moirano F, Bedogni C & Messina G. **Methods of Disinfecting Stethoscopes: Systematic Review.** Int. J. Environ. Res. Public Health 2015. 17, 1856.

20. Bearman G, Bryant K, Leekha S, Mayer J, Munoz-Price LS, Murthy R et al. **Healthcare personnel attire in non-operating room settings.** *Infect Control Hosp Epidemiol.* 2014. 35:107–21.
21. Longtin Y, Schneider A, Tschopp C, Renzi G, Gayet-Ageron A, Schrenzel J et al. **Contamination of stethoscopes and physicians' hands after a physical examination.** *Mayo Clin Proc* 2014. 89:291–9.
22. O'Flaherty, N & Fenelon, L. **The stethoscope and healthcare-associated infection: A snake in the grass or innocent bystander?** *J. Hosp. Infect.* 2015. 91, 1–7.
23. Sanders S. **The stethoscope and cross-infection.** *British J Gen Pract* 2003. 53:971–972.
24. World Health Organization. **Report on the Burden of Endemic Health Care-Associated Infection Worldwide.** 2011. WHO Document Production Services: Geneva, Switzerland. pp. 1–34.
25. Nerandzic MM, Cadnum JL, Eckart KE & Donskey CJ. **Evaluation of a hand-held far ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens.** *BMC Infect Dis* 2012. 12:120.
26. Parmar, RC, Valvi, CC, Sira, P, & Kamat, JR. **A prospective, randomised, double-blind study of comparative efficacy of immediate versus daily cleaning of stethoscope using 66% ethyl alcohol.** *Indian J. Med. Sci.* 2004. 58:423–430.
27. Datta, P, Kaur, M, Rawat, S, Gupta, V & Chander, J. **Stethoscope, "the friendly foe"—A study to evaluate bacterial contamination of stethoscopes and disinfection practices.** *J. Infect. Dev. Ctries.* 2018. 12:887–893.
28. Gurevich I, Dubin, R and Cunha BA. **Dental instrument and device sterilization and disinfection practices.** *Journal of Hospital Infection.* 1996. 32:295–304.
29. Grandiere-Perez, L, Bovet, J, Beaudron, A, Saulnier, P, Bianchi, S, Delemotte, M & Ramanantsoa, C. **Efficacy of an ethanol-based hand sanitizer for disinfection of stethoscopes.** *J. Hosp.Infect.* 2015. 91: 183–184.
30. Mehta,AK, Halvosa,JS, Gould,CV & Steinberg, JP. **Efficacy of alcohol-based hand rubs in the disinfection of stethoscopes.** *Infect.Control Hosp.Epidemiol.* 2010.31:870–872.
31. Schroeder, A, Schroeder, MA & D'Amico, F. **What's growing on your stethoscope? (And what you can do about it).** *J.Fam.Pract.* 2009.58: 404–409.
32. Hill, C, King, T, & Day, R. **A strategy to reduce, M.R.SA colonization of stethoscopes.** *J.Hosp.Infect.* 2006.62:122–123.
33. Leprat, R, Minary, P, Devaux, V, de Waziere, B, Dupond, JL & Talon, D. **Why, when and how to clean stethoscopes.** *J.Hosp.Infect.* 1998. 39:80–82.
34. Giese, N and Darby, J. **Sensitivity of microorganism to different wavelengths of UV light: Implications on modeling of medium pressure UV systems.** *Water research.* 2000. 34:4007–4013.
35. Nerandzic MM, Cadnum JL, Eckart KE & Donskey CJ. **Evaluation of a hand-held far ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens.** *BMC Infect Dis.* 2012. 12:120.
36. Leontsini F, Aris Papapetropoulos A & Vantarakis, A. **Stethoscopes As Vectors Of Multi-Resistant Coagulase Negative Staphylococci In A Tertiary Hospital.** *Int J Med Sci Public Health* 2013. 2:324–329.
37. Brook I. **Bacterial flora of stethoscopes' earpieces and otitis externa.** *Ann OtolRhinolLaryngol.* 1997. 106(9):751–2.
38. Messina, G, Burgassi, S, Messina, D, Montagnani, V & Cevenini, G. **A new UV-LED device for automatic disinfection of stethoscope membranes.** *Am J Infect Control.* 2018. 43, e61–e66.
39. Messina G, Fattorini M, Nante N, Rosadini D, Serafini A, Tani M & Cevenini G. **Time Effectiveness of Ultraviolet C Light (UVC) Emitted by Light Emitting Diodes (LEDs) in Reducing Stethoscope Contamination.** *Int J. Environ. Res. Public Health.* 2016. 13 940