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ORIGINAL ARTICLE

THE USE OF FLUORESCENT MARKING TECHNIQUE AS AN INDICATOR OF CLEANLINESS AND DISINFECTION IN THE NEONATAL INTENSIVE CARE UNIT

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The authors declare that the data presented are original material and have not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all the authors, who have met the requirements for authorship.

ABSTRACT

Background: Environmental surfaces harbor pathogens that transmit them and there is a need for environmental cleaning and disinfection to prevent the spread of infection.

Objective: This study aimed to determine if the use of fluorescent marking (FM) technique in high touch areas can be used as an index of cleanliness and disinfection as determined by aerobic colony count.

Methods: This was an experimental study done at the University of the Philippines Philippine General Hospital Neonatal Intensive Care Unit (NICU). A total of 40 surfaces were swabbed for cultures with aerobic colony count (ACC) then adjacent areas are marked with fluorescent gel. After cleaning and disinfection, checking for residual fluorescent markings with congruent environmental culture with an aerobic colony count of the same surface was done. The rate of removal and colony count were then compared to assess the specificity and sensitivity of the fluorescent marking technique as a gauge of cleanliness of high touch surface areas. Any residual fluorescence of the marked areas was considered unclean and an aerobic colony count of < 2.5 - 5CFU/ml^2 were considered an acceptable level of cleanliness.

Result: A total of 40 high contact surfaces were sampled from 5 areas were collected. Prior to cleaning, 60% (24) of the surfaces (60%) did not contain microorganisms. After cleaning, the (FM) had 38% and in the ACC 83% were assessed to be clean. The sensitivity of FM is 85.71% and specificity of 42.42%. The positive predictive value (PPV) is 24% with the positive likelihood ratio (positive LR) of 1.49 and the negative predictive value (NPP) is 93.33%.

Conclusion: The use of Fluorescent Marking technique in high touch areas as an index of cleanliness and disinfection is a good marker for cleanliness and disinfection. Furthermore, it is a simple, rapid, inexpensive and has potential to increase awareness of the environment that can be utilized as an objective parameter to assess cleanliness and disinfection.

KEYWORDS:
Nosocomial infection, fluorescent marker, healthcare-associated infection, environmental culture

INTRODUCTION

Reducing the spread of healthcare-associated pathogens to patients constitutes one of the most challenging aspects of health care epidemiology.[1, 2] According to the World Health Organization (WHO), the prevalence of healthcare-associated infection in developed countries varies between 3.5% and 12%.[3] In European countries, the prevalence range from 4.6% to 9.3%[4], while prevalence in the United States was 4.5% in 2002. This corresponded to 9.3 infections per 1,000 patient-days and 1.7 million affected patients.[5] In low- and middle-income countries, the prevalence of healthcare-associated infection varies between 5.7% and 19.1%.[3]

Among the Department of Health retained hospitals, the net infection rates revealed that 36.67% had 0.0% net infection rate, and 63.33% had an average net infection rate below 1%.[4] In contrast the University of the Philippines Philippine General Hospital Department of Pediatrics has reported a nosocomial infection rate of 9.20% in 2014 and 9.77% for the first half of 2016.[5]

Several factors can cause healthcare-associated infections. Some determinants are more specific to settings with limited resources.[3] Microorganisms dwell in inanimate objects and serve as a source of contamination between the healthcare workers and patient.[6] A study of Morgan et al showed that contaminated environmental surfaces act as reservoirs for patient-to-patient transmission via the hands of healthcare workers.[7]

Similarly, Curtis J. Donskey, et al noted that current guidelines for pathogens emphasize the importance of environmental disinfection as a control measure[8]. Furthermore, Axel Kramer et al concluded that most common nosocomial pathogens may survive or persist on surfaces for months and can be a continuous source of transmission if no regular preventive surface disinfection is performed.[6]

Objective parameters to determine the degree of cleanliness are important interventions as part in preventing healthcare-associated infections. In the NICU, visual inspection is the most utilized modality to assess cleanliness and is an inexpensive and rapid way. However, visual inspection may be crude and unreliable.[9] Presently numerous studies have utilized fluorescent markers as a method in checking and improved strategies for environmental cleaning.[10-15] Similar to visual inspection, it is simple, inexpensive and used an objective parameter in determining cleanliness and is underutilized or not all. The goal of this study is to compare the proportion of surface assessed as clean at baseline (pre-cleaning) and post cleaning of selected high touch areas at the NICU as determined by aerobic colony count and to determine the diagnostic accuracy of the fluorescent marking technique for the assessment of cleanliness of selected high touch areas at the NICU in terms of sensitivity, specificity, positive predictive value and negative predictive using aerobic colony count as the gold standard.

METHODOLOGY

Study design

This study was an experimental study done at the University of the Philippines, Philippine General Hospital's Neonatal Intensive Care Unit (NICU).

Sample size

A minimum of 40 surfaces is required for this study. The sample size computation is based on the assumption that surfaces with the complete and partial removal of the fluorescent marker are 90% and 50% clean, respectively when assessed using the culture-based technique. The computed sample size has a level of significance of 5% and 80% power.

Study Procedure

The fluorescent gel/liquid used was Glo Germ™ that was used to determine surface cleaning effectiveness to avoid transmission or spread of microbes. It is a viscous, translucent solution formulated using a stable, nontoxic base, to which was added a chemical marker that fluoresces under

black light. [16] Approximately 0.2 - 0.5 mL of solution was applied to each surface to create a well-circumscribed target with diameters of approximately 1- 1.5 cm.

The time of cleaning was congruent with the cleaning schedule; procedures, paraphernalia for cleaning, and disinfecting solutions followed standards prescribed by the Hospital Infection Control Unit (HICU).

The specified target areas were adapted from the list of frequently contaminated surfaces provided by the United States CDC. [17] For each of the 5 locations, 8 areas were identified such that 40 high touch surfaces were swabbed for environmental cultures and aerobic colony count (ACC).

The microbiologic sampling method was adapted from the study of Snyder et al[9]; the primary investigator used 2 sterile cotton-tipped swabs moistened with sterile water or sterile NSS rubbed over an approximately 2 × 2 inch area. The area was covered with a back-and-forth pattern and subsequently in an overlapping but perpendicular back-and-forth pattern, performed with a twisting motion to expose the entire swab to the surface. The specimen was brought to the microbiology laboratory that was swabbed onto a primary media (Blood Agar Plate) to determine ACC using the standard method. After samples for cultures were taken, adjacent areas of about 1-2cm from the area of swabbing were marked with fluorescent gel by the principal investigator.

Cleaning was done by the trained cleaning crew who remained blinded to the fluorescence marking procedure. The crew used paraphernalia and methods prescribed by HICU as follows: after donning clean gloves, clean towels were moistened with sodium hypochlorite (bleach) with a dilution of 1:100, moderate pressure were applied on surfaces of about 5-10 seconds.

After cleaning, a research assistant checked for removal of fluorescent markings. Another environmental culture with an aerobic colony count of the same surface was done to determine the post-cleaning state of the unit. The rate of removal and colony count were then compared to assess the specificity and sensitivity of the fluorescent marking technique as a gauge of cleanliness of high touch surface areas.

The following definitions of cleanliness were used as outcome measurements:

a. The absence of fluorescence is defined as a clean surface, while a fully intact or a partially removed mark is defined as dirty.[9]
b. An aerobic colony count of 5CFU/ml^2 from the environmental cultures is considered an acceptable level of cleanliness. If a surface has been found to contain 6 CFU/mL^2, this was considered to be positive for microbial growth (unclean)^2

Ethical considerations

This protocol was approved by University of the Philippines Manila Research Ethics Board (UPMRB) PGH Review Panel. The study was conducted with the approval from the UPMREB PGH Review Panel.

This research did involve any patients. However, medical devices and surfaces utilized around the patients are involved. There was no direct contact of the fluorescent marker with any patient. Cleaning personnel was informed of any areas found to be unclean so that appropriate remedial measures can be implemented.

Statistical Analysis

Descriptive statistics were used to summarize the aerobic colony count of the high-touch surfaces. Frequency and proportion were used for nominal variables. Fisher's exact/Chi-square test was used to compare the number of areas that were cleaned before and after disinfection. Diagnostic accuracy test was used to determine the sensitivity, specificity, predictive value and likelihood ratio of the Fluorescent Marking (FM) scan using the Aerobic Colony Count (ACC) as the gold standard. All valid data were included in the analysis. Missing

variables were neither replaced nor estimated. The null hypothesis was rejected at 0.05α-level of significance. STATA 12.0 was used for data analysis.

The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of the fluorescent marking technique as an indicator of disinfection were determined for diagnostic accuracy test using.

RESULTS

A total of 40 surfaces were sampled from the hospital (Table 1). Five areas were analyzed: (1) bed rails/cribs, (2) tray tables, (3) IV poles or grab areas, (4) IV pump controls and (5) telephone areas. The presence of microorganisms was determined by swabbing the surfaces and inoculating the swabs on the appropriate culture medium to determine the aerobic colony count (ACC).), while areas with 5 CFU/mL^2 and below were considered to be without growth (clean), following the parameters as set in the study by Dancer, 2016.

Pre-cleaning monitoring showed that 24 surfaces (60%) were noted to be clean and 16 were deemed unclean. Among the unclean surfaces were syringe pumps (4 out of 5), infusion pumps (3 out of 5), IV poles (3 out of 8), incubator port locks (2 out of 3), crib (1 out of 5), nurse tray table (1 out of 3), patient tray table (1 out of 6) and refrigerator handle (1 out of 1). The isolated microbes were Acinetobacter species, Diptheroids, Sarcina lutea, Coagulase-negative Staphylococcus, Staphylococcus aureus, and Nocardia species.

After cleaning, high touch surfaces showed 25 surfaces (62.5%) remained positive with FM, of which 13 (32.5%) had intact FM and 12 (30%) surfaces had partial removal, and 15 (37.5%) surfaces had complete removal of FM. Majority of the areas with positive fluorescent markings were infusion pumps (5 out of 5), syringe pumps (4 out of 5), incubator controls (3 out of 3) and incubator port locks (3 out of 3). The isolates post cleaning were Acinetobacter species, Coagulase-negative Staphylococcus, *Sternotrophomonas maltophilia*, Nocardia species, *Sarcina lutea, Staphylococcus aureus,* and Diptheroids.

The proportion of surfaces considered to be clean and unclean was compared per area. For all of the five areas analyzed, there was no significant difference in the proportion of clean and unclean surfaces at baseline and after cleaning except for the IV pump controls.

The overall number of clean and unclean surfaces shows that the number of surfaces assessed to be clean was significantly different at baseline and after disinfection (p=0.026) were more surfaces were clean after the disinfection process.

Using the fluorescent marker (FM) to assess the cleanliness of the surfaces, only 15 out of 40 surfaces (38%) areas were considered to be clean after the areas were disinfected. In this analysis, all areas that were partially positive for FM were considered to be unclean.

To determine the diagnostic accuracy of FM, the number of surfaces assessed to be clean using this technique was compared with the number of surfaces clean as assessed by ACC (Table 2). The sensitivity of FM, or the probability that the presence of the FM results is unclean or ACC > 5 CFU/ml^2 on the surface, is 85.71% (95% C.I. 42.13-99.64). The specificity of FM, or the probability that the absence of FM results is clean or ACC < 5 CFU/ml^2 on the surface, is 42.42% (95% C.I. 25.48-60.78).

The positive predictive value (PPV), or the probability of being unclean as assessed by the presence of FM on surfaces is 24% (95% C.I. 9.36-45.13). The negative predictive value (NPP), or the

Table 1. Assessment of the cleanliness pre and post-disinfection of select high-touch surfaces in the hospital as assessed by aerobic colony count and the fluorescent marking technique (n=40)

Surface sampled	Number sampled	Before cleaning	After cleaning	P-value	Number (%) cleaned by FM
		Frequency (%)			
Bed rails/crib/incubator portlock	11			1.000	2 (18.18)
With Growth		3 (27.27)	4 (36.36)		
Without growth		8 (72.73)	7 (63.64)		
Tray table	9			0.471	6 (66.67)
With Growth		2 (22.22)	0 (0)		
Without growth		7 (77.78)	9 (100)		
IV pole (grab area)	8			0.569	5 (62.5)
With Growth		3 (37.5)	1 (12.5)		
Without growth		5 (62.5)	7 (87.5)		
IV pump control	10			0.02	1 (10)
With Growth		7 (70)	1 (10)		
Without growth		3 (30)	9 (90)		
Multi-module monitor controls	2			1.000	1 (50)
With Growth		1 (50)	1 (50)		
Without growth		1 (50)	1 (50)		
Total	40			0.026*	15 (37.5)
With Growth		16 (40)	7 (17.5)		
Without growth		24 (60)	33 (82.5)		
Statistical Test Used: Fisher's exact Test; * - Chi-square test					

Table 2.Diagnostic accuracy of the fluorescent marker in detecting clean surfaces using the aerobic colony count as the gold standard

	With microorganism (n=7)	Without microorganism (n=33)	Total
	Frequency (%)		
FM unclean	6 (57.58)	19 (85.71)	25 (37.5)
FM clean	1 (42.42)	14 (14.29)	15 (67.5)
Total	7 (100)	33 (100)	40 (100)

Sensitivity	85.71 (42.13 to 99.64)	Positive LR	1.49 (0.98 to 2.27)
Specificity	42.42 (25.48 to 60.78)	Negative LR	0.34 (0.05 to 2.16)
PPV	24 (9.36 to 45.13)		
NPV	93.33 (68.05 to 99.83)		
PPV, positive predictive value; NPV, negative predicted value; LR, likelihood ratio			

the probability of being clean as assessed by the absence of FM on surfaces is 93.33% (95% C.I. 68.05-99.83). The positive LR was 1.49. This value tells us that the presence of FM is 1.49 times more likely that a surface is clean. As a rule, the higher is the computed positive LR from 1 as a reference point, the stronger is the evidence for the cleanliness of a surface. The negative LR was 0.34. This implies that the absence or complete removal of FM on the surface is 0.34 less likely to be unclean among the surfaces.

DISCUSSION

Increasing evidence from Center for Disease Control and Prevention has recommended greater attention to the cleaning and disinfection of equipment and environment. [18] High-touch surfaces require more frequent cleaning and disinfection [19] and done at least daily and more frequently if the risk of environmental contamination is higher.[20] Presently, UP PGH Infection Control and Prevention Manual have existing protocols for daily and terminal cleaning[21]. Compared to the Tasmanian Infection Prevention and Control Unit, they have utilized the use of fluorescent markers (FM) as a means to monitor cleaning process.[22]

This research employs a fluorescent gel method that demonstrates a lack of attention to those surfaces in the near-patient zone[13]. Furthermore, a fluorescent gel application can provide a more standardized approach to process evaluation compared to visual inspection[23] and a favorable impact preventing transmission of the pathogen.[24] The cost is P 1,292.75 per 2 ounces (60ml) bottle or less than P 10.00 per surface application (about 0.2 to 0.5ml).

In this study, the use of FM as an index of cleanliness and disinfection compared to ACC have a good sensitivity of 85.71% but low specificity. And the visibility of the FM under a UV light provides a better objective parameter compared to visual inspection similar to a study by Snyder et al.[9]

The study showed much higher sensitivity and a lower specificity compared to a study by Snyder et al involving 15 high touch surfaces showing a specificity of 56% and sensitivity of 51% in determining cleanliness against visual inspection, ATP bioluminescence, and ACC. [9]

Similarly, Boyce et al compared FM against ACC and ATP criteria and showed that 378 (76%) of 500 surfaces were classified as having been cleaned according to a fluorescent marker, compared with 384 (77%) according to ACC and 225 (45%) according to ATP. They concluded that FM is useful in determining how frequently high-touch surfaces are wiped during terminal cleaning, however, contaminated surfaces classified as clean according to fluorescent marker after terminal cleaning were significantly less likely to be classified as clean according to ACC and ATP assays. [25]

In comparison to the studies conducted by Goodman et al and Carling [12, 26] involved multiple sampling of surfaces and interventional strategies to improve cleaning were not demonstrated in this study due to a minimal and single sampling of surfaces.

Although minimal agreement of the two techniques (ACC and FM) in assessing the cleanliness, a significant increase in cleanliness from a baseline of 60% to 83% was noted in the ACC and comparison of the surfaces per area showed a significant difference at baseline and after disinfection.

Furthermore, a study by Philip Carling, MD, demonstrated the use of fluorescent marking provides objective documentation of opportunities to improve thoroughness of environmental cleaning leading to programmatic interventions, resulting in substantial improvements in cleaning, reduction of surface healthcare-associated pathogens and decreased acquisition of MRSA and VRE. [26]

Similar to this study, Snyder et al showed that fluorescent marker was noted to be simple, rapid and easy to use compared to environmental

cultures that are expensive, results made available after 2 days and require laboratory support.[9] Although, the FM can raise awareness of the environment and can be used as an objective parameter to assess cleanliness, FM inability to identify and quantify bacterial load is a disadvantage. This was exhibited when the cleaning crew was made aware of the areas where fluorescent markers were not removed.

Several limitations of the study were observed. Primarily, single sampling was done with on a single unit (NICU) due to financial constraints. Majority of the surfaces sampled were noted to be clean as determined by ACC, compared to other studies [8, 12, 25, 26] which had numerous units and multiple sampling utilizing FM compared to ACC and/or ATP bioluminescence as the standard of cleanliness.

Second, the NICU was the setting due to the increased admission and occupancy rate and potential risk for nosocomial outbreaks.[27] 1-2 cleaning crews were assigned per shift with a 45 patient capacity, and during the study, actual patients were 96. Additional cleaning crews were recruited in order to compensate for the workload at the time of the study. The Revised Organizational Structure and Staffing Standards for Government Hospitals CY 2013 Edition have general guidelines for staffing[28] however it does not specifically mention nor have a formula as to how many personnel should be manning a critical unit especially in an event of increased admission rates. According to the National Guidelines for Clean Hospitals in India, adequately staffed sanitation department is one of the most important factors that govern the success of environmental cleaning and staffing levels must be appropriate to each department of the healthcare organization with the ability to increase staffing in the event of any exigency.[29] General staffing levels may be calculated by adding the average time taken for a worker to complete individual tasks.[20] However, manpower requirement is recommended according to the type of patient care area, in Intensive Care Unit (e.g. NICU) one sanitary attendant for up to six ICU beds in each shift and thereafter additional for every six beds in the morning shift but can be halved by the evening and night shifts. Furthermore, a dedicated group of 4 to 8 sanitary attendants will be utilized for intensive cleaning and washing of patient care areas and other areas.[37]

Third, post-cleaning inspection yielded positive fluorescence mainly on the incubator port locks, IV grab areas and control units of equipment. These areas were shown to the cleaning crew to make them aware and be cleaned accordingly. It was noted that they were cognizant that areas with fluorescence were not cleaned thoroughly due to fear of destroying machines because of the liquid nature of the cleaning material and areas were not known as high touch areas.

CONCLUSION

In conclusion, the use of Fluorescent Marking technique in high touch areas as an index of cleanliness and disinfection is a good marker for cleanliness and disinfection. Furthermore, the use of a fluorescent marker is a simple, rapid, inexpensive and has potential to increase awareness of the environment can be utilized as an objective parameter to assess cleanliness.

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