Clinical and Experimental Health Sciences

Effectiveness of the Bicinchoninic Acid Method in Patient Unit Cleaning in Intensive Care

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ABSTRACT

Objective: This study was conducted to identify the effectiveness of the Bicinchoninic Acid/(BCA) method applied for evaluate the cleaning the beds of the patients with infections or colonization requiring strict contact isolation after discharge.

Methods: This is an experimental study and it was used the ORION checklist. In this study, 480 BCA and 480 microbiological samples were taken from 40 patient units before and after cleaning and the results were compared. The cleaning procedure was evaluated by examining whether there was post-cleaning gel residue in the areas stained with fluorescent gel before the cleaning.

Results: When post-cleaning BCA and post-cleaning microbiological sampling data were compared, no statistical difference was found. When the data of the areas stained with fluorescent gel before and after the cleaning were compared, it was observed that there was a statistical difference. It was revealed that the bedside and the bed controller were mostly contaminated in both methods.

Conclusions: It was concluded that BCA was an effective method that could be used to evaluate the cleaning applied to the infected patient unit. It is thought that cleaning only areas that are considered to be contaminated after evaluating the cleaning with an effective method will prevent contamination due to cleaning and will provide more positive results in terms of time, labor, and cost. The control of cleanliness using objective methods can help maintain a safe environment.

This study is registered to ClinicalTrials.gov with the number ID:NCT04212130.

Keywords: Intensive care, patient unit cleaning, bca method, microbiological sampling, fluorescent gel

1. INTRODUCTION

Florence Nightingale attached the highest priority to the control of environmental factors and hygiene in the nursing care process, which gained a professional identity with her(1). She emphasized that sanitation was an indispensable basic factor in the protection and improvement of health(2). Environmental cleaning is one of the vital components of infection control(3). In Intensive care the cleaning of frequently touched environmental surfaces and the monitoring and verification of cleaning results are important for patient safety(4–6). There are many studies indicating that environmental cleaning in hospitals is not always sufficient(7–11).

The evaluation of the effectiveness of environmental cleaning and disinfection procedures has received great interest in recent years(12,13). The effectiveness of environmental cleaning can be evaluated using different methods. Visual evaluation, ATP (Adenosine Triphosphate) measurement with fluorescent gel, protein tests or microbiological sampling methods can be used to measure/evaluate the effectiveness of the cleaning and disinfection procedures(11).

Despite the increase in the number of intensive care beds in recent years, the length of stay of the patients in the intensive care unit has been prolonged due to the increasing service quality and improving health services, so the need for intensive care beds is increasing day by day. Therefore, the effective use of intensive care beds is very important(14). Due to high demands for intensive care beds(15) and it is very important to perform effective cleaning so that patients can be admitted as soon as possible and to evaluate the cleaning with a fast and safe method(7–9,11).

Nurses, who have always been at the forefront and the largest group of healthcare workers from past to present, have attempted to develop strategies to identify and reduce preventable sources of contamination in hospitals and other healthcare settings(1,16,17).

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This study was conducted to identify the effectiveness of the BCA method applied to evaluate the cleaning the beds and medical equipment of the patients with infection or colonization requiring strict contact isolation after discharge.

Hypotheses of the Study

H^0 = The BCA method has no effect on the evaluation of patient unit cleaning performed in intensive care.

H^1 = The BCA method has an effect on the evaluation of patient unit cleaning performed in intensive care.

2. METHODS

2.1. Type and Sample of the Study

This is an experimental study. In this study was used the "Guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection checklist". The patient units of patients with infections or colonization requiring strict contact isolation after discharge constituted the sample of the study. In this study, when the values of alpha 0.05, β= 0.10, 1-B 0.90 were taken, it was decided to include 32 patient units in the study, and the power of the test was found to be p=0.9657. Data collection was maintained until the BCA protein pen and fluorescent gel were exhausted, and a total of 43 patient units were included in the study. During the data collection stage, while two patient units were excluded from the study since the patients were admitted before the data collection process was completed, one patient unit was excluded from the study due to the disruption of blinding. Consequently, the study was conducted with the data of 40 patient units. 480 BCA and 480 microbiological samples were taken before and after cleaning from a of 40 patient units

2.2. Data Collection Tools

In the study, while the data on the effectiveness of cleaning performed in the patient unit were collected by the fluorescent gel marking method, the data on the presence of biological agents on the surface after cleaning were collected by the BCA and microbiological sampling methods.

2.3. Data Collection

The data of the study were collected between 01.12.2019 and 31.03.2020. The number of patient units included in the study is shown in the ORION flow diagram (Figure 1)

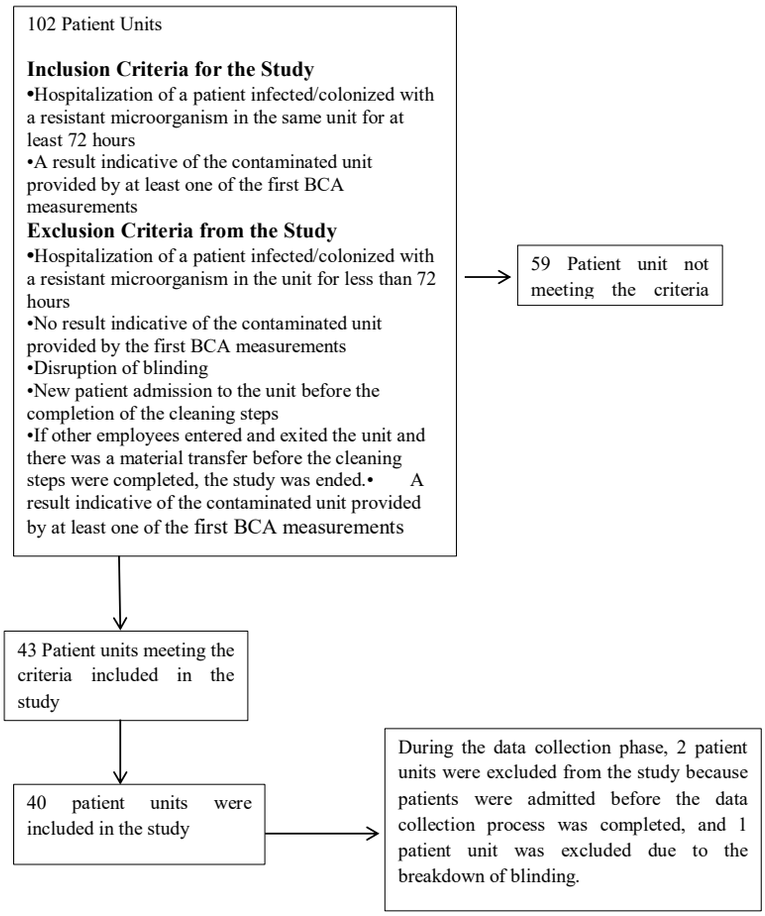

Figure 1. *ORION flow diagram*

2.4. Implementation of the Study

The cleaning staff who volunteered to participate in the study were trained, and the cleaning instruction of the institution was used as the training material. As a result of the preliminary application carried out before the study, dining tables and shelving units were excluded from the sample since both the BCA and microbiological examination tests gave clean results before and after the cleaning. As it was indicated in the study conducted by Adams et al. (2017)(18), the fact that contamination was less on surfaces that were frequently touched by healthcare workers but away from the patient is similar to the results of our preliminary application. Therefore, samples were taken from (1).bedsides, (2).patient beds, (3).monitors, (4).mechanical ventilators, (5).infusion pumps, and (6).bed controllers from infected patient units in intensive care while obtaining the data of the study. The implementation process of the study is presented in below.

Implementation Stages of the Study	
1	When the infected patient unit was emptied, the first BCA swab samples were collected from the planned areas. The compliance of the unit with the study inclusion criteria was evaluated when at least one of the BCA swabs was found to be contaminated. The units that were found to be contaminated were included in the study
2	Swabs were collected for microbiological sampling and sent to the microbiology laboratory
3	The areas to be cleaned with a fluorescent gel were stained and marked, and it was waited for the gel to dry for 3-5 minutes
4	After the measurements, the whole unit was cleaned in accordance with the cleaning standards of the institution
5	After the cleaning, it was waited for the setting to dry for 30 minutes, and within this period, the doors of the unit were closed, and a warning note was written to prevent entrance and exit to the unit
6	After the waiting period, the cleanliness of the unit was checked visually. After the visual evaluation, the gel applied areas were checked with a UV lamp. The cleaning of the areas with gel residues was repeated
7	The swab samples were collected from the same areas again for post-cleaning BCA and microbiological sampling. The study was ended in the units that were considered to be clean according to the BCA measurement results. In a case when the BCA measurement results were found to be contaminated after the first cleaning, the second cleaning was performed.
During the application process, the 30-minute waiting period for the area to dry between two cleaning procedures was determined in parallel with the study carried out by Donskeys, 2013(9) to reduce the possibility of contamination of the cleaned surfaces	

2.5. Data Evaluation Criteria

The effectiveness of cleaning was evaluated with BCA swab samples in accordance with the instructions and reference parameters of the device used for BCA measurement, and the results below 5 micrograms were considered clean. The absence of gel residues in the setting as a result of checking the cleaned areas with a UV lamp was considered as an indicator of effective cleaning. The failure to produce bacteria in microbiological samples or the bacteria produced below 2 colonies per cm^2 was considered to be an indicator that the unit was clean.

2.6. Objectivity of the Study and Ensuring Blinding

The cleaning of the infected units was performed by the same people so that it would not be affected by the individual differences and sensitivities of the cleaning staff. Since the control stage with a UV lamp was performed with the cleaning staff, the staff were prevented from guessing the areas by changing the stained areas in each unit to eliminate the possibility of guessing the stained areas. The study was conducted in a single-blind manner.

2.7. Inclusion Criteria for the Study

- Hospitalization of a patient infected/colonized with a resistant microorganism in the same unit for at least 72 hours
- A result indicative of the contaminated unit provided by at least one of the first BCA measurements

2.8. Exclusion Criteria from the Study

- Hospitalization of a patient infected/colonized with a resistant microorganism in the unit for less than 72 hours
- No result indicative of the contaminated unit provided by the first BCA measurements
- Disruption of blinding
- New patient admission to the unit before the completion of the cleaning steps
- If other employees entered and exited the unit and there was a material transfer before the cleaning steps were completed, the study was ended.

2.9. BCA Test Measurements

There are many types of protein tests, which are one of the cleaning evaluation methods, and the Bicinchoninic Acid (BCA) method is one of these tests(19). The BCA test, which was first defined by Smith et al. (1985), is based on the biuret test. This test is based on the conversion of Cu (II) to Cu (I) under alkaline conditions. Cu (I) is then reacted with BCA. As a result of this reaction, a dark purple color is formed and changes the color of the solution in which the reaction takes place(19). The test gives results in 1-5 minutes.

In the study, surface protein residues were detected by the BCA method (Terragene, Chemdye®P RO1 MICRO, Bionova MiniPRO, Santa Fe, Argentina). The PRO1 MICRO measuring device can measure between 0.3 micrograms and 10 micrograms. This system can detect a low amount of protein residues(20). The measuring range of the PRO1 MICRO hygiene system complies with international standards such as ISO 15883-1, DGSV (Germany), HTM 01-01 and HTM 01-05 (England)(20).

2.10. Evaluation of Data

The data obtained from the study were loaded into the SPSS v.22 package program and statistically analyzed. In the evaluation of the data, the McNemar test was used when the data obtained from the same regions were compared with dependent groups at different times, and the chi-square test in multiple cells was used when the groups were compared in terms of a variable obtained by counting. The level of significance was accepted to be 0.05.

2.11. Ethical Aspect of the Study

Each stage of the study was conducted in accordance with the ethical principles. Before starting the application, written permissions were obtained from the ethics committee of Cumhuriyet University (dated 04.07.2019, decision number 2019-17/15) and the institution where the study would be conducted (dated 05.07.2019, numbered 692).

3. RESULTS

When pre-cleaning BCA and pre-cleaning microbiological sampling results were compared, while 78.75% (n=189) of the samples collected from the patient unit by BCA were found to be contaminated, 46.66% (n=112) of the samples collected from the patient unit by microbiological sampling were contaminated (Table 1). When the initial BCA and microbiological sampling contamination rates were compared, it was found that the difference was not statistically significant (p=0.129).

When the BCA data obtained from the same areas before and after cleaning were compared, it was determined that 230 of a total of 240 samples, namely 95.83% of them, were cleaned after the first cleaning, there was a difference between BCA levels before and after cleaning, and this difference was statistically significant (p=0.001) (Table 1).

When the microbiological sampling results obtained from the same areas before and after cleaning were compared, it was determined that the patient units were cleaned after the first cleaning by 96.7%. It was determined that there was a difference between the microbiological sampling results obtained from the same areas before and after cleaning and that this difference was statistically significant (p=0.019) (Table 1).

When post-cleaning BCA and post-cleaning microbiological sampling data were compared, no statistical difference was found (p=0.230). While 230, namely 95.84% of the samples obtained from the patient units by the BCA method, were found to be clean, 232, namely 96.67% of the samples obtained by microbiological sampling, were clean. No statistically significant difference was found between the methods in terms of cleaning values (p=0.292) (Table 1).

Table 1. *Comparison of BCA and microbiological sampling results*

Variables	Clean: n (%)	Contaminated: n (%)	Total: n (%)	Statistical Result
Pre-Cleaning BCA	51 (21.25)	189 (78.75)	240 (100)	
Pre-Cleaning Microbiological Sampling	128 (53.3)	112 (46.7)	240 (100)	p=0.129
Pre-Cleaning BCA	51 (21.25)	189 (78.75)	240 (100)	
BCA After the First Cleaning	230 (95.8)	10 (4.2)	240 (100)	p=0.001
Pre-Cleaning Microbiological Sampling	128 (53.33)	112 (46.67)	240 (100)	
Microbiological Sampling After the First Cleaning	232 (96.7)	8 (3.3)	240 (100)	p=0.019
BCA After the First Cleaning	230 (95.83)	10 (4.17)	240 (100)	
Microbiological Sampling After the First Cleaning	232 (96.7)	8 (3.3)	240 (100)	p=0.292

Mc Nemar test used

The contamination rates of the areas where the samples were collected before and after cleaning are presented in Figure 2. When the table was examined, according to the pre-cleaning BCA method, mostly contaminated areas were bedsides by 100%, pumps by 95%, beds by 90%, mechanical ventilators (MV) by 80%, and bed controllers by 67.5%, and it was determined that the monitor was least contaminated by 40%. When the cleaned area and pre-cleaning microbiological sampling results were examined, it was observed that the bedside (72.5%) and the bed controller (62.5%) were mostly contaminated. When the contamination rates of the areas where BCA swab samples were collected after the first cleaning were examined, it was determined that all of the MV, bed controllers, and monitors were cleaned after the first cleaning ($p<0.05$), and the lowest cleaning rate was 17.5% for pumps.

It was observed that while fluorescent gel residues remained mostly on the bedsides by 60%, the least fluorescent gel residue was on the bed controller by 2.5% (Table 2).

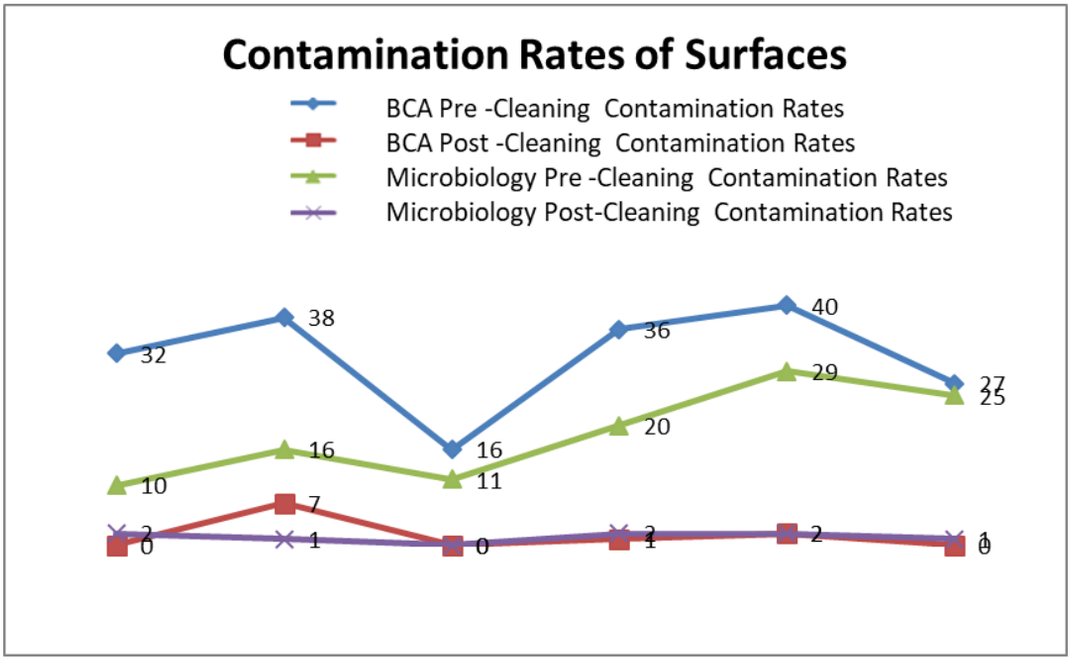

Figure 2. *Contamination rates of surfaces*

Table 2. *Comparison of the cleaned area and the fluorescent gel results*

		Fluorescent Gel Results		Total n(%)	Statistical Result
		Clean n(%)	Contaminated n(%)		
Cleaned Area	**MV**	33(82.5)	7(17.5)	40(100.0)	
	Pump	36(90.0)	4(10.0)	40(100.0)	
	Monitor	38(95.0)	2(5.0)	40(100.0)	
	Bed	23(57.5)	17(42.5)	40(100.0)	
	Bedside	16(40.0)	24(60.0)	40(100.0)	
	Bed controller	39(97.5)	1(2.5)	40(100.0)	
Total		185(77.1)	55(22.9)	240(100.0)	X^2=60,97* p =0,001

*Chi-square test used and * when the overall results are compared*

4. DISCUSSION

When the data obtained as a result of our study were examined, it was observed that while fluorescent gel residues remained mostly on the bedsides, the least fluorescent gel residue was on the bed controller (Table 2). In the study conducted by Choi et al. (2010) in which they performed microbiological sampling from the patient's environment in intensive care during the fight against the Acinetobacter outbreak, bedsides were among the most contaminated areas(21), as in our study. When the fluorescent gel residue rates of the surfaces after cleaning were examined, it was observed that the bedside was the area that was the most difficult to clean (Table 2). The low rate of cleaning at the bedside was associated with the cleaning staff having problems reaching narrow and irregular surfaces such as the joint of the bed and the bedside during cleaning works. It was

observed that the surface with the highest cleaning rate was the bed controller, which can be explained by the fact that the bed controller has a flat surface and is easily accessible. In general, when the surfaces stained with fluorescent gel after the cleaning and their cleaning rates were examined, it was concluded that cleaning staff tended to less frequently wipe the areas that were difficult to reach and the surfaces that were far from the patient. In a study conducted to evaluate the cleaning as a result of the increase in infection rates in the ICU, when the samples collected from the bedsides were evaluated with ATP, it was determined that the bedsides were not completely cleaned although there was a statistically significant difference between the ATP values after cleaning(10). This study also supports our results.

In some studies, it was emphasized that the bedsides were mostly contaminated surfaces(22–24). In our study, when contamination rates in the units were evaluated according to the criteria of the proximity of surfaces to the patient and the staff contact, it was observed that contamination rates were the highest on the bedsides and bed controllers that were closest to patients and mostly touched by healthcare personnel. However, the level of contamination was low in areas such as monitors that were far from patients but touched by healthcare personnel (Figure 2). Based on these data, it can be concluded that the surfaces that were closest to the patient and mostly touched by healthcare personnel were more contaminated than other surfaces. In their study conducted in the intensive care unit, Adams et al. (2017) found that the dining table was the area that was most frequently touched but had the lowest bio-load(18). These results support the data we obtained in our preliminary study.

When pre-cleaning BCA and pre-cleaning microbiological sampling results were compared with post-cleaning BCA and post-cleaning microbiological sampling data, no statistically significant difference was found between the methods (Table 1). The results of the experimental study conducted by Perçin and Renders (2018) revealed that the BCA method was a sensitive method with regard to presenting quantitative and qualitative data by detecting the protein residues in the setting(20). When BCA and microbiology data were compared both before and after the first cleaning, the fact that no difference was found between them supports that the BCA method can be used to evaluate environmental contamination in patient units. Although there was no statistically significant difference between BCA and microbiology data before cleaning, when the data were evaluated numerically, it was observed that 189 (78.75%) and 112 (46.66%) of the surfaces were contaminated according to pre-cleaning BCA data and microbiological data, respectively, and it is remarkable that there was a statistically significant difference between numerical data (Table 1). In line with these data, it is observed that the BCA method revealed the contamination rate more than the microbiology method. The fact that higher contamination rates were found in the BCA data can be attributed to the fact that the BCA method revealed not only the bacteriological load but also the entire biological load on the surface. The fact that the BCA method detects the presence of blood residues or body fluids in the environment and the conditions that are not infected but may become pathogenic over time if they are not cleaned can be considered as another indicator that BCA can be used to evaluate the effectiveness of cleaning. These data support our H^1 hypothesis. Furthermore, the cleaning of surfaces can be evaluated within 2-3 days by the microbiology method. Patients cannot be admitted to the unit during this long evaluation period, which causes the unit not to be used effectively. The failure to use the unit effectively leads to patient victimization and financial losses in institutions. The fact that the BCA method provides results in a short period of 15 minutes suggests that the BCA method is a method that can be used primarily in the evaluation of environmental cleaning in the clinic since it can prevent the specified problems.

When the data obtained from our study were examined, although the microbiology samples were clean before cleaning, the growth of microbiology samples after the first cleaning on the same surface suggests that the cleaning cloth used may have been contaminated and may not have been cleaned effectively in transitions between surfaces. After the second cleaning, it was determined that the same surface was clean by both the BCA and microbiology methods. In their study on the control of the cleaning of the patient environment with different cleaning agents, Bergen et al. (2009) indicated that there might be environmental contamination with cleaning cloths(25). In the study conducted by Gavalda et al. (2015) to determine the surface contamination that occurs within the first hour after cleaning in the intensive care unit, the reason for environmental contamination was associated with the contamination of cleaning cloths(7). This result supports the data in our study. In the study conducted by Gan et al. (2017), it was concluded that the use of three cleaning cloths instead of one in the cleaning of the same patient unit increased the effectiveness of the cleaning(8).

5. CONCLUSIONS

In this study, the following results were obtained. It was concluded that:

1. No difference was found between the first cleaning and the second cleaning applied to the patient unit. Although repeated cleaning is not required, cleaning should be repeated only in areas that are found to be contaminated as a result of controlling cleaning with objective methods.
2. Similar results were obtained with the BCA and microbiology methods in the evaluation of terminal cleaning, and cleaning can be controlled effectively and in a shorter time with the BCA method compared to the microbiology method.
3. When the surfaces stained with fluorescent gel and the cleaning rates are examined, cleaning staff tend to less

frequently wipe the rough areas that are difficult to reach and far from the patient.

4. In patient units, the surfaces that were the closest to patients and mostly touched by the staff were more contaminated than other surfaces.
5. The failure to comply with cleaning protocols during unit cleaning and to clean cleaning cloths effectively after surface passages may lead to environmental contamination originating from the cleaning cloth.

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Conflict of Interest

There is no conflict of interest between the authors.

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