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Evaluating the Effects of Peracetic Acid and Chlorine Dioxide Disinfectants on Staphylococcus Aureus Isolated from Surfaces of NICU

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A R T I C L E I N F O

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ABSTRACT

Introduction: Today, disinfection of surfaces by using antimicrobial agents is critical for the prevention and control of pathogens and reduction of infection in hospital. The aim of this study was to investigate and compare the effects of two disinfectants against *Staphylococcus aureus* isolated from the NICU of Shahid Sadoughi Hospital of Yazd in 2017.

Materials and Methods*:* In this descriptive cross sectional study, bacterial culture of samples collected from different surfaces of the NICU and *S. aureus* isolates were identified using conventional biochemical tests. Peracetic acid and chlorine dioxide various concentrations were used as disinfectants. Their effects against *Staphylococcus aureus* were determined by Standard disc diffusion method. Data were analyzed by linear mixed models in SPSS version 23.

Results*:* 39.39% of samples were found to be S. aureus infected. The mean diameter of growth inhibition zone for peracetic acid 0.1% was significantly lower than that for peracetic acid 0.2%, and peracetic acid 0.1% was significantly higher than that for chlorine dioxide ($P < 0.001$). The comparison of growth inhibition zone diameters for peracetic acid 0.1% and chlorine dioxide disinfectants showed that the average diameter of the inhibition zone created by peracetic acid 0.1% was significantly higher than that created by chlorine dioxide. The most effective disinfectant on S. aureus strains isolated was peracetic acid 0.2% and the least effective disinfectant was chlorine dioxide.

Conclusion: In health care facilities with *S. aureus* infection, peracetic acid 0.2% can be used effectively to reduce nosocomial infection rate.

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Introduction

Contaminated surfaces have always been considered as one of the ways of transmission of diseases in health care facilities. Proper disinfection and sterilization can be an effective way to control hospital infections[1]. Nosocomial infections are one of the common problems of hospitals that increase the burden of diseases in the community and mortality in hospitalized patients [2].

The incidence rate of these infections in NICUs has been reported from 5.9% to 31.8% [3, 4]. Factors such as prematurity, low weight, long hospital stay, use of invasive methods such as tracheal tube, ventricular shunt, vascular catheter, and intravenous feeding with fat emulsions play an important role in the development of these infections and the difference in their incidence rates in neonates [5, 6]. One of the most distinct and most frequently occurring bacterial infections in the hospitals around the world is *Staphylococcus aureus* infection [7]. This bacterium is a gram-positive, catalase-positive, obligate anaerobic and sporeless coccus [8, 9]. Many factors can contribute to the transmission of *Staphylococcus aureus* . One of the most important factors for the spread of nosocomial infections is inappropriate use of antimicrobial agents [10]. Proper use of disinfectants and antiseptic agents for medical equipment and surfaces can be very effective way to reduce exposure population [11]. Having enough knowledge about the principles of disinfection, antisepsis and sterilization is a key factor. Unfortunately, due to inappropriate selection of disinfectants, inappropriate physical conditions of the setting and lack of relevant knowledge and training among the staff, the effects of these substances have been declining, leading to unhealthy condition and consequently increased of Nosocomial infections [12].

Peracetic acid (PA), with the chemical formula $C_2H_4O_3$, is a combination of acetic acid and peroxyhydroxide that is called with different commercial names around the world. Peracetic acid with oxidizing the outer membrane can kill bacterias, endospores, yeasts, and fungal spores.

PA belongs to the organic peroxides family and is produced by combination of acetic acid and hydrogen peroxide in aqueous environment, and exhibits high antimicrobial properties because of its high oxidation potential [13]. Chlorine dioxide (ClO_2) is a strong oxidizing substance and serves as a highly effective disinfectant for hospital equipment [14]. The prevention and control of hospital infection in infants is very important, because their immune system has not yet been fully activated and therefore microbial agents may develop severe and fatal infections in them. Also premature and low birth weight infants with acute medical problems stay for weeks to months in the NICU, during which they may contact with medical equipment, antibiotic-resistant hospital flora, and invasive procedures.Therefore NICU should be more studied to ensure that disinfection process is safe for infants and staffs. [15].

Materials and Methods

Studied disinfectants

Peracetic acid at concentrations 0.1% and 0.2% (Merck Co., Germany) and Chlorine dioxide 1% (Dorna Daroye, Yazd, Iran) were used as disinfectants.

Sample collection

This descriptive cross sectional study was performed on samples from the NICU of Shahid Sadoughi Hospital in Yazd in 2017. First, 93 samples was calculated by using the G-Power software version 3192 given the effect size of 0.3, the significance level of 0.5 and the power of 80%.

Sampling procedure was performed within three months (from November to February 2017) in three randomly selected days at 1-month intervals (to be repeated).

. 33 places in different parts of the NICU with the highest exposure to patients and staff were selected. Then, surfaces of 10 cm x 10 cm were marked with tape strips. At 11-12 o'clock, a sterile swab was moisturized with sterilized physiological serum, and all marked surfaces were sampled [16]. Then, samples were poured into a tube containing 3 ml of the prepared and labelled tryptic soy broth (TSB) culture medium (Darvash Company, Iran),

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and then were immediately transferred to the Microbiology Laboratory of Shahid Sadoughi University of Medical Sciences.

All sampling steps were carried out near an alcohol light. Sampling from all selected places was carried out according to the above method. Sampling was performed in triplicate at 1-month intervals and without any prior coordination with the staff of the NICU.

Sample culture and identification of Staphylococcus aureus isolates

100 µl of each sample was transferred to the already prepared culture media blood agar- Eosin Methylene Blue Agar (EMB) (Darvash Company, Iran) and cultured linearly by using a sterile loop near the flame. Plates were incubated at 37 °C for 24 hours. The colonies present in the culture media were gram stained. In the case that clustered gram-positive cocci were observed, the colonies were identified using conventional biochemical tests such as catalase, coagulase, and Mannitol fermentation in Mannitol salt agar (Darvash Company, Iran) [17].

Antimicrobial effect of disinfectants

Susceptibility of bacterial isolates against disinfectants was measured by the Kirby-Bauer method and according to the CLSI protocol [18]. In brief, sterilized blank disks were dipped with 20 µl of the disinfectant and after complete absorption, disks were left at 37 °C to dry completely. A bacterial suspension of the fresh (24-h) culture of *S. aureus* isolates, equivalent to 0.5 McFarland opacity (1.5×10^8 CFU/ml), was prepared and cultured on the Mueller-Hinton agar (Darvash Company, Iran) by using a sterile swab. Then, disks containing disinfectants were placed on the culture media at a distance of 24 mm from each other by means of sterile pins, and stored at 37 °C for 18-24 h. After incubation, the bacterial growth inhibition zone around the disk was measured in mm. The susceptibility measurement was conducted in triplicate with the three disinfectants for all isolates and the mean diameter of the growth inhibition zone was calculated after each test [2]. In this study, the growth inhibition zone diameter of 6 mm was considered to represent no effect, 7-10 mm low effect, 11-15 mm average effect and more than 15 mm strong effect [2]. To control the test, a disk containing physiological serum was used, and to control the sterility, a blank disk without any additive was used. Finally, the data were tabulated and analyzed using the mixed linear model and descriptive tables in the SPSS version 23.

Ethical issues

This study was conducted after its protocol was approved by the Medical Ethics Committee of Shahid Sadoughi University of Medical Sciences and Health Services (IR.SSU.SPH.REC.1395.9)

Results

39 isolates (39.39%) if all samples (99 samples) were found *S. aureus* positive in three steps of sampling. 11 samples (28.20%) were found in the first sampling, 15 (38.46%) in the second sampling and 13 (33.33%) in the third sampling. Comparison of the inhibition zone diameters of the three disinfectants was performed using a linear mixed model. The mean (± standard deviation) values of the inhibition zone diameters for the three disinfectants are shown in Table 1. The mean inhibition zone diameters obtained for the peracetic acid 0.1% and 0.2% and chlorine dioxide 0.1% were 36.228 (mm), 45.319(mm) and 4.424(mm) respectively.

Table 1: Descriptive Indicators Estimated Based on Model for Growth Inhibition Zone for Three Disinfectants

Disinfectant	Mean(mm)	SD
Peracetic acid 0.1%	36.228	1.625
Peracetic acid 0.2%	45.319	1.659
Chlorine dioxide 1%	4.424	1.625

The paired comparison of the effect of disinfectants on the inhibition zone showed that the mean diameter of the inhibition zone created by the peracetic acid 0.1% was significantly lower than

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that created by the peracetic acid 0.2% (P = 0.000), and this variable for peracetic acid 0.2% was significantly higher than that for chlorine dioxide 1% (P = 0.000) (Table 2).

The comparison of the diameters of growth inhibition zones created by the peracetic acid 0.1% and 0.2% and chlorine dioxide 1%, showed that the mean diameter of the growth inhibition zones created by peracetic acid 0.1% were significantly higher than that created by the chlorine dioxide 1% (P = 0.000).

Table 2: Comparison of the effects against disinfectants (peracetic acid 0.1% and 0.2% and chlorine dioxide)

Disinfectant(I)	Disinfectant(J)	Mean (I-J)	SD	*df*	P-value	95% CI	
						Lower limit	Upper limit
Peracetic acid 0.1%	Peracetic acid 0.2%	-9.091*	1.371	72.531	0.000	-12.452	-5.730
	Chlorine dioxide 1%	31.804*	1.588	86.291	0.000	27.927	35.681
Peracetic acid 0.2%	Peracetic acid 0.1%	9.091*	1.371	72.531	0.000	5.730	12.452
	Chlorine dioxide 1%	40.895*	1.371	72.531	0.000	37.534	44.256
Chlorine dioxide 1%	Peracetic acid 0.1%	-31.804*	1.588	86.291	0.000	-35.681	-27.927
	Peracetic acid 0.2%	-40.895*	1.371	72.531	0.000	-44.256	-37.534

* Adjustment for multiple comparisons: Bonferroni.

Discussion

The results of this study indicated that the peracetic acid has a strong antibacterial effect on the gram-positive bacterium *S. aureus*, which is consistent with other studies [13, 19, 20]. In the other hand chlorine dioxide was found to cause the lowest mean diameter of the inhibition zone for *S. aureus*.

The study of Mary-Garvry et al. showed that peracetic acid has a high level of antimicrobial activity [21], which is consistent with our study. The study of Lynam et al. also revealed that resistance to peracetic acid was not observed among the important nosocomial bacterial pathogens [23]. The results of this study are consistent with one study conducted in Imam Khomeini Hospital of Urmia [24] and the study of Samarghandi et al [25]. Babaie et al. investigated the replacement potential of aldehyde with peracetic acid and hydrogen peroxide for disinfection of surfaces in the CCU and ICU of Shahid Rajaee Cardiovascular Research Center, Tehran, and found that hydrogen peroxide was significantly better than peracetic acid [26]. Vizcaino-Alcaide et al. compared the effects of the disinfectants peracetic acid and glutaraldehyde 20% and their results showed that peracetic acid is a safe disinfectant and a good alternative to glutaraldehyde 20% for high-level disinfection [27]. Another study has proposed the replacement of peracetic acid with chlorine dioxide for disinfection of sewage [28], which is consistent with the current study. Antibacterial activity of peracetic acid has been investigated and its inhibitory effect on the growth of many bacteria such as *Mycobacterium*, *Pseudomonas*, *Enterococci* and *Staphylococci* has been demonstrated [19, 20]. In the present study, two concentrations (0.1% and 0.2%) of this disinfectant were investigated. The study of Moradi indicated that gram-positive bacteria exhibit higher susceptibility to peracetic acid than gram-negative ones [13]. This difference can be attributed to the different wall structures of the two groups of bacteria, because it has been established that the *numerous* peptidoglycan layers in gram-positive bacteria walls cause a higher susceptibility to bactericidal drugs and agents compared to gram-negative ones. Gram-positive cell envelope is relatively simple, consisting of two to three layers: a cytoplasmic membrane, one or more thick peptidoglycan layers, and in some bacteria an extracellular layer called the bacterial capsule [29]. Frata et al. studied *S. aureus* contamination on the surfaces of a hospital clinic, and concluded that disinfecting and cleansing the surfaces with collaboration and training of hospital staff, is

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effective to reduce the *S. aureus* burden [30]. Investigations in the Burns Ward of Shahid Sadoughi Hospital in Yazd showed that all disinfectants studied were effective on isolated microorganisms (including *Pseudomonas aeruginosa*, Enterobacter species, Bacillus species, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, Enterococci, and Proteus and Acinetobacter species), and there was a significant difference between the mean number of bacteria before and after disinfection [31]. One study was performed on the surfaces of dental units in restorative department in 2015-2016; and its results showed that all specimens before disinfection had *S. aureus* contamination. There were no significant differences in the effects on gram-positive bacteria among the three materials used (Septi Surface، H_2O_2, Anius DDSH), although all caused a significant reduction in surface contamination [32], which is consistent with our study with respect to the effect of chlorine dioxide on the gram-positive bacterium *S. aureus*, because in our study, the chlorine dioxide 1% solution only reduced the surface contaminations.

Conclusion

Peracetic acid is an effective compound for disinfection and removal of the microorganisms on the surfaces studied. This compound can greatly help control *S. aureus*. According to the results of this study, it is strongly recommended to use peracetic acid 0.2% as a disinfectant for the surfaces in hospitals and health care centers where the gram-positive bacterium *S. aureus* is widely present.

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Conflict of interest

We have no competing interests.

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