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Determining the Efficacy of Some Hospital Disinfectants Against Methicillin-Resistant Staphylococci Isolated From Different Wards of an Educational Hospital

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Abstract

Objectives: Methicillin-resistant staphylococci are important causes of nosocomial infections. Due to the presence of these bacteria in hospitals as a significant challenge in hospital infection control, the identification of effective disinfectants against methicillin-resistant staphylococci is necessary. This study was designed to evaluate the effectiveness of common hospital disinfectants against methicillin-resistant staphylococci.

Materials and Methods: In this cross-sectional study, the effectiveness of 4 surface disinfectant cleaners (Deconex 50 AF, Microzed GP-H, Peranacid M1 and Surfocept quick) against methicillin-resistant staphylococci (10 *Staphylococcus aureus*, 10 *Staphylococcus saprophyticus*, and 55 *Staphylococcus epidermidis*) was evaluated using broth dilution method, disc diffusion assay, and cell viability assay.

Results: The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Peranacid and Surfocept against the isolates were higher than those of Deconex and Microzed. The results of disk diffusion assay showed that staphylococcus strains exhibited greater inhibition zone diameter than other disinfectants at different concentrations of Deconex and Microzed. According to the results of cell viability assay, *S. aureus*, *S. epidermidis*, and *S. saprophyticus* isolates did not grow at three concentrations (0.5%, 1% and 2%) of Deconex, Microzed, and Peranacid, respectively.

Conclusions: Deconex and Microzed had more antimicrobial properties than the 2 other agents and methicillin-resistant staphylococcus isolates had a higher resistance to both Peranacid and Surfosept.

Keywords: MRAS, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, Disinfectants, Nosocomial infections

Introduction

Disinfectants are widely used in health centers to control the growth of bacterial pathogens in inanimate objects (1). These compounds are used daily for sterilization or disinfection of medical devices and different wards of the hospitals. On the other hand, the inappropriate usage of many of these materials, poor efficacy of disinfectants, failure to provide effective concentrations of disinfectants, inappropriate physics of hospitals, and different responses of various pathogens have decreased the efficiency of disinfectants against hospital microorganisms (2). Hospital infections are common problems in hospitals and health centers, and one of the major factors regarding the spread of nosocomial infections is the inappropriate usage of disinfectants (3). The prevalence of nosocomial infections in Iran is 4.5%, and bloodstream infections, surgical site infections, and pneumonia are among the most common infections (4).

The most common bacteria causing nosocomial infections include *Acinetobacter baumannii*, *Clostridium difficile*, *Escherichia coli*, *Klebsiella pneumonia*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, Vancomycin-resistant Enterococci (VRE) and *Burkholderia cepacian* (5). Staphylococci play a major role in nosocomial infections, which contribute to various infections in patients admitted to different wards of the hospitals (6-8). *S. aureus* is one of the most important bacteria that cause infectious diseases including food poisoning, scalded skin syndrome, furuncles, impetigo, folliculitis, and abscesses by producing enterotoxins and exfoliative toxins (9,10). On the other hand, coagulase-negative staphylococci have been one of the important causes of bacteremia, endocarditis, wound infection, urinary tract infections, pneumonia, skin infection, and soft tissue infection during the last decade (11,12). At first, methicillin-resistant staphylococci were identified at hospitals, while nowadays they are found both in the community and hospitals. These bacteria are among

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the important clinical and epidemiological problems of hospitals (13). Due to the increasing spread of methicillin-resistant staphylococci and the importance of these bacteria in infections, especially nosocomial infections, the identification of effective disinfectants against methicillin-resistant staphylococci is important. In this study, the efficacy of some hospital disinfectants against methicillin-resistant staphylococci was investigated

Materials and Methods

Isolation and Confirmation of Staphylococcus Isolates

In this cross-sectional study, 17 *Staphylococcus aureus*, 44 *Staphylococcus saprophyticus*, and 140 *Staphylococcus epidermidis* isolates were collected from different wards such as maternity, internal, dialysis, surgical room, neonatal intensive care unit (NICU), intensive care unit (ICU), and emergency of an educational hospital (Khorasan-Razavi province, Gonabad, Iran) from 2016 to 2017. Samples were obtained from the places with the most contact with patients and healthcare workers in different wards of the hospital by sterile and moistened swabs. Then, swabs were placed in brain-heart infusion broth medium (Merck Co., Germany). Later, the characterization and identification of bacteria were carried out based on standard procedures. Finally, the isolates were recognized by Gram staining, colony characteristics, catalase, coagulase, DNase, and novobiocin sensitivity tests and fermentation of mannitol (14).

Determination of Methicillin-Resistant Isolates

Methicillin-resistant isolates were recognized using both phenotypic and genotypic methods. In phenotypic method, oxacillin screen agar test was carried out based on Clinical and Laboratory Standards Institute (CLSI) guidelines (15). *S. aureus* ATCC 33591 was used for quality control. The isolates, identified as Methicillin-resistant isolates by phenotypic method, were confirmed using polymerase chain reaction (PCR) assay for detection of *mecA* gene. The primer sequences for *mecA* gene, annealing temperature, and expected size of amplicon for PCR assay are presented in Table 1.

Bacterial DNA was extracted by a simple and rapid boiling procedure (16). Briefly, 1.5 µL of the bacterial culture was centrifuged at 8000 rpm for 3 minutes. Then, 200 mL of lysis buffer including 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA was added to the pellets. Micro-tubes were incubated in boiling water for 10 minutes and centrifuged at 10 000 rpm for 2 minutes. The supernatant was transferred into the sterile micro-tubes and was put at -20°C for PCR testing.

PCR was carried out with 1X PCR buffer, 2 mM of MgCl2, 0.2 mM of deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 0.25 µM of each primer (forward and reverse primers), 1.5 U of Taq DNA polymerase (Jena Bioscience, Germany), and 3 µL of template DNA in a total volume of 25 µL. The micro-tubes were placed in a thermocycler (Bio-Rad, US). PCR products were detected by electrophoresis on 1% agarose gel and examined under UV illumination. *S. aureus* ATCC 33591 was used as positive control.

Antimicrobial Susceptibility Testing

Methicillin-resistant isolates were assessed for antibiotic sensitivity using the disk diffusion method. Antibiotic susceptibility was tested by employing the following disks (MAST Co., UK): penicillin (10U), clindamycin (2 µg), ciprofloxacin (5 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), and nitrofurantoin (300 µg). Vancomycin susceptibility was determined using agar dilution assay. The procedures were carried out and defined based on the CLSI guidelines (15). The bacteria were evaluated for inducible clindamycin resistance by disk diffusion using the D-zone test and interpreted according to CLSI guidelines (15). In this test and other following tests, *S. aureus* ATCC 25923 was used for quality control.

Disinfectants

Several common hospital disinfectants were chosen for testing. The following disinfectants (surface disinfectant cleaner) were used in this study: Deconex 50 AF (Borer Chemie AG, Switzerland), Microzed GP-H (Saziba Co. Iran), Peranacid M1 (Dornadarouyeh Co. Iran), and Surfosept Quick (Rezarad Co. Iran).

Determination of MICs and MBCs

The MICs of disinfectants were determined using the broth dilution method and based on CLSI guidelines (15). Before MIC determination, all the bacteria were cultured on blood agar base (Merck Co., Germany) containing 5% sheep blood. 40 g of medium was suspended in 1 L demineralized water and autoclaved at 121°C for 15 minutes. It was cooled to 45-50°C and 5%-8% sterile blood was added to the culture medium. Finally, it was poured into plates at 35°C for 24 hours. Test solutions

Table 1. Primers and Conditions Used for Detection of *mecA* Gene

Target Gene	Primer Name	Sequence (5' To 3')	Length (Base)	Annealing Temperature (°C)	Amplicon Size (bp)	References
mecA	*mecA* -F	"AAA ATC GAT GGT AAA GGT TGG C"	22	50	533	(17)
	mecA -R	"AGT TCT GCA GTA CCG GAT TTG C"	22			

containing different concentrations of each disinfectant were prepared through serial (from 9.5×10^{-6} to 5% v/v) dilution in Mueller-Hinton broth (Merck Co., Germany). The strains were inoculated into tubes with various concentrations of each disinfectant (final concentrations, 5×10^5 cfu/mL) and incubated at 35°C for 24 hours. The concentration that completely inhibited bacterial growth was considered as the MIC value. The determination of minimum bactericidal concentrations (MBCs) was performed by sub-culturing 10 µL from each tube that exhibited no growth on Mueller-Hinton agar culture plates and then incubated at 35°C for 24 hours. The concentration that revealed no visible bacterial growth was taken as MBC.

Disc Diffusion Assay

This assay was performed according to CLSI guidelines (15). The isolates were inoculated on Mueller-Hinton agar culture plates and the disks (6.0 mm in diameter, Whatman filter paper no. 1, Whatman International Ltd, UK) containing different concentrations of each disinfectant (0.5, 1 and 2%) were put on the surface of the agar. The agar plates were placed at 35°C for 24 hours and the inhibition zone was recorded.

Cell Viability Test

The viability of isolates was assessed by determining colony-forming units (CFUs). At first, all the isolates were cultured on blood agar base (Merck Co., Germany) containing 5% sheep blood and incubated at 35°C for 24 hours. Then, 10 µL from 1.5×10^5 cfu/mL was treated with various concentrations of each disinfectant (0.5, 1 and 2%) at 35°C for 10, 30 and 60 minutes (18). Afterwards, 50 µL of the treated culture was inoculated on the surface of a Tryptic soy agar (Merck Co., Germany) and the colonies were counted after 24 hours of incubation at 35°C. The viability of the strains was analyzed by comparing the number of CFUs on agar plates with the control plates.

Statistical Analysis

To analyze the data, SPSS version 22.0 was used, with a significance level of $P < 0.05$. Since the data were not normally distributed (based on Shapiro–Wilk test), the MICs and MBCs against various bacterial groups were compared by the Kruskal–Wallis test.

Results

In the present study, *S. aureus*, *S. saprophyticus* and *S. epidermidis* were isolated from different wards (dialysis, maternity, and NICU) of the hospital (Table 2). The results showed that 10 (58.8%), 10 (22.7%), and 55 (39.2%) isolates of *S. aureus*, *S. saprophyticus* and *S. epidermidis* were methicillin-resistant, respectively, and most of them were isolated from NICU and maternity wards (Table 2). In all methicillin-resistant strains, the *mecA* gene was present (Figure 1).

According to the results of antibiotic susceptibility tests, MRSA and methicillin-resistant *S. epidermidis* (MRSE) isolates were highly resistant to penicillin (100% and 81.8%, respectively). There was a high resistance in methicillin-resistant *S. saprophyticus* (MRSS) isolates to penicillin (80%) and erythromycin (80%) (Table 3).

D phenotype was observed in only one of the isolates of *Staphylococcus* strains.

The MICs and MBCs values are shown in Table 4. The MIC of Deconex was higher against MRSA isolates than for *S. epidermidis* and *S. saprophyticus* isolates, whereas MICs of Microzed and Surfocept were higher against *S. epidermidis* and *S. saprophyticus* isolates than against other isolates, respectively. The MIC of Peranacid was higher against MRSA and *S. saprophyticus* isolates than against *S. epidermidis* isolates. According to these results, Deconex and Microzed had the highest antimicrobial activity, respectively.

Regarding MRSA isolates, there was a significant difference between Deconex and Peranacid ($P<0.001$) and Microzed and Peranacid ($P=0.025$) in MICs. There

Table 2. The Frequency of *Staphylococcus* Isolates in Hospital Wards

Ward	No. of *Staphylococcus saprophyticus*		No. of *Staphylococcus epidermidis*		No. of *Staphylococcus aureus*		Total
	MRSS	MSSS	MRSE	MSSE	MRSA	MSSA	
Maternity	6 (17.1%)	10 (28.6%)	7 (20.0%)	9 (25. 7%)	1 (2.9%)	2 (5.7%)	35 (17.4%)
Internal	1 (3.0%)	8 (24.2%)	10 (30.3%)	12 (36.4%)	1 (3.0%)	1 (3.0%)	33 (16.4%)
Dialysis	0 (0.0%)	1 (5.6%)	5 (27.8%)	8 (44.4%)	2 (11.1%)	2 (11.1%)	18 (9.0%)
Surgical room	0 (0.0%)	1 (4.3%)	6 (26.1%)	16 (69.6%)	0 (0.0%)	0 (0.0%)	23 (11.4%)
NICU	2 (5.0%)	6 (15.0%)	14 (35.0%)	15 (37.5%)	3 (7.5%)	0 (0.0%)	40 (19.9%)
ICU	1 (4.5%)	3 (13.6%)	5 (22.7%)	10 (45.5%)	1 (4.5%)	2 (9.1%)	22 (10.9%)
Emergency	0 (0.0%)	5 (16.7%)	8 (26.7%)	15 (50.0%)	2 (6.7%)	0 (0.0%)	30 (14.9%)
Total	10 (5.0%)	34 (16.9%)	55 (27.4%)	85 (42.3%)	10 (5.0%)	7 (3.5%)	201 (100%)

MSSA, methicillin-susceptible *S. aureus*, MRSA: methicillin-resistant *S. aureus*, MSSE: methicillin-susceptible *S. epidermidis*, MRSE: methicillin-resistant *S. epidermidis*, MSSS: methicillin-susceptible *S. saprophyticus*, MRSS: methicillin-resistant *S. saprophyticus*, ICU: intensive care unit, NICU: neonatal intensive care unit.

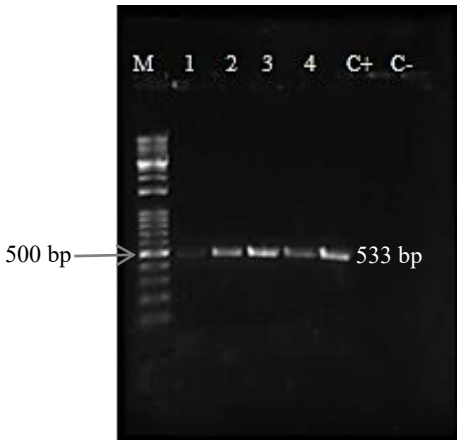

Figure 1. Detection of *mecA* Gene in Isolates by PCR. M: DNA ladder (100 bp), 1 to 4: methicillin-resistant staphylococci, C+: positive control, and C-: negative control

Table 3. The Antibiotic Resistance Rates of Methicillin-resistant Staphylococcus Strains (%)

Antibiotics	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Ciprofloxacin	70	58.1	50
Trimethoprim/ sulfamethoxazole	60	50.9	70
Tetracycline	80	61.8	60
Clindamycin	60	38.1	50
Erythromycin	70	65.4	80
Gentamicin	60	52.7	60
Penicillin	100	81.8	80
Chloramphenicol	40	54.5	50
Vancomycin*	0	0	0
Nitrofurantoin	0	0	0

*Vancomycin susceptibility profile was determined using agar dilution method.

was a significant difference between Deconex and Peranacid (P=0.013) in MBCs. A statistically significant difference was found in MICs between Deconex and Microzed (P=0.037), Deconex and Peranacid ($P<0.001$) and Deconex and Surfocept ($P<0.001$) in MRSE isolates. Moreover, a significant difference between Deconex and Peranacid ($P<0.001$) and Deconex and Surfocept ($P<0.001$) was seen in MBCs. In methicillin-resistant *S. saprophyticus* isolates, there was a significant difference in MICs and MBCs between Deconex and Surfocept ($P<0.001$) and Deconex and Peranacid ($P<0.001$).

The results of disk diffusion assay demonstrated that *Staphylococcus* strains had greater inhibition zone diameter at different concentrations of Deconex and Microzed compared to other disinfectants. At 2% concentration of Microzed, 4, 22 and 2 strains of *S. aureus*, *S. epidermidis* and *S. saprophyticus*, respectively had inhibition zone diameters greater than 15 mm. Moreover, at 2% concentration of Deconex, 1, 14 and 4 strains of *S. aureus*, *S. epidermidis* and *S. saprophyticus*, respectively had inhibition zone diameters greater than 15 mm.

According to the results of cell viability assay, *S. aureus*, *S. epidermidis*, and *S. saprophyticus* isolates did not grow at 0.5%, 1% and 2% concentrations of Deconex, Microzed and Peranacid for 10, 30 and 60 minutes, respectively. At 2% concentration of Surfosept, *Staphylococcus* strains had no growth, but the growth was observed at other concentrations (Table 5).

Discussion

Environmental surfaces in hospital are mostly contaminated with microbial pathogens. These surfaces are the sources for transmission of pathogens to human (19). Disinfection and sterilization are two important methods for controlling infections in the hospital setting. In addition, due to increasing antibiotic resistance of bacteria, disinfection and decontamination of healthcare equipment and hospital environment are essential factors in controlling the spread of antibiotic resistance (20,21). The environmental contamination has an important role in the transmission of pathogens such as methicillin-resistant staphylococci, especially among patients (22). methicillin-resistant staphylococci are major problems in healthcare settings worldwide and the emergence of these bacteria underscores the use of appropriate disinfectants

Table 4. MIC and MBC (v/v %) of Disinfectants Against Methicillin-Resistant *Staphylococcus* Isolates

Disinfectant	*Staphylococcus aureus*, n=10		*Staphylococcus epidermidis*, n=55		*Staphylococcus saprophyticus*, n=10	
	MIC	MBC	MIC	MBC	MIC	MBC
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Deconecx 50 AF	9×10^{-4} (1.5×10^{-4} - 3×10^{-3})	9×10^{-4} (3×10^{-4} - 4.8×10^{-3})	1.5×10^{-4} (6.6×10^{-5} – 3×10^{-4})	3×10^{-4} (1.3×10^{-4} – 7.5×10^{-4})	3×10^{-4} (1.3×10^{-4} – 6×10^{-4})	2.2×10^{-4} (1.3×10^{-4} – 3.7×10^{-4})
Microzed GP-H	3×10^{-4} (1.3×10^{-4} – 7.5×10^{-4})	1.8×10^{-3} (5×10^{-4} – 6×10^{-3})	1.8×10^{-3} (6×10^{-4} – 6×10^{-3})	3.6×10^{-3} (1.0×10^{-3} – 9.7×10^{-3})	1.2×10^{-3} (6×10^{-4} – 3×10^{-3})	3.6×10^{-3} (2.1×10^{-3} – 6×10^{-3})
Peranacid M1	9.7×10^{-3} (4.2×10^{-3} – 2.4×10^{-2})	9.7×10^{-3} (4.2×10^{-3} – 2.4×10^{-2})	7.2×10^{-3} (2.1×10^{-3} – 2.4×10^{-2})	9.7×10^{-3} (2.4×10^{-3} – 2.4×10^{-2})	9.7×10^{-3} (4.2×10^{-3} – 2.4×10^{-2})	9.7×10^{-3} (4.2×10^{-3} – 3.9×10^{-2})
Surfocept quick	2.4×10^{-3} (5×10^{-4} – 1.2×10^{-2})	3.6×10^{-3} (1.0×10^{-3} – 1.2×10^{-2})	7.2×10^{-3} (2.1×10^{-3} – 2.4×10^{-2})	1.4×10^{-2} (4.8×10^{-3} – 3.9×10^{-2})	9.7×10^{-3} (4.2×10^{-3} – 2.4×10^{-2})	1.4×10^{-2} (4.8×10^{-3} – 4.8×10^{-2})

Table 5. Cell Viability of Methicillin-resistant Staphylococcus Strains as Measured by Conventional Plate Count Method (cfu, %) Treated With Various Concentrations of Surfosept

Group	*Staphylococcus aureus* (n=10)			*Staphylococcus epidermidis* (n=55)			*Staphylococcus saprophyticus* (n=10)		
	10 min	30 min	60 min	10 min	30 min	60 min	10 min	30 min	60 min
Control	100	100	100	100	100	100	100	100	100
Surfosept 0.5%	58.6±2.4*	46.5±2.2	20.8±3.1	9.8±2.0	5.3±1.2	0	31.2±3.4	16.7±2.0	7.0±2.3
Surfosept 1%	12.9±1.4	8.0±2.0	0	4.9±1.3	3.0±1.0	0	0	0	0
Surfosept 2%	0	0	0	0	0	0	0	0	0

*Means ± standard deviation.

for the prevention of infection (23). Therefore, the use of effective disinfectants in clinical environments is a key factor in controlling and preventing the spread of infection (24). In this study, the efficacy of some hospital disinfectants against methicillin-resistant staphylococci isolated from different wards of an educational hospital has been studied.

In the present study, due to the fact that *S. saprophyticus* is the normal flora of the genitourinary tract in some women, most strains of methicillin-resistant *S. saprophyticus* were isolated from the maternity ward, not from surgical room, dialysis and emergency wards. Most strains of MRSA and MRSE were isolated from the NICU. Due to the importance of NICU as a special department, the isolation of these pathogenic strains from this department should be considered.

In this study, the lowest number of *Staphylococcus* strains belonged to the dialysis, ICU and surgical room, respectively, which could be related to the sensitivity of these wards in terms of the type of hospitalized patients and the guidelines for infection control. Due to the importance of *S. aureus* in causing post-surgical infections, no isolates were taken from the surgical room.

According to the results of antimicrobial susceptibility, MRSA isolates had high resistance to penicillin, tetracycline and ciprofloxacin, which is similar to the results of many previous studies (25-28). However, coagulase-negative staphylococci strains were resistant to penicillin, tetracycline and erythromycin. Similar to other studies, all strains had high sensitivity to vancomycin and nitrofurantoin (26,27,29-31). It was found that 2.27% of *S. saprophyticus* isolate had D phenotype.

It was revealed that Deconex and Microzed had lower MIC and MBC and higher efficacy than the other two disinfectants. Nevertheless, Deconex and Microzed had lower MIC against *S. epidermidis* and *S. aureus* isolates, respectively. The Surfosept had the lowest MIC against *S. aureus*. Peranacid had equal MIC values against both *S. aureus* and *S. epidermidis* strains. Similarly, Rutala et al reported that commercial agents including quaternary ammonium compounds are highly effective against MRSA (32). However, some studies suggested that methicillin-resistant staphylococci and vancomycin-resistant enterococcus (VRE) do not show decreased susceptibility to disinfectants used in the hospital (33,34).

The results of the cell viability assay with different concentrations of disinfectants at different times showed that the strains were grown only in the presence of Surfosept. *S. aureus* strains showed more resistance to Surfosept and *S. epidermidis* strains showed the least resistance. In disk diffusion assay, the Microzed and Peranacid had the highest and lowest antimicrobial activities, respectively. Comparison of the results of the disc diffusion test and cell viability assay showed that *S. epidermidis* strains exhibited higher resistance to Surfosept compared to other strains. In a study by Meade and Garvey, the effectiveness of new chemical disinfectants against pathogens was investigated. MRSA showed the same sensitivity to disinfectant agents compared with methicillin-susceptible *S. aureus* based on the results of disk diffusion test. Additionally, a great reduction in cell viability was seen for chemical disinfectants (35).

Presterl et al compared the efficacy of some disinfectants such as povidone iodine, alcohol and H_2O_2 against *Staphylococcus epidermidis*. According to their study, alcohol and H_2O_2 were more effective against *S. epidermidis* biofilms whereas povidone iodine was less effective (36).

According to the results of the present study, the MIC of the disinfectants used against MRSA strains isolated from the dialysis ward was higher than the other MRSA strains isolated from the other wards. In addition, these strains showed a smaller inhibition zone diameter. Additionally, 3 strains of 6 methicillin-resistant *S. saprophyticus* strains taken from maternity ward showed higher resistance to disinfectants. Resistance to disinfectants among gram-positive cocci such as staphylococci is problematic. Although resistance to these agents has not been determined in similar way to drug resistance, several studies have reported a low susceptibility of pathogenic bacteria to disinfectants (37).

In our study, some differences were seen in the effectiveness of disinfection agents. These differences can be related to genetic alterations taking place in these strains. It is, therefore, important to investigate the exactitude of genes that mediates resistance to disinfectants so as to prepare information for the prevention of the spread of diseases caused by methicillin-resistant staphylococci that

are resistant to some disinfection agents.

A limitation of this study is that the tests were carried out with bacteria under in vitro conditions, and the bacterial responses may be somewhat different for hospital environments.

It is suggested that, to control nosocomial infections in hospital environments, strong disinfectants such as Deconex and Microzed be used, contact time be considered according to guidelines, surfaces be cleaned before using disinfectants and genetic alterations related to the differences in the effectiveness of disinfections be studied.

Conclusions

The findings of the present study indicated that Deconex and Microzed had more antimicrobial properties than the two other agents against methicillin-resistant staphylococcus isolates and these bacteria had a higher resistance to both Peranacid and Surfosept.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

The project was confirmed by the Ethics Committee of Gonabad University of Medical Sciences with the code of IR.GMU.REC.1396.90.

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