Original Article

Evaluation of Efficacy of the Current Disinfectants on Gram-Negative Bacteria Isolated from Hospital in Yazd in 2014

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Abstract

Background and Purpose: The burn unit is a suitable environment for growing of bacteria such as Pseudomonas, Acinetobacter, and Staphylococcus that appropriate disinfection can reduce these pathogens. The aim of this study was to evaluate the effect of different disinfectants on Gram-negative bacteria isolated from the surface of accidents and burn hospital in Yazd.

Materials and Methods: In this study, 240 samples were randomly collected from different parts of accidents and burn hospital before and after disinfection. The samples were cultured on blood agar and Eusion-Metilen-Blue agar media in the Microbiology Laboratory of Medicine School of Shahid Sadoughi University in Yazd and Colony counting were determined. Identification was done by biochemical tests after incubation at 37° C for 48 hours. The studied disinfectants were Deconex 50AF, Descoscid, Epimax SC, and Silvosept. At last, data were analyzed with using paired t-test.

Results: The Gram-negative bacteria were isolated from burn unit before disinfection included Pseudomonas aeruginosa, Escherichia coli, Proteus spp., Klebsiella spp., Acinetobacter spp., and Enterobacter spp. According to the results, all disinfectants reduced the pollution before and after disinfection; nevertheless, this reduction at the time of using Epimax SC and Silvosept only showed a significant difference for P. aeruginosa (P = 0.001 and 0.003) and for E. coli (P = 0.020 and 0.005), respectively.

Conclusion: All disinfectants were effective on Gram-negative bacteria isolated from surfaces and had shown a significant difference only between P. aeruginosa and E. coli number before and after disinfection by Epimax SC and Silvosept. The most effective disinfectant on P. aeruginosa and E. coli was Epimax SC and Silvosept, respectively.

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Key words: Burn Unit, Disinfectant, Gram-negative Bacteria

1. Introduction

The increasing nosocomial infections are one of the main problems that the education-health centers and also hospitals are currently faced with it. Environmental surfaces in contact with patients are infected by epidemiological important microorganisms which survive on different levels of the hospital (1). Many Gram-negative bacteria such as Acinetobacter can remain resistant from 3 days to 5 months on dry surfaces (2,3). Burn unit is a suitable environment for the growth of bacteria such as Pseudomonas, Enterobacter, and Staphylococcus (4).

Burned patients are highly susceptible to infection due to losing skin that is a border to prevent the penetration of microorganisms. In addition, the immune system of these patients is very active in response to burn injuries and changes occur in their immune system (5). At the first burn wound is sterile; nonetheless, the bacteria grow in it within 12 hours (6).

Since Pseudomonas aeruginosa is a bacterium with low required food for growth and can remain in the environment and easily be transferred to susceptible patients. According to available reports, this bacterium is the first bacteria for creating nosocomial infections in burn medical centers; therefore, these infections are often in burned patients (7). In another study in 2002, Weis et al. in Germany found Staphylococcus, Enterococcus, Escherichia coli, Pseudomonas, Acinetobacter, and Enterobacter in burn patients (6). Acinetobacter baumannii is also an important pathogen of burn wound infections, especially in immunocompromised patients and patients who are in the intensive care unit (ICU) (8).

The pollution of environmental surfaces may play a role in transfer of pollutions (9-11). The infection incidence in healthcare and education-health centers causes contaminants enter into the environment from the various ways and mechanisms; therefore, several factors can have a role to the infection transfer (12). An important factor in the spread of nosocomial infections is an improper use of disinfectants (13). Disinfection is the process to remove all or most of pathogenic microorganisms on inanimate surfaces except bacterial spores. Disinfection should be done following the cleanup (14) because the cleaning methods by detergents may be insufficient to kill the pathogens in the hospital environment (1). Sanitizers or disinfectants are used for sterilization and disinfection of medical devices, the operating rooms and maternity, burn sections, physical surfaces, and hallways every day (15). Effective use of disinfectants is an important factor in the prevention of nosocomial infections (10). Activity against key pathogens and lethal speed are the most important factors in disinfectant choice (16).

The use of safe and effective disinfectant solution with minimal damage to equipment and personnel is one of the fundamental principles of disinfection. None of the disinfectants are suitable for all different needs, the choice of disinfectant is also important in medical centers. Therefore, an investigation to determine the effects of different disinfection is necessary to select a suitable disinfectant (13). In this study, the disinfection effects of Deconex 50AF (Irenic Company, Switzerland), Descoscid (Altonafarin Company, Germany), Epimax SC (Emad Company, Iran), and Silvosept (Chitotech Company, Iran) evaluated and compared on Gram-negative bacteria (P. aeruginosa, Acinetobacter, E. coli, Proteus, Klebsiella, and Enterobacter) that were isolated from different parts of Shahid Sadoughi Burn Hospital in Yazd in 2014.

2. Materials and Methods

Sampling

This is a descriptive-analytical study and sampling was random. To evaluate the disinfectants efficacy (Deconex 50 AF,

Epimax SC, Descoscid, and Silvosept) using a statistical formula and to determine the sample size on $\alpha = 0.05$, $P = 0.800$, $d = 0.15$, the sample size was calculated equivalent 30. The location of the samples was selected and marked according to the highest level of patient contact and personnel from different parts of the hospital rooms, isolation room, dressing room, and the hospital corridor of burn injuries in the Medical University of Shahid Sadoughi in Yazd in 2014.

Sampling was carried out at the end of the business day and before disinfecting the surfaces, a sterile cotton swab moistened with sterile saline, and sampled from the entire areas and placed in the tube containing 3 ml of tryptic soy broth medium. Then, the marked places were disinfected by a disinfectant according to the instructions and after finishing contact time and drying disinfectant; sampling was done again according to the mentioned methods and cultured.

2.2. Sample culture

For culturing the samples, a tube containing the sample was mixed by Vortex, and then 100 µl of sample was transferred to plates containing blood agar medium and Eusion-Metilen-Blue agar medium by a sampler and was cultured in the whole culture medium by a sterile loop. They were incubated at 37° C for 48 hours (17).

After incubated, plates containing cultured samples were investigated (before and after disinfection by disinfectants). If there are suspected colonies of Gram-negative bacteria, they were identified by Gram-stain and routine biochemical tests such as determining the fermentation of glucose, lactose and sucrose in the triple sugar iron medium, urea hydrolysis, producing indole from tryptophan, use of citrate, moving, producing hydrogen sulfide, determining the method of fermentation in methyl red Voges-Proskauer, oxidase production, and oxidation-fermentation test in the OF (17).

All culture media used in this study were manufactured by Merck, Germany. It should be noted that all of the samples was performed with the alcoholic lamp. A total of 30 samples were taken before and 30 after disinfection. Physiologic serum was used as positive controls. In this study, each disinfectant used according to the manufacturer's recommended concentration for surfaces disinfection.

2.3. Comparison of studied bacteria colony count

After identification of bacteria, the numbers of bacterial colonies were counted before disinfection and by considering the dilution factor, their number was determined in 1 ml (cfu/mL). If investigated colony exists on the plate, this method also was used after disinfection for counting. This method was used to compare the number of investigated bacteria in all places and all of the above disinfectants.

2.4. Statistical analysis

To analyze the data, the descriptive tables were used and for the normal data, paired t-test (paired t-test) was used.

3. Results

In this study, 240 samples (for each disinfectant, 30 samples before and 30 samples after disinfection) were taken from different parts of the bedroom (the handle of the refrigerator, closet, food table, the lights of the service, the handle of the door and bed), isolation room (liquid dish, the head of tap, tap, switch, the handle of the refrigerator, oxygen flowmeter, earphone, commode, dining table, door frames), dressing room (armrest, bed, the handle of the shower, tap, liquid dish, earphone), and corridor (the door and the handle of male and female's WC, the liquid dish of female's WC, the tap of female's WC, earphone, nursing stations) in burn injuries hospital. The results are shown in table 1.

Table 1. Comparison of the average (cfu/mL) of the isolated Gram-negative bacteria before and after use of Deconex 50AF, Descoscid, Epimax SC, Silvosept

Pathogen bacteria	Disinfectant											
	Deconex 50AF**			Descoscid**			Epimax SC***			Silvosept****		
	Mean ± SD		P value	Mean ± SD		P value	Mean ± SD		P value	Mean ± SD		P value
	Before (N = 30)	After (N = 30)		Before (N = 30)	After (N = 30)		Before (N = 30)	After (N = 30)		Before (N = 30)	After (N = 30)	
E. coli	32.85 ± 6.06	0 ± 0	0.180	18569.20 ± 3448.31	0.18 ± 0.03	0.317	4.24 ± 1.75	0.60 ± 0.17	0.200	5.21 ± 2.26	0.57 ± 0.13	0.005
P. aeruginosa	15 ± 4	9.12 ± 1.66	0.122	_*	_*	_*	18569.23 ± 3449.82	0.77 ± 0.20	0.001	18.65 ± 5.50	1.83 ± 0.46	0.003
Klebsiella sp.	1.85 ± 0.45	3.65 ± 0.66	0.490	2.12 ± 0.62	1.48 ± 0.27	0.060	3.8 ± 0.96	0 ± 0	0.102	_*	_*	_*
Proteus sp.	_*	_*	_*	0.18 ± 0.03	0.4 ± 0.1	0.317	_*	_*	_*	_*	_*	_*
Acinetobacter sp.	_*	_*	_*	_*	_*	_*	0.37 ± 0.06	0 ± 0	0.317	1.82 ± 0.33	0 ± 0	0.317
Enterobacter sp.	_*	_*	_*	_*	_*	_*	9.28 ± 1.72	0 ± 0	0.317	0.76 ± 0.20	0 ± 0	0.157

*There were no bacteria before disinfection, **Between isolated Gram-negative bacteria before and after disinfection did not show a significant difference, ***Only between P. aeruginosa and E. coli was a significant difference before and after disinfection, ****Only between P. aeruginosa and E. coli was a significant difference before and after disinfection. P. aeruginosa: Pseudomonas Aeruginosa, E. coli: Escherichia coli

4. Discussion

In the present study, Gram-negative bacteria isolated from different burn parts were P. aeruginosa, E. coli, Proteus species, Klebsiella, Acinetobacter, and Enterobacter before disinfection. According to the average of Gram-negative bacteria isolated before and after disinfection, all studied disinfectants caused reducing existing pollution. Nevertheless, this reduction at the time of using Epimax SC and Silvosept only showed a significant difference for P. aeruginosa (0.001 and 0.003) and E. coli (0.020 and 0.005), respectively.

de Andrade et al. (18) study, in 2000, was performed to evaluate the microbial situation of hospital mattress before and after disinfection to identify bacteria that are important in the epidemiology of nosocomial infections (Staphylococcus and Pseudomonas). It was found that 500 of 1040 total culture plates from 52 mattresses were obtained positive results (48%/1). A study by Shams et al. (19), in 2010, was performed to evaluate the contamination of ICUs in the Hamadan Hospitals. They showed that the most commonly isolated bacteria were Gram-negative bacilli such as E. coli, Enterobacter, Klebsiella, and Pseudomonas, and at the next level, Gram-positive cocci were Micrococcus and Staphylococcus epidermidis. In general, Gram-negative bacteria were the dominant factors of infection in studied hospitals which corresponded with the results of the present study. In a study that was done in England on the sensitivity of nosocomial Gram-negative bacteria to disinfectant showed that tetravalent ammonium compounds on the bacteria have been less effective than chlorhexidine (20).

Ehrampoush et al. (21) in another study, in 2010, evaluated the combination of hydrogen peroxide and silver on the steel surface contaminated by some pathogens. They concluded this compound prevent the growth of pathogenic bacteria on the surface as well.

In a study in Hamadan Hospitals by Youssefi Mashouf et al. that was done, in 2008, for investigation of bacterial contamination in operating rooms and comparing Deconex SB and Hayzhen in reducing pollution, it was found the rate of infection in operating rooms of hospital was relatively high and Deconex SB was relatively better of Hayzhen in reducing bacterial contamination. Their results showed that both solutions had appropriate disinfection effect after disinfection on bacteria isolated from operating rooms (12). These findings are consistent with our study. Zazouli et al. (22), in 2015, investigated the effects of common disinfectants used for E. coli bacteria separated from two teaching hospitals in Sari. The results showed that 48 samples of 120 samples (40%) were contaminated, and 15 (13%) of these isolates were positive for E. coli. The results also showed that the most effective disinfectants against E. coli were Cidex, Deconex Plus53, and Creolin, respectively. Another study that was done at teaching hospitals in Hamadan represented the most effective disinfectants were Creolin and Cidex that showed no significant differences on Staphylococcus and Pseudomonas (13).

Yosefimashouf et al. (23), in 2014, evaluated the bacterial contamination of ophthalmic biomicroscopy apparatus (slit-lamp) in eye centers of Farshchian Hospital in Hamadan and assessment of the current disinfectants. They concluded the efficacy of sodium hypochlorite was more effective than chlorhexidine. Moreover, after disinfection, bacterial contamination was considerably decreased. Saboori et al. (24), in 2006, investigated the effect of Micro10^{+} and Deconex 53 Plus dentistry tools and showed that Deconex 53 Plus (1%) and Micro10^{+} (2%) had an acceptable disinfection effect on dentistry tools during 1 hour. Valizadeh et al. (25) investigated the efficiency of Epimax S and Epimax SC on dominant pathogens in ICU of Urmia. The results showed that there is a significant difference between Epimax S

and Epimax SC in nosocomial infection control. Johnson et al. (26), in 2005, studied the efficacy of an alcohol/chlorhexidine hand hygiene program in a hospital with high rates of nosocomial methicillin-resistant Staphylococcus aureus (MRSA) infection and showed 40% MRSA reduction and 90% E. coli and Klebsiella spp. reduction. Sharkhizan et al. (27), in 2014, studied the efficacy of new disinfectants including Sanocil, Alprocide, Bibfort, Javel-dose compared with Micro10 and Deconex on isolated organisms from dentistry units. Their results showed that out of 120 cultured samples, 98 positive cultures (81.6%) were obtained that 254 strains and 14 bacteria species were isolated that 72.1% of them were Gram-positive and 27.9% were Gram-negative bacteria. The results also revealed that the most effective disinfectants on pathogenic strains were Deconex and Alprocide.

Gram-negative bacteria of the Enterobacteriaceae such as Klebsiella, Enterobacter, Serratia, and Proteus are resistant to disinfectants especially ammonium chloride and phenol compounds. In the present study, the more efficiency of Silvosept and Epimax SC in the elimination of Gram-negative bacteria (E. coli and P. aeruginosa) is probably due to the basis of their composition. Silvosept has a combination of nano-colloidal silver, Epimax SC has a basic combination of hydrogen peroxide, ammonium tetravalent, and alcohol that in this study, and Deconex 50AF has less effect on Gram-negative bacteria due to its ammonium chloride combination.

Therefore, it is clear that having enough and complete information about microorganisms of different parts of the hospital and recognition of disinfectant, disinfection methods, percent and effectiveness of disinfectants that used on them, caused the increasing the authorities knowledge and ability for eliminating the sources of contamination and reducing nosocomial infections.

Conflict of Interests

Recommended concentrations of disinfectants for use are based on controlled conditions. Obviously, the real conditions are never controllable, thus human errors and unexpected conditions will provide the possibility of survival and microbes reproduction.

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