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Original Article

Efficacy of Disinfectants Against Pathogenic Bacteria in Intensive Care Units of Abadan and Khorramshahr Hospitals, Iran

Sara Kooti[1#], Hossein Moradikazerouni[2#], Seyed Mohammad Ali Malaekeh[3], Mostafa Alijanzadeh[4], Somayeh Rahimi[5,6*]

[1]Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Research Development Unit, Valiasr Educational Hospital, Abadan University of Medical Sciences, Abadan, Iran
[3]Department of Laboratory Sciences, School of Allied Medical Sciences, Abadan University of Medical Sciences, Abadan, Iran
[4]Shahid Rajaee Port and Maritime Administration, Bandar Abbas, Iran
[5]Department of Environmental Health Engineering, Abadan University of Medical Sciences, Abadan, Iran
[6]Research Center for Environmental Contaminants (RCEC), Abadan University of Medical Sciences, Abadan, Iran

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***Corresponding author:**
Somayeh Rahimi,
Email: Rahimi_s97@yahoo.com
#These authors contributed equally to the study.

Abstract

Background: Effective surface disinfection techniques are necessary to decrease the risk of dissemination of hospital-acquired infections (HAIs), which pose a serious concern in the healthcare industry. The aim of this study was to evaluate the efficacy of four disinfectants (Lysoformin 3000, Epimax Surclean, Minuten Spray, and Epimax quick) in reducing pathogenic bacteria on surfaces in the intensive care units (ICUs) of Abadan and Khorramshahr hospitals.

Methods: A descriptive-analytical approach was used with non-probability convenience sampling in three hospitals. High patient and staff contact areas were taken into consideration when choosing sampling locations. Before and after each agent's disinfection, 240 samples in total were gathered and then subjected to culture and biochemical testing to determine the presence of pathogens. Paired t-tests were utilized for statistical analysis.

Results: Before disinfection, the mean colony counts of pathogenic bacteria were determined for each disinfectant in different ICU locations. After disinfection, significant reductions in pathogen counts were observed for all four disinfectants. Commonly identified Gram-negative bacteria included *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*, while *Staphylococcus aureus* and *Enterococcus* were prevalent among Gram-positive bacteria.

Conclusion: Effective disinfection is critical in healthcare settings to reduce the risk of infection transmission. The findings highlight the importance of selecting appropriate disinfectants tailored to specific circumstances and pathogens. Continued research and improvement of disinfection protocols are essential to meet the evolving challenges of infection control. This study contributes to the knowledge base for improving the safety of healthcare environments and reducing HAIs.

Keywords: Disinfectants, Intensive care units, Bacteria, Hospitals

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Introduction

The global prevalence of recurrent and emerging diseases, changing disease dynamics, the emergence of microbial resistance, and the growing demand for healthcare services have made hospital-acquired infections (HAIs) an inescapable problem (1). These infections represent a significant challenge within the healthcare landscape, leading to increased healthcare expenditures (2). HAIs, or nosocomial infections, are diseases that patients contract while they are in the hospital, either during their admission or within 48–72 hours after admission. They can appear up to six weeks after they are discharged (3,4). According to the World Health Organization, these infections affect an estimated 50% of hospitalized patients worldwide, posing a significant challenge to communities and healthcare facilities (3,5,6). These infections occur in various hospital settings, such as pediatric, burn, special care, and surgical units (1). They include bacterial infections (68%), *Candida* infections (9%), and viral infections (22%) (1). The consequences of these infections are urinary tract, respiratory tract, bloodstream, and surgical site infections. These infections frequently result in extended hospital stays and sometimes have fatal consequences (7-9). HAIs can be caused by medical procedures, lax hospital hygiene standards, poor personal hygiene among staff, and failure to follow hospital hygiene and safety protocols (10,11). While the complete eradication of such infections may be an unattainable goal, adherence to established standards and guidelines can effectively mitigate and manage infectious risks (12,13).

A key component of hospital protocols is the cleaning/disinfection of surfaces (5). Hospital surfaces play an important part in the infection cycle, serving as potential reservoirs for disease-causing bacteria and facilitating the spread of infectious agents throughout the healthcare environment (14). To stop further infectious consequences, crack the network of infection transmission, and slow the progression of disease, effective surface cleaning is crucial. Continued advancements in surface disinfection methods in healthcare facilities are essential to achieving this goal. Even the most thorough cleaning may leave behind microscopic traces of body fluids that can carry infectious agents (15,16).

Disinfectant chemicals are routinely used in hospitals for surface disinfection and are classified into low-level, intermediate-level, and high-level disinfectants (17). These agents are utilized daily to sterilize or disinfect medical equipment, operating rooms, maternity wards, burn units, dressings, injections, and floors and surfaces in hospital corridors (18). The primary goal of using disinfectant chemicals in hospitals is to lower the threat of nosocomial and environmental infections. Several disinfection methods and disinfectants are recommended to achieve this goal (19, 20). Given the evolving resistance patterns of hospital-acquired pathogens, it is imperative to periodically evaluate the effectiveness of disinfectants and chemicals against multidrug-resistant bacteria (21).

This study aims to evaluate the effectiveness of two instrument disinfectants, Lysoformin 3000 and Epimax quick, and two surface disinfectants, Minutes spray and Epimax Surclean, against pathogens isolated from the special care wards of three hospitals connected to Abadan University of Medical Sciences.

Materials and Methods

Study Design and Sampling

In this descriptive-analytical study, non-probability convenience sampling was employed to gather data from the special care units of Shahid Beheshti and Taleghani Hospitals in Abadan and Valiasr Hospital in Khormashahr. Specific areas for sampling within patient rooms and nursing stations were selected based on the highest frequency of contact for both patients and staff. These targeted areas included refrigerator handles, cabinets, dining tables, service lamps, beds, room door handles, chair handles, and telephones. A total of 240 samples were gathered from the above-mentioned hospitals. In general, 30 pre-disinfection and 30 post-disinfection samples were collected for each of the four types of disinfectants. Each disinfectant was sampled on a different day. Several disinfectants were used in compliance with the recommended contact times provided by the manufacturer, including Lysoformin 3000 (10 minutes), Epimax Surclean (5 minutes), Minute Spray (3 minutes), and Epimax Quick (1 minute). To reduce variability, experienced observers kept an eye on adherence to contact times throughout disinfection cycles. Following application, surfaces were left to air dry in accordance with instructions (22).

Using sterile swabs wet with sterile saline, the samples were taken from all assigned areas prior to surface cleaning at the conclusion of the workday. Next, these samples were put in tubes with 3 mL of the Tryptic Soy Broth medium. Following the necessary contact time and drying of the disinfectant material, the designated areas were subsequently cleaned in accordance with the manufacturer's instructions, and sampling was performed once more using the same technique.

Cultivation and Isolation of Bacteria

All samples were moved right away to the Microbiology Research Laboratory of Abadan University of Medical Sciences. In the laboratory, the tubes containing the samples were vortexed, and 100 µL of each sample was cultured on blood agar and eosin-methylene blue media. Then, the culture plates were incubated at 37 °C for 48 hours. After the incubation period, Gram staining and biochemical tests (triple sugar iron, sulfide-indole-motility, citrate, and oxidase) were used to check the plates for the presence or absence of pathogens. The total number of pathogenic bacteria in the culture plates before disinfection was determined, taking into account the dilution factor, to determine the number of pathogens per mL. The same counting procedure was utilized if the pathogen of interest was still present in the plates after disinfection.

This method was used to compare the pathogen colony counts in all designated areas and for all types of applied disinfectants. As a negative control, sterile saline (0.9% NaCl) was employed to take environmental influences and naturally occurring bacterial degradation into consideration. Parallel surfaces were treated with sterile saline in place of the disinfectant for each test.

Statistical Analysis

The data were analyzed using SPSS, version 21 (SPSS Inc., Chicago, IL, USA). Descriptive tables and a paired t-test were utilized for data analysis.

Results

This study assessed the effectiveness of four different disinfectants (Lysoformin 3000, Epimax Surclean, Minuten spray, and Epimax quick) in reducing the presence of pathogenic bacteria in the intensive care units (ICUs) of Taleghani, Shahid Beheshti, and Valiasr Hospitals. Prior to disinfection, the average colony counts of pathogenic bacteria in the ICU areas were 317.35, 41719.16, 23818.44, and 63.17 for Lysoformin 3000, Epimax Surclean, Minuten spray, and Epimax quick, respectively (Table 1). After disinfection, the colony counts decreased significantly to 9.08, 2.14, 3.02, and 0.98 for Lysoformin 3000, Epimax Surclean, Minuten spray, and Epimax quick, respectively (Table 1). These results indicated a statistically significant reduction in pathogens and pathogenic bacteria for all four disinfectants tested ($P<0.05$). A mean decrease from 305.42 ± 45.21 CFU/mL to 298.15 ± 38.76 CFU/mL ($P=0.47$) was observed in the negative control (sterile saline), demonstrating that there was little spontaneous decay during the disinfection period. The decreases in bacterial load found for all disinfectants were caused by their antimicrobial action rather than natural degradation, as the negative control (sterile saline) did not exhibit any statistically significant reduction in bacterial load ($P>0.05$, Table 1).

The most prevalent Gram-negative bacteria identified in the culture results were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*, while the most common Gram-positive bacteria were *Staphylococcus aureus* and *Enterococcus* (Table 2).

The average number of pathogens isolated from different surfaces in the ICUs of the three hospitals before and after disinfection is provided in Table 3. The results revealed differences in surface efficacy (e.g., phones vs. beds), and possible explanations were offered pertaining to material and use.

Table 1. Mean Colony Counts of Isolated Pathogens Bacteria Before and After Disinfection

Disinfectants	Before Disinfection (Mean ± SD)	After Disinfection (Mean ± SD)	PV
Lysoformin 3000	317.35 ± 38.43	9.08 ± 0.63	0.001
Epimax Surclean	41719.16 ± 12359.01	2.14 ± 0.77	0.001
Minuten spray	23818.44 ± 5071.35	3.02 ± 0.32	0.001
Epimax quick	63.17 ± 11.32	0.98 ± 0.12	0.001
Negative control	305.42 ± 45.21	298.15 ± 38.76	0.47

Note. ICU: Intensive care unit; SD: Standard deviation.

Discussion

The transmission of infectious diseases in healthcare settings is a critical concern, mainly through the transmission of airborne pathogens and direct and indirect contact (23). Considering that pathogens can remain on surfaces for a long time, their elimination can be extremely difficult (24). In particular, hospital surfaces are recognized as potential sources of infection due to the presence of vulnerable patient populations and high numbers of infected patients (25). Surface contact can facilitate the transfer of pathogens among patients, their relatives, and healthcare personnel. This transmission not only contributes to antibiotic resistance in pathogens but can also lead to serious infections and even death in patients. Hospital surfaces can be divided into accessible and inaccessible groups. Accessible surfaces, which have the most contact with patients and caregivers, include items such as refrigerator handles, cabinets, dining tables, service lights, beds, room door handles, chair arms, telephones, keyboards, computer mice, and the like (24). Inaccessible surfaces encompass items such as windows, doors, ceilings, lamps, and some wall surfaces. Despite the use of convenience sampling, the study's emphasis on high-contact surfaces, which are generally acknowledged to be important disease reservoirs, guarantees its applicability to actual infection control procedures. Studies have shown that surfaces that are closer and more accessible to patients tend to harbor higher levels of pathogens. The presence of high levels of pathogens around patients, the spread of antibiotic-resistant pathogens, and the resulting infections underscore the importance of effective surface decontamination and disinfection. There is a need for continued research to explore new and improved methods to enhance surface disinfection in healthcare settings (26). Lysoformin 3000 produced a ~2.5-log reduction (317.35 to 9.08 CFU/mL), indicating opportunities for procedure optimization. The Centers for Disease Control and Prevention recommend a ≥ 3-log reduction for high-touch surfaces. Real-world factors, such as insufficient surface covering or interference from organic loads, could be the cause of discrepancies (27, 28). The prevalence of HAIs in Iran was 6.4%, with the highest rates in Sanandaj (15.6%), Qom (8.0%), Tehran (7.1%), and Torbat Heydarieh (7.0%), but the lowest in Urmia (4.0%) (1).

Considerable efforts have been made to study disinfectants and their effectiveness. The results of these studies revealed that despite the diligent work of hospital cleaning and disinfection teams and the implementation of best hygiene practices, microbial loads in patient care environments often exceed safe levels, posing a serious risk to patients and medical staff. In some cases, even after repeated disinfection attempts, pathogen concentrations do not reach zero (29). The importance of choosing the right disinfectants and materials for effective infection control cannot be overstated (30). Testing the efficacy of disinfectant solutions used in healthcare settings is one approach to ensuring proper decontamination. It is important to note that the results of these tests may differ from laboratory conditions because real-world environments have their own variables, including potential human errors in the decontamination process (31). Therefore, the selection of appropriate decontaminants in healthcare settings is critical, prompting researchers to investigate the efficacy of various disinfectants.

High humidity levels in ICUs (common in tropical climates such as Abadan) can increase the wet contact time of disinfectants, potentially increasing efficacy. However, excessive humidity can also dilute disinfectants or promote biofilm formation (26). Comparing porous surfaces (e.g., chair handles) to nonporous materials (e.g., stainless steel), the former may retain infections and decrease disinfection penetration. Schmidt et al (29) found reduced efficacy on textured surfaces, which is in line with our results. Slower bacterial decrease was observed on high-touch surfaces (e.g., telephones and doorknobs),

Table 2. Pathogens Isolated From Various Surfaces in the Intensive Care Unit

Hospital	Beheshti Hospital	Taleghani Hospital	Valiasr Hospital
Pathogen Category	**Gram-Negative**	**Gram-Positive**	**Gram-Negative**
Pseudomonas aeruginosa	*S. aureus*	*P. aeruginosa*	*Staphylococcus aureus*
-	-	*Acinetobacter baumannii*	-
-	Candida	-	*P. aeruginosa*
Enterobacter aerogenes	-	*Klebsiella pneumonia*	-
Enterococcus faecalis	-	*Escherichia coli*	-
Proteus mirabilis	-	*Proteus mirabilis*	-
K. pneumoniae	-	*Enterococcus faecalis*	-

Table 3. Mean Total Pathogen Contamination Before and After Disinfection in Different Areas of the ICU

Hospital	Location	Lysoformin 3000 (Mean ± SD)	Epimax Surclean (Mean ± SD)	Minuten Spray (Mean ± SD)	Epimax Quick (Mean ± SD)
Beheshti hospital	Patient room	3.21 ± 0.19	0.13 ± 1.08	0.00 ± 0.00	0.03 ± 0.00
-	Nursing station	0.6 ± 2.36	0.5 ± 0.13	1.10 ± 0.65	0.01 ± 0.00
Taleghani hospital	Patient room	0.9 ± 1.47	0.9 ± 0.04	1.04 ± 0.68	0.23 ± 0.03
-	Nursing station	1.00 ± 0.05	0.19 ± 2.18	0.23 ± 4.16	0.00 ± 0.00
Valiasr hospital	Patient room	0.00 ± 0.00	0.05 ± 0.4	0.63 ± 1.56	1.32 ± 0.89
-	Nursing station	4.36 ± 0.19	1.15 ± 2.36	0.14 ± 1.57	0.35 ± 0.03
Total	-	9.08 ± 0.63	2.14 ± 0.77	3.02 ± 0.32	0.98 ± 0.12
P value	-	0.03*	0.04*	0.14	0.01*

Note. ICU: Intensive care unit; SD: Standard deviation.
*Statistically significant

most likely as a result of quick recontamination in between cleanings. This emphasizes the necessity of more regular cleaning cycles, suggested by the Centers for Disease Control and Prevention, in high-traffic locations (32). The investigation was conducted in ICUs in Abadan and Khorramshahr, where typical humidity levels (60–80%) and temperatures (25–35˚C) may encourage microbial resistance or enhance the evaporative loss of disinfectants.

Environmental elements, including mechanical removal during swabbing or spontaneous bacterial decomposition, had little impact, as observed by a slight decrease in the negative control (sterile saline). This demonstrates that the antibacterial qualities of all disinfectants were responsible for the notable drops in bacterial counts ($P < 0.05$). Despite the studied disinfectants' notable effectiveness, incorrect or continuous usage may favor resistant microorganisms. For instance, it is known that *P. aeruginosa* and *S. aureus* can become resistant to specific disinfectants by means of processes like biofilm formation or efflux pump activation (33,34). Several previous studies have evaluated the efficacy of disinfectants against healthcare-associated pathogens. Their results underscore the importance of selecting effective disinfectants that are tailored to specific circumstances and pathogens present (17,35-42). In this study, the most prevalent Gram-negative bacteria in the sample cultures were *P. aeruginosa*, *Klebsiella*, and *E. coli*, while *S. aureus* predominated among the Gram-positive bacteria. Bacterial counts before and after each of the four disinfectants examined in the study differed significantly. The 98.5% decrease observed with Epimax Quick aligns with the findings of Schmidt et al, demonstrating the 99% effectiveness of quaternary ammonium compounds against *Enterococcus* (29). According to Amodio et al, the lesser reduction for Lysoformin 3000 (97%) in comparison to hydrogen peroxide-based treatments (99.9%) indicated limits particular to the formulation (38). All four disinfectants were effective against the pathogens present on the surfaces. This emphasizes the importance of testing disinfectant solutions as a critical component of monitoring decontamination and controlling HAIs. While concentrations recommended for commercial disinfectants are valuable under controlled conditions, real-world conditions are often unpredictable. This study was conducted in ICUs in Abadan and Khorramshahr, where average temperatures (25–35 ˚C) and humidity (60–80%) can increase evaporative loss of disinfectants or promote microbial resistance. High humidity, for instance, may lengthen the wet contact time but also dilute treatments that are based on alcohol. Despite the lack of systematic documentation, the possible impact of environmental circumstances highlights the necessity of climate-controlled validation in subsequent research.

It is well known that the detected pathogens, such as *P. aeruginosa* and *S. aureus*, can become resistant to antibiotics and disinfectants by means of enzymatic inactivation or altered membrane permeability (43,44). For example, *Enterococcus* species may be able to tolerate quaternary ammonium compounds, and *P. aeruginosa* biofilms may decrease disinfectant penetration. Further testing is necessary to prove efficiency against disinfectant-resistant strains and multidrug-resistant organisms, even though our investigation showed considerable reductions in colony counts. This is in line with the World Health Organization guidelines, indicating that medicines with demonstrated efficacy against high-risk multidrug-resistant organisms in ICUs should be given priority.

Conclusion

Therefore, it is essential to emphasize the selection of appropriate disinfectants, correct and effective methods of use, accurate identification of infection reservoirs, and continuous and thorough infection control training for healthcare workers. To improve generalizability, future research should use stratified sampling or randomized controlled trials. These measures should be prioritized to improve infection control in healthcare settings.

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Authors' Contribution

Conceptualization: Somayeh Rahimi.
Investigation: Sara Kooti and Hossein Moradikazerouni.
Project administration: Sara Kooti, Hossein Moradikazerouni, Seyed Mohammad Ali Malaekeh, Mostafa Alijanzadeh, and Somayeh Rahimi.
Software analysis: Hossein Moradikazerouni and Somayeh Rahimi.
Data analysis: Hossein Moradikazerouni, Seyed Mohammad Ali Malaekeh, Mostafa Alijanzadeh, and Somayeh Rahimi.
Writing–original draft: Sara Kooti and Hossein Moradikazerouni.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Ethics Committee of Abadan University of Medical Sciences (ID: IR. ABADANUMS.REC.1395.150) and was financially supported by this university.

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