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Biocides Susceptibility of Clinically Isolated *Escherichia coli* in Presence and Without Organic Material

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ABSTRACT

Background and Aim: Hospital-acquired infections are considered a major health concern worldwide because of the increasing morbidity and mortality rate. Hence, selecting the most efficient disinfectants in a clinic is crucial. This study evaluated the antibacterial efficacy of seven mostly used disinfectants against clinically isolated *Escherichia coli*.

Materials and Methods: For this purpose, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of Aniosyme DD1 0.5%, Steranios 2%, Aniospray 29 and Surfanios 0.5% at Hazrat Valiasr hospital and PROSEPT® Floor 0.75%, PROSEPT® Instru 0.05% and PROSEPT® Med used at Ayatollah Mousavi hospital were determined both in the presence and absence of bovine serum albumin (BSA) using microbroth dilution assay.

Results: The results indicate that PROSEPT® Med had the lowest MIC and MBC values, followed by Aniospray 29 and PROSEPT® Instro, and the presence of BSA reduced antibacterial activities of disinfectants.

Conclusion: The disinfectants applied at Ayatollah Mousavi hospital generally had higher antibacterial activities. Due to the importance of nosocomial pathogens at healthcare centers, selecting the most potent, fast-acting, and efficient disinfectants to prevent hospital-acquired infections is essential.

Keywords: Disinfectants, *Escherichia coli*, Hospital-acquired infections, Minimum bactericidal concentrations, Minimum inhibitory concentrations

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1. Introduction

In recent years, hospital-acquired infections (HAIs) have become a global health concern that can lead to higher morbidity and mortality rates, length and costs of hospitalization, and greater use of antibiotics **(1)**. Healthcare-associated infections result in almost 7.1 million annual cases and 99,000 annual deaths in the United States **(2)**. HAIs develop two to three days after patient admission. The symptoms of the related infection should not be observed in the patient at the time of hospitalization, and the relevant disease should not be in the incubation period **(3, 4)**. If nosocomial pathogens are found at a certain body site of an asymptomatic patient, including blood or cerebrospinal fluid, the person might be considered infected. Similarly, healthcare staff may acquire HAIs **(5, 6)**. Almost all microorganisms can cause infections in hospitalized patients; however, very few organisms are considered nosocomial pathogens **(7, 8)**.

Compared with bacteria involved in almost 90% of HAIs, fungi, viruses, mycobacterium, and protozoans are less responsible for nosocomial infections **(9, 10)**. The microorganisms frequently contributing to HAIs include *Staphylococcus aureus*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Bacillus cereus*, *Streptococcus* spp., enterococci, *Legionella*, coagulase-negative staphylococci and members of the

Enterobacteriaceae family. Based on the data, *Escherichia coli*, *P. aeruginosa*, *S. aureus* and enterococci play an important role **(9)**. As an arising nosocomial bacteria, *E. coli* is capable of causing severe health problems. It is oxidase negative, a facultatively anaerobic, rod-shaped gram-negative organism that is generally found in the gut of healthy individuals and other animals. A wide range of diseases, including urinary tract infections, pneumonia, neonatal meningitis, gastroenteritis, and septicemia, are caused by this microorganism **(11)**. Due to certain virulence factors such as capsule, sequestration of growth factors, endotoxins, antigenic phase variation, and antimicrobial-resistant some strains of *E. coli* can cause infection **(12)**. Of major concern is the rise of multidrug-resistant strains of *E. coli* which have been isolated from the environment, animals, and hospitalized patients worldwide. In particular, the emergence of fluoroquinolones resistant, Carbapenemases, and extended-spectrum β-lactamases producing strains has challenged the infection treatment **(13)**. Thus, the infections caused by this antimicrobial-resistant microorganism are difficult to treat, as the proper antibiotic selection is limited **(14)**. Hospital environments and surfaces naming ceilings, floors, windows, walls, doors, and medical equipment can transmit nosocomial pathogens to patients **(15)**. Hence, decontaminating, including sterilization as well as applying disinfectants, is crucial to reduce the microbial spread and cross-infection risk. Common disinfectants include glutaraldehyde, sodium hypochlorite, phenolic compounds, iodophors, 70% ethyl alcohol and 92% isopropyl alcohol, peracetic acid/ hydrogen peroxide (0.5 to 2%) in addition to sodium hypochlorite (1%), quaternary ammonium compounds and a chlorhexidine **(16)**. However, selecting the right disinfectant is challenging since a broad range of products is available on the market. In addition, various nosocomial pathogens show different susceptibility patterns to disinfectants **(17)**. It has also been shown that these microorganisms have a high resistance to the lethal effects of disinfectants and can grow inside some of them, which is the cause of infection transmission and cross-infection in medical centers **(18)**. Therefore, it is essential to identify microorganisms and evaluate microbial sensitivity to the available disinfectants by various techniques. The aim of the present study was to determine susceptibility of isolated *E. coli* strains from two university hospitals in Zanjan, Ayatollah Mousavi and Hazrat Valiasr hospitals, to seven widely used disinfectants which are routinely applied for disinfection of surfaces, floors and facilities both in presence and without organic substances.

2. Materials and Methods

Bacterial Isolation and Identification

One hundred clinical strains of *E. coli* were randomly selected and isolated from hospitalized patients and outpatients at Ayatollah Mousavi and Hazrat Valiasr hospitals of Zanjan city of Iran from June 2019 to November 2020. The isolated *E. coli* were further identified on selective media of Eosin Methylene Blue agar **(19)**.

Disinfectants Selection

Aniosyme DD1 0.5%, Steranios 2%, Aniospray 29 and Surfanios 0.5% at Valiasr hospital and PROSEPT® Floor 0.75%, PROSEPT® Instru 0.05% and PROSEPT® Med at Ayatollah Mousavi hospital were used as selected disinfectants to test the *in vitro* susceptibility of *E. coli* isolates. Table 1 shows the compositions and final concentrations according to the manufacturer's literature.

Table 1. Composition, application and preparation of disinfectants used at Hazrat Valiasr and Ayatolah Musavi hospital

Disinfectant	Composition in 100 g	Applications	Preparation
Aniosyme DD1	- Quaternary ammonium propionate - Polyhexamethylene biguanide hydrochloride - Enzymatic complex - Surface-active agents - Stabilising agents - Sequestrating agents	- Reinforced pre-disinfection and cleaning of instruments - Cleaning in ultrasonic trays - Collection of contaminated instruments	0.5% (Pour a 25 ml dose in 5 liters of cold or tepid water)
Steranios 2%	- 2% glutaraldehyde, buffered at pH 6 in the presence of surface effects catalysor - STERANIOS 2% NG and STERANIOS 2% ECS contain two compounds limiting glutaraldehyde evaporation, when associated	- Medical devices - Surgical, medical, endoscopic - Heat-sensitive equipment	Ready-to-use
Aniospray 29	- Hydroalcoholic solution (ethanol 55%) - Quaternary ammonium propionate	- Previously cleaned, non-immersible medical devices resistant to alcohol	Ready-to-use

Disinfectant	Composition in 100 g	Applications	Preparation
	- Fragrance	(stethoscopes, cables and connectors, pressure sensors, blood sugar testers,…)	
Surfanios	- N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine - Didecyldimethylammonium chloride - Excipients	- Floors - Walls - Medical equipment and non-invasive medical devices	0.0025 (20ml for 8L)
PROSEPT® Floor	- 10 g quaternary ammonium compounds - Perfumes - Dyes	- Large surfaces of medical devices	0.75%
PROSEPT® Instru	- 9.9 g of alkylamine - 3.6 g of dialkyldimethylammonium chloride - Cleaning booster - Auxiliaries	- Mirrors - Polishers - Instruments - Silicone parts - Plastic spatulas - Acrylic glass slabs	0.5 %
PROSEPT® Med	- 44.9 g isopropanol - 30.0 g 1-propanol - 0.15 g dialkyldimethylammonium chloride - Water - Natural moisturizing factors - Perfume	Surgical and hygienic hand and forearm	Ready-to-use

Disinfectants Susceptibility Assay

The disinfectant susceptibility of *E. coli* was determined using a slightly modified microdilution method suggested by NCCLS (National Committee for Clinical Laboratory Standards) guidelines **(20)**. To perform the technique, ninety-six wells of a microtiter plate were filled with 100 µL freshly prepared Mueller Hinton Broth (MHB). Next, the columns were filled with 100 µL 0.5 McFarland adjusted microbial suspension and 100 µL disinfectant dilution sequentially from the lowest to the highest dilution of the biocide agent. The last two columns were used as growth control (100 µL MHB + 100 µL bacterial suspension) and sterile control (200 µL MHB), respectively. The microtiter plate was incubated at 37°C for 24h and visually examined for any turbidity. The lowest concentration of the disinfectant that inhibited bacterial growth, causing no turbidity, is considered a minimum inhibitory concentration (MIC). 10 µL suspension from clear wells was sub-cultured on the surface of Mueller Hinton Agar (MHA) and incubated at 37°C for 24h to determine the minimum bactericidal concentration (MBC). The efficacy of disinfectants can be influenced by the presence of organic materials. Disinfectants may be inactivated or blocked from attaching microbial membrane receptors by interfering with organic materials **(21-23)**. An actual dirty situation was mimicked by adding 0.5% w/v of bovine serum albumin (BSA) into intended wells, determining MIC and MBC to examine the influence of proteins on the disinfection process. The tests were performed in triplicate **(24)**.

3. Results

MIC, MICal, MBC, and MBCal Values of Hospital Disinfectants

One hundred *E. coli* samples were isolated from Hazrate Valiasr and Ayatollah Mousavi university hospitals, identified by inoculation onto EMB agar based on microbial laboratory methods. MIC and MBC (without bovine serum albumin), MICal and MBCal (with bovine serum albumin) of seven commonly used hospital biocides against clinical isolates of *E. coli* were evaluated.

The obtained values are shown in Table 2. PROSEPT® Med and Aniospray 29 showed the lowest mean MIC values at 0.263×10^{-2} and 0.504×10^{-2} v/v %, respectively. According to Table 2, bacteriostatic activities of other biocides are classified as follows; PROSEPT® Instru> Aniosyme DD1> PROSEPT® Floor> Surfanios> Steranios. Furthermore, antibacterial properties were studied under dirty conditions. In the presence of BSA, higher MIC values of Aniosyme DD1, Surfanios, Steranios 2%, Aniospray 29, PROSEPT® Floor, and PROSEPT® Med were obtained, representing a reduction in the bacteriostatic activities. The MICal values of PROSEPT® Instru did not exhibit any difference; while, that of Steranios 2% had experienced about a four-fold increase (from 13.637×10^{-2} to 61.75×10^{-2} v/v %). As shown in Table 2, PROSEPT® Med and Aniospray 29 had the highest bactericidal activities with MBC values at 0.419×10^{-2} and 0.728×10^{-2} v/v %, respectively. The bactericidal potency of the remaining biocides is as follows; PROSEPT® Instru> Aniosyme DD1> PROSEPT® Floor> Surfanios> Steranios. To simulate the so-called 'dirty' situations, MBCal values were also measured. In the presence of BSA, higher MBC values of Surfanios, Steranios 2%, and PROSEPT® Instru were obtained. Antibacterial efficiency of Aniosyme DD1, Aniospray 29, and PROSEPT® Floor did not alter in the stimulated dirty condition, and MBCal values of PROSEPT® Med were reduced (Table 2).

Table 2. Median and mean MIC, MBC, MICal and MBCal values (v/v %) of selected disinfectants against *E. coli* isolates, the data shown are multiplied in 10^2

	MIC		MBC		MICal		MBCal	
Disinfectant	**Median**	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	**Mean ± SD**
Aniosyme DD1	0.78	1.04 ± 0.22	1.5	1.56 ±0.52	1.5	1.64 ± 0.51	1.5	1.469 ± 0.523
Surfanios	1.5	1.283 ± 0.54	1.5	2.03 ± 0.49	1.5	1.8 ± 0.50	1.5	2.478 ± 0.48
Steranios 2%	12	13.637 ± 3.9	12	15.67 ± 1.05	12	61.75 ± 0.44	12	20.7 ± 3.425
Aniospray 29	0.39	0.504 ± 0.28	0.39	0.73 ± 0.19	0.78	0.655 ± 0.12	0.78	0.727 ± 0.258
PROSEPT® Floor	1.1	1.27 ± 0.56	1.1	1.74 ± 0.51	1.1	1.595 ± 0.52	1.1	1.635 ± 0.518
PROSEPT® Instru	0.78	0.72 ± 0.14	0.78	1.08 ± 0.26	0.39	0.696 ± 0.27	0.78	1.064 ± 0.573
PROSEPT® Med	0.19	0.263 ± 0.129	0.39	0.419 ± 0.295	0.39	0.435 ± 0.05	0.19	0.226 ± 0.027

Minimum Inhibitory Concentration (MIC)
Minimum Bactericidal Concentration (MBC)
Minimum Inhibitory Concentration in the presence of bovine serum albumin (MICal)
Minimum Bactericidal Concentration in the presence of bovine serum albumin (MBCal)

MICs and MICals distribution of disinfectants are given in Tables 3 and 4. PROSEPT® Instru and PROSEPT® Med had the lowest MIC values, inhibiting 4 and 1 isolates in order, while other disinfectants did not show antibacterial effects at this concentration (0.048 × 10^{-2} v/v %). Interestingly, Steranios 2% demonstrated the highest MIC value at 50 × 10^{-2} v/v %. By comparing these tables, it can be deduced that the presence of BSA generally decreased the antibacterial activities of the biocides.

Also, distributions of MBCs and MBCals are presented in Tables 3 and 4. As shown in Table 3, the lowest MBC values belong to PROSEPT® Instru and PROSEPT® Med, which killed 8 and 5 isolates respectively at 0.097 × 10^{-2} v/v %, showing their potent bactericidal activities. This is while Steranios 2% tended to have the highest MBC amount (50 × 10^{-2} v/v %). As tables 2 illustrated, adding BSA to the wells raised the median MBCs of Aniospray 29 (from 0.39 × 10^{-2} to 0.78 × 10^{-2} v/v %); whilst, that of PROSEPT® Med decreased from 0.39 × 10^{-2} to 0.19 × 10^{-2} v/v %. The median MBCs of the other biocides didn't change; however, a decrease in their bactericidal properties is evident (Tables 3 and 4).

Table 3. Distribution of MIC (%), MBC (%) and MBCal (%) of various disinfectants by microtiter method

disinfectant	Number of strains at each MIC (%) of disinfectant											
	0.5×10^{-1}	2.5×10^{-1}	1.2×10^{-1}	6.25×10^{-2}	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}
AnDD1	0	0	0	0	6	27	48	19	0	0	0	0
Surf.	0	0	0	1	7	43	43	6	0	0	0	0
Stera.	1	11	85	3	0	0	0	0	0	0	0	0
Anspy	0	0	0	0	3	4	15	43	35	0	0	0
Pinstro	0	0	0	0	0	26	27	24	8	11	4	0
Pmed	0	0	0	0	0	1	6	22	51	19	1	0

Continuation of Table 3 from the previous page. Distribution of MIC (%), MBC (%) and MBCal (%) of various disinfectants by microtiter method

disinfectant	Number of strains at each MBC (%) of disinfectant											
	0.5×10^{-1}	2.5×10^{-1}	1.2×10^{-1}	6.25×10^{-2}	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}
AnDD1	0	0	0	2	18	40	32	8	0	0	0	0
Surf.	0	0	0	7	33	47	21	2	0	0	0	0
Stera.	4	17	78	1	0	0	0	0	0	0	0	0
Anspy	0	0	0	0	5	12	23	50	10	0	0	0
Pinstro	0	0	0	0	10	31	30	12	9	8	0	0
Pmed	0	0	0	0	0	5	18	31	41	5	0	0

Disinfectant	Number of strains at each MBC al (%) of disinfectant											
	0.5×10^{-1}	2.5×10^{-1}	1.2×10^{-1}	6.25×10^{-2}	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}
AnDD1	0	0	0	4	4	20	20	12	0	0	0	0
Surf.	0	0	0	8	12	44	32	4	0	0	0	0
Stera.	12	16	60	12	0	0	0	0	0	0	0	0
Anspy	0	0	0	0	0	12	43	31	8	4	0	0
Pinstro	0	0	0	0	4	12	32	24	24	4	0	0
Pmed	0	0	0	0	0	0	24	36		0	0	0

Aniosyme DD1 (AnDD1), Surfanios (Surf.), Steranios (Stera), Aniospray (Anspy), Procept instro (Pinstro), Procept med (Pmed) Procept floor (Pfloor)

Table 4. Distribution of MIC (%), MBC (%) and MBCal (%) of Procept floor by microtiter method

disinfectant	Number of strains at each MIC (%) of disinfectant									
	8.1×10^{-1}	3.9×10^{-2}	6.4×10^{-2}	3.2×10^{-2}	1.1×10^{-2}	8.5×10^{-3}	9.2×10^{-3}	4.1×10^{-3}	3.7×10^{-4}	6.3×10^{-1}
Pfloor	0	0	3	5	46	44	1	1	0	0
disinfectant	**Number of strains at each MBC (%) of disinfectant**									
	8.1×10^{-1}	3.9×10^{-2}	6.4×10^{-2}	3.2×10^{-2}	1.1×10^{-2}	8.5×10^{-3}	9.2×10^{-3}	4.1×10^{-3}	3.7×10^{-4}	6.3×10^{-1}
Pfloor	0	0	7	15	53	23	2	0	0	0
disinfectant	Number of strains at each MBC al (%) of disinfectant									
	8.1×10^{-1}	3.9×10^{-2}	6.4×10^{-2}	3.2×10^{-2}	1.1×10^{-2}	8.5×10^{-3}	9.2×10^{-3}	4.1×10^{-3}	3.7×10^{-4}	6.3×10^{-1}
Pfloor	0	0	4	16	51	29	0	0	0	0

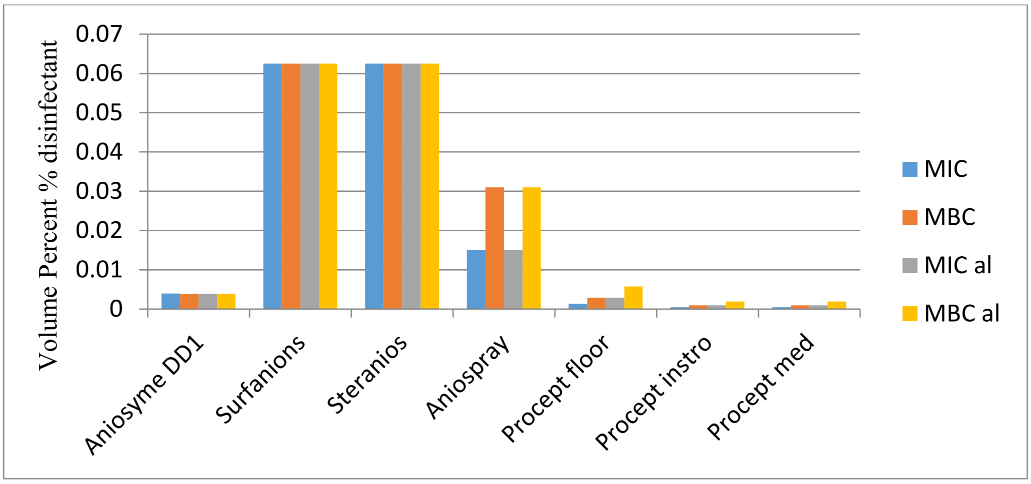

Figure 1. The investigated criteria of MIC, MBC, MICal, MBCal for hospital disinfectants against E. coli isolates

Sample-hospital

	Sample-hospital	Source		Sample-hospital	Source
1	*E. coli* 1M	catheter	**48**	*E. coli* 13V	wound
2	*E. coli* 2M	bedsore	**49**	*E. coli* 12V	bedsore
3	*E. coli* 3M	blood	**50**	*E. coli* 13V	UTI
4	*E. coli* 4M	catheter	**51**	*E. coli* 14V	catheter
5	*E. coli* 5M	catheter	**52**	*E. coli* 15V	catheter
6	*E. coli* 6M	sepsis	**53**	*E. coli* 16V	catheter
7	*E. coli* 7M	catheter	**54**	*E. coli* 17V	wound
8	*E. coli* 8M	UTI	**55**	*E. coli* 18V	UTI
9	*E. coli* 9M	UTI	**56**	*E. coli* 19V	bedsore
10	*E. coli* 10M	UTI	**57**	*E. coli* 20V	UTI
11	*E. coli* 11M	UTI	**58**	*E. coli* 21V	UTI
12	*E. coli* 12M	UTI	**59**	*E. coli* 22V	UTI
13	*E. coli* 13M	blood	**60**	*E. coli* 23V	UTI
14	*E. coli* 14M	UTI	**61**	*E. coli* 24V	UTI
15	*E. coli* 15M	sepsis	**62**	*E. coli* 25V	sepsis
16	*E. coli* 16M	-	**63**	*E. coli* 26V	UTI
17	*E. coli* 17M	bedsore	**64**	*E. coli* 27V	catheter
18	*E. coli* 18M	bedsore	**65**	*E. coli* 28V	catheter
19	*E. coli* 19M	UTI	**66**	*E. coli* 29V	catheter
20	*E. coli* 20M	UTI	**67**	*E. coli* 30V	catheter
21	*E. coli* 21M	UTI	**68**	*E. coli* 31V	catheter
22	*E. coli* 22M	UTI	**69**	*E. coli* 32V	catheter
23	*E. coli* 23M	UTI	**70**	*E. coli* 33V	catheter
24	*E. coli* 24M	catheter	**71**	*E. coli* 34V	catheter
25	*E. coli* 25M	catheter	**72**	*E. coli* 35V	wound
26	*E. coli* 26M	catheter	**73**	*E. coli* 36V	UTI
27	*E. coli* 27M	catheter	**74**	*E. coli* 37V	UTI
28	*E. coli* 28M	catheter	**75**	*E. coli* 38V	wound
29	*E. coli* 29M	UTI	**76**	*E. coli* 39V	wound
30	*E. coli* 30M	-	**77**	*E. coli* 40V	meningitis
31	*E. coli* 31M	bedsore	**78**	*E. coli* 41V	sepsis
32	*E. coli* 32M	UTI	**79**	*E. coli* 42V	UTI
33	*E. coli* 33M	-	**80**	*E. coli* 43V	bedsore
34	*E. coli* 34M	catheter	**81**	*E. coli* 44V	catheter
35	*E. coli* 35M	catheter	**82**	*E. coli* 45V	-
36	*E. coli* 1V	meningitis	**83**	*E. coli* 46V	bedsore
37	*E. coli* 2V	-	**84**	*E. coli* 47V	UTI
38	*E. coli* 3V	bedsore	**85**	*E. coli* 48V	UTI
39	*E. coli* 4V	sepsis	**86**	*E. coli* 49V	catheter
40	*E. coli* 5V	UTI	**87**	*E. coli* 50V	catheter
41	*E. coli* 6V	UTI	**88**	*E. coli* 51V	catheter
42	*E. coli* 7V	UTI	**89**	*E. coli* 52V	catheter
43	*E. coli* 8V	bedsore	**90**	*E. coli* 53V	UTI
44	*E. coli* 9V	UTI	**91**	*E. coli* 54V	sepsis
45	*E. coli* 10V	UTI	**92**	*E. coli* 55V	UTI
46	*E. coli* 11V	blood	**93**	*E. coli* 56V	UTI
47	*E. coli* 12V	UTI			

4. Discussion

Various factors such as the biological fluids of patients and contaminated appliances can cause contamination of hospital environments, increasing the prevalence of HAIs. Thus, it is essential to select not only the most potent but also efficient disinfectants to control and prevent the spreading of nosocomial infections **(25-27)**. This study aimed to investigate the antimicrobial efficacy of seven selected disinfectants used at Hazrat Valiasr and Ayatollah Mousavi hospitals against 100 isolates of *E. coli* according to MIC, MBC, MICal, and MBCal values. The obtained data revealed that selected disinfectants might have a different range of antimicrobial activities and different effects against isolated microorganisms. Hence, it is crucial to evaluate the MIC and MBC values of applied disinfectants to determine their effectiveness against nosocomial pathogens.

Based on our findings, PROSEPT® Med had the lowest values of MIC and MBC, followed by Aniospray 29 and PROSEPT® Instru in order. This is while those of Steranios 2% were the highest values with the widest range. In contradiction to our findings, Nabizadeh reported the lowest MIC and MBC values of Steranios 2%, which were determined by the microdilution method. This is probably due to the different selection of disinfectants: Steranios 2%, Deconex HLDPA, and Microzed Quatenol, along with different bacterial cell structures of tested microorganisms (*Enterococcus faecalis* and *Burkholderia cepacia*) **(17)**. To the best of our knowledge, antimicrobial activities of PROSEPT® Med, Aniospray 29, and PROSEPT® Instru against *E. coli* were not studied; however, disinfectant susceptibility of *E. coli* was reported by some authors. Xia and coworkers studied the susceptibility of 510 collected *E. coli* isolates against cetyltrimethylammonium bromide, cetylpyridinium chloride, CHX, benzalkonium chloride, and triclosan. The MICs of the five disinfectants were determined using the agar dilution method, and CHX showed the lowest MIC values, while triclosan had the widest range of MICs **(28)**. In another similar research by Oosterik, susceptibility of a selection of 97 *E. coli* isolates to various antibiotics, and the five most applied disinfectants in the poultry industry were determined. MIC and MBC values of glyoxal, formaldehyde, hydrogen peroxide, glutaraldehyde, and a quaternary ammonium compound were reported as concentration ranges by the microbroth dilution assay. The results revealed that alkyldimethylbenzylammonium chloride had the lowest MIC and MBC values. In addition, phenotypic resistance to the disinfectants was not observed, while tested antibiotics were selected for resistance to *E. coli* **(29, 30)**. Oosterik's findings correlate well with our results, demonstrating that disinfectants containing quaternary ammonium compounds (PROSEPT® Instru, PROSEPT® Med, Aniospray 29, PROSEPT® Floor, and Surfanios) had greater antimicrobial effects against *E. coli* isolates **(31)**. The physical and ionic stability of bacterial membrane can be disrupted by quaternary ammonium compounds **(32)**.

Since organic materials are present in the bodies of the patient and hospital environments that inhabit nosocomial pathogens such as E. coli, their effects on the antimicrobial properties of biocides should be taken into account **(24)**. Thus, this study was extended to evaluate the reduction effects of BSA as an organic material on the antimicrobial efficiency of the disinfectants. With the exception of the MIC value of PROSEPT® Instru, which nearly stayed the same in the presence of BSA, those of Aniosyme DD1, Surfanios, Steranios 2%, Aniospray 29, PROSEPT® Floor, and PROSEPT® Med experienced an increase of 6.44%, 68.3%, 351.81%, 29.96%, 29.15%, and 65.39% respectively. Considering bactericidal effects of the disinfectants, BSA increased MBC amounts of Surfanios, Steranios 2%, and PROSEPT® Instru to 39.89%, 32.08%, and 19.81%, respectively. However, those of Aniosyme DD1, Aniospray 29, and PROSEPT® Floor did not show a significant alteration, and MBC of PROSEPT® Med reduced to 40.06%. These results agree with the study conducted by Vickery. The study investigated antibacterial activities of hydrogen peroxide (Oxivir and 6% H_2O_2 solution), peracetic acid (Surfex and Proxitane), and chlorine (Chlorclean and sodium dichloroisocyanurate tablets) against *S. aureus* dry-surface biofilm with and without organic soil (5% bovine calf serum (BCS) and 10% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) **(33)**. The findings showed that the presence of organic material highly affected bactericidal efficacies of Proxitane, Oxivir, Chlorclean, sodium dichloroisocyanurate tablets, and H_2O_2 solution, whereas those of Surfex did not change **(34)**.

5. Conclusion

This study was performed to determine the lethal concentrations of clinically used disinfectants. In conclusion, PROSEPT® Med was the most effective biocide against *E. coli* followed by Aniospray 29 and PROSEPT® Instru. Generally, the disinfectants used at Ayatollah Mousavi hospital showed higher antibacterial effects than those used at Hazrat Valiasr hospital. Furthermore, our findings suggest that the presence of organic materials, including exudation and blood, may reduce disinfectants' efficacy, increasing the survival rate of microbes. Thus, the susceptibility profile of nosocomial microbes against biocides should be monitored in the presence and absence of organic materials routinely in medical centers.

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Authors' Contribution

All authors participated in conducting the project and approval the final manuscript.

Ethical Approval

The study was approved by the ethics committees of the Zanjan University of Medical sciences. Ethical Code: ZUMS.REC.1394.280.

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Conflict of Interest

The authors have no conflict of interest to declare relevant to this article's content.

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