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Original Article

Antibacterial Activity of Germicide-P®: A Persulfate Based Detergent/Disinfectant on Some Hospital Isolates

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Abstract

A novel chemical detergent/disinfectant formulated based on persulfate salts, germicide P®, is available for disinfection of surfaces and medical devices at the room temperature. Because of the resistance of hospital-resident microorganisms, the antimicrobial activity of this compound is evaluated in the present study. The tests conducted with this disinfectant included bactericidal tests against 25 clinical isolates of gram negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* as well as 25 clinical isolates of gram positive bacteria of *Staphylococcus aureus* with and without 5% bovine serum albumin (BSA) as dirty condition. All of the experiments also performed using standard strains and serial dilution method applied to find minimum bactericidal concentration (MBC) of germicide P ® against the selected bacteria at different exposure times. Also, to determine the ability of germicide P® for disinfection of surfaces, standard surface tests were conducted using all of the clinical isolates as well as standard strains. According to the *in vitro* results of the tests, germicide P® found to be a promising product with the MBC of 1/10 after 5 min. against all of the tested bacteria at the room temperature, however, the results of the surface tests indicated that a significant reduction in the efficacy of germicide P® was observed and the MBC dropped to 1/5. In conclusion, surface antisepsis without intervention of organic materials in the concentration range of 1/5 was achieved with this new disinfectant.

Keywords: Antimicrobial activity; Disinfectant; Germicide P; Hospital bacteria; Persulfate compound.
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1. Introduction

The hospital environment serves as a reservoir for nosocomial pathogens and many nosocomial infections (NIs) are likely to result from inadequate use of disinfectant followed by patient-to-patient transmission [1]. NIs remain a major global concern. Overall, national prevalence rates of NIs have been described as ranging between 3.5 and 9.9% [2], but they vary significantly between

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departments, patient groups, types of surgical procedures, and the use of different medical devices, etc. [1, 3, 4]. The most common NIs are lower respiratory tract infections, urinary tract infections, surgical-site infections, and primary septicemia [1, 5-7]. They lead to additional days of hospital stay and treatment [8-10], increased risk of death [11], and treatment costs [1, 12, 15]. The overall financial burden incurred by NIs has been estimated to be remarkable, for example: $4.5 billion per year in the United States alone [1, 2]. Approximately one-third of all NIs are regarded as preventable by disinfection using sufficient disinfectant products [16]. High-level disinfection is a key measure in the control of hospital-acquired infections. Several products can serve this objective, and selection depends on efficiency, security and cost [2]. Germicide-P® is a newly formulated disinfectant product based on persulfate salts (Fazel Derakhshan Co., Iran). According to the company's preliminary investigations, Germicide-P® has shown to be an effective disinfectant in veterinary applications. The purpose of this study was to determine the *in vitro* germicidal activity of Germicide-P® against some hospital bacterial isolates.

2. Materials and methods

2.1. Bacterial strains

The following standard strains as well as clinical isolates were obtained from blood cultures or urine specimens from Sina Hospital of Tabriz, Iran: *Pseudomonas aeruginosa* (25 clinical isolates), *P. aeruginosa* (ATCC 9027), *Escherichia coli* (25 clinical isolates), *E. coli* (ATCC 10536), *Staphylococcus aureus* (25 clinical isolates) and *S. aureus* (ATCC 6538). All of the test organisms were identified using USP standard identification methods and preserved at -20 ° C until the assays were performed.

2.2. Disinfectant product

Germicide-P® (Fazel Derakhshan Pharmaceutical Co., Iran) supplied as stock product. The dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80 from Germicide-P® were prepared using sterile distilled water as diluents.

2.3. Antimicrobial evaluation tests

The antibacterial activity of Germicide-P® was monitored by standard suspension test in 5, 15, 30, 60 and 120 min. contact times using 0.5 McFarland concentration of each bacterial strain and minimum bactericidal concentrations (MBC) were determined at different exposure times and various dilutions [17, 18]. Also to determine the effect of organic materials on the disinfectant efficacy of Germicide-P®, the same disinfectant test was carried out in the presence of 5 % bovine serum albumin.

2.4. Surface test

Surface test was performed according to Association of Official Analytical Chemists (AOAC) use-dilution method [19]. Briefly, stainless steel ring carriers were inoculated by soaking them in a 48 h broth culture with 10^7 to 10^8 cfu/ml of each bacterial strain for 15 min., yielding 10^5 to 10^6 cfu/carrier. The carriers were removed with a hooked inoculating needle and allowed to dry for 40 min. at 36± 1 ºC in Petri dish matted with two filter paper sheets. After drying, the inoculated carriers were placed individually into the disinfectant and exposed for 15 min. The rings were removed carefully and placed into tubes containing 10 ml of neutralizing broth (Letheen Broth/DIFCO). After 20 min., each carrier was removed to other new tubes with the same culture broth, in order to assure the neutralizing process. All tubes were incubated at 36± 1 ºC for 48 h. Fifteen carriers were used for each experiment. *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 10536) and *S. aureus* (ATCC 6538) were included in the

Table 1. Mean percentage of test strains growth after various contact times with Germicide-P®.

Test strains (Clinical isolates)	Contact time (min.)	Mean bacterial growth in serial dilutions of Germicide-P® (%) 1:5	1:10	1:20	1:40
P. aeruginosa	5	0	0	100	100
	15	0	0	80	100
	30	0	0	75	100
	60	0	0	72	100
	120	0	0	71	100
S. aureus	5	0	0	81	100
	15	0	0	68	100
	30	0	0	64	100
	60	0	0	60	100
	120	0	0	60	100
E. coli	5	0	0	53	100
	15	0	0	43	100
	30	0	0	29	100
	60	0	0	27	100
	120	0	0	27	100

study as the reference strains. The performance standard of the method is: Only 1 positive/15 replicates of carriers is admitted to consider that the disinfectant is efficient to kill the test bacteria. Isolates showing two or more positive carriers were considered less susceptible to the disinfectant than the reference strain [18, 19].

3. Results

3.1. Minimum bactericidal concentration (MBC) of Germicide-P®

Table 1 shows the results of serial dilution test to determine MBC of Germicide-P® against the selected bacterial strains at different exposure times.

3.2. Activity of Germicide-P® in the surface test condition

The results of experiment to show the effectiveness of Germicide-P® to kill the selected bacterial isolates in the surface tests are presented in Table 2.

3.3. The influence of organic materials on disinfectant activity of Germicide-P®

To determine the effect of organic materials on the disinfectant efficacy of Germicide-P® against standard bacterial strains of *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 10536) and *S. aureus* (ATCC 6538), the disinfectant test in the presence of 5 % BSA was carried out and the results are summarized in Table 3.

4. Discussion

The main aim of the application of disinfectants in hospital environments is to reduce the risk of endemic and epidemic nosocomial infections in patients. A great number of disinfectants are used in the health care settings with various advantages and disadvantages [1]. Germicide-P®, a new disinfectant formulated based on persulfate salts, was evaluated in this study for its disinfectant effectiveness. The strains of *E. coli*, *S. aureus* and *P. aeruginosa* used in this study are the ones proposed in the European Standard UNE-EN 1276 [20] for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. According to the experiments to determine the contact time and MBC of germicide-P®, the 1:10 dilution of Germicide-P® in sterile water was effective after 5 min. contact time in the case of all isolates, whereas 1:20 dilution killed 47 and 19 % of *E. coli* and *S. aureus*

Table 2. The killing effect of Germicide-P® in the surface test condition.

Test strains	No. of clinical isolates	Susceptibility of bacterial strains in serial dilutions of Germicide-P® (%)		
		1:5	1:10	1:20
P. aeruginosa	25	76	71	66
S. aureus	25	98	94	79
E. coli	25	86	74	68

isolates, respectively, and failed to kill any of *P. aeruginosa* isolates after 5 min. Only 73, 40 and 29 % killing was achieved after 120 min for *E. coli, S. aureus* and *P. aeruginosa*, respectively, indicating the more susceptibility of *E. coli* and *S. aureus* to Germicide-P® compared to *P. aeruginosa.*

Gram-negative bacteria are generally less susceptible to biocides than Gram-positive species. Such resistance is likely to be intrinsic, due to outer membrane that acts as a protective barrier. Due to the capacity of surviving in unfavorable environmental conditions and to the high resistance to antibiotic agents, antiseptics and disinfectants, *P. aeruginosa* continues to be an important pathogen in hospital acquired infections, mainly respiratory and urinary infections. The transmission of this bacterium is almost always related to contamination of medical-surgical instruments and respiratory apparatus [19]. Vess and co-workers [21] demonstrated that *Pseudomonas* spp. survive during long periods on the surfaces of polyvinyl chloride (PVC) pipes, showing tolerance to the treatment with different disinfectants (synthetic phenols, QACs, formaldehyde and chlorine), and could become a potent reservoir of microbial contamination. Fernandé z-Astorga and collogues [22] have reported that the high resistance of *Pseudomonas* spp. to cationic agents seems to be associated with the chemical composition of the external membrane. Our study also demonstrated that *P. aeruginosa* strains were more resistant to the tested disinfectant.

Hospital isolates of gram-negative bacteria belonging to the *Enterobacteriaceae* family, such as *Klebsiella, Enterobacter, Serratia* and *Proteus,* have shown resistance to disinfectants, mainly quaternary ammonium compounds and phenols [19]. However, according to a study done by Guimaraes and coworkers [19] as well as Homand and colleagues [23] *E. coli* strains were more susceptible to disinfectants than other *Enterobacteriaceae* tested. The results of our study also were in good agreement with the previous findings and our *E. coli* strains showed more susceptibility to the Germicide-P®.

There are several reasons for testing hospital-acquired pathogens in the surface test; the most important one is that biocides are well known to be less effective on surfaces than in suspension although surface disinfection may well be the more clinically relevant process [19]. Based on the results of surface test of Germicide-P®, the antibacterial efficacy of Germicide-P® on the selected strains was remarkably modified in which 100% susceptibility of all tree bacteria in 1/5 dilution decreased to 76, 98 and 86% in the case of *P. aeruginosa* and *S. aureus* and *E. coli*, respectively. However, the activity of Germicide-P® was not significantly influenced by the added organic load, according to the figures pertaining to the additional organic load test and the differences between MBC of Germicide-P® with and without organic load are meaningless indicating that the mechanism of antibacterial activity of this disinfectant is not remarkably influenced by the presence of organic materials.

In conclusion, germicide P® found to be a promising product with the MBC of 1/5 after 5 min against all of the tested bacteria at the room temperature in *in vitro* condition and *E. coli* isolates were the most susceptible isolates and surface antisepsis without intervention of

Table 3. Mean percentage of test strains growth with and without BSA in the contact time of 15 min.

Test strains	Mean bacterial growth (%) at different Germicide-P® dilutions			
	1:5 / 1:5$_D$*	1:10 / 1:10$_D$	1:20 / 1:20$_D$	1:40 / 1:40$_D$
P. aeruginosa	0 / 0	0 / 0	80 / 76	100 / 100
S. aureus ATCC 6538	0 / 0	0 / 0	68 / 69	100 / 100
E. coli ATCC 10536	0 / 0	0 / 0	43 / 43	100 / 100

* D: Dirty conditions of 5 % bovine serum albumin.

organic materials in the same concentration range was achieved with this new disinfectant.

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