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Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination of Disinfectants in the Pharmaceutical Industry against Pathogens

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ABSTRACT

Background: Using disinfectants that inactivate microorganisms in various industries, from the pharmaceutical to the food service industry, has been increasing over the last few years. Antimicrobial disinfectants are used as primary treatment options against pathogens on surfaces in healthcare facilities to help prevent healthcare-associated infections. The aim of this study was to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the disinfectants used and study the corrosion of surfaces in contact with these disinfectants.

Methods: In this study, MICs and MBCs of the disinfectants against *B. cereus, S. cerevisiae, P. aeruginosa*, and *S. epidermidis* were measured by the microdilution method. Ethanol, percidine, benzalkonium chloride, and deconex 50ff were examined for antimicrobial effects.

Results: The findings showed that *S. cerevisiae* had the lowest level of disinfectant resistance, whereas *B. cereus* consistently exhibited higher resistance. Additionally, when compared to other disinfectants at lower concentrations, deconex 50ff was the most potent disinfectant that resulted in microbial growth suppression. Deconex 50 FF had MICs of 0.101, 12.939, 3.235, and 1.617mg/L for *S. cerevisiae, B. cereus, P. aeruginosa*, and *S. epidermidis*, respectively. In contrast, MBC of *S. cerevisiae, B. cereus, P. aeruginosa,* and *S. epidermidis* were 0.404, 25.879, 12.939, and 6.470 mg/L, respectively. Macroscopic analysis of stainless steel and plastic sandwiches treated with the specified disinfectant concentrations revealed no corrosion.

Conclusion: Considering the importance of these microbial strains in healthcare-associated infections, using these effective disinfectants is recommended in the pharmaceutical industry.

1. Introduction

Pharmaceutical companies apply clean, hygienic, and controlled conditions to create sterile drug products. Disinfectants must meet strict cleanliness standards, control microbial buildup, and be non-toxic to the pharmaceutical substances they manufacture [1]. Among the disinfectants most widely used in pharmaceutical companies are peracetic acid (PAA), benzalkonium chloride (BC), 60-80 % ethanol, and deconex 50ff. The antimicrobial activity of peracetic acid is also based on the oxidation of cell components [2]. Compounds derived from quaternary ammonium, like BC, are cationic surfactants that destroy the lipid bilayer membrane and have an antimicrobial effect on several types

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of microorganisms [3]. Quaternary ammonium salt-based Deconex 50ff antimicrobial agents are applied as sprays to sensitive surfaces of electronic devices, cameras, work tables, and dentistry units in healthcare facilities [4]. Inflammable disinfectants like ethanol alcohol can denature the proteins of microorganisms, disrupt cellular metabolism, impact the cytoplasm, and cause cell lysis, but they are less antiseptic than other disinfectants. For disinfectants to be effective, they must be used in appropriate doses. Application at sublethal concentrations is ineffective and can even be counterproductive. Low-dose biocides are associated with increased bacterial biofilm-forming capacity, resistance to these agents, and antibiotic resistance. For this reason, knowledge of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of disinfectants is important [2]. According to previous studies, ethyl alcohol concentrations between 60 and 90 % were the most efficient against healthcare infections, apart from spores [5]. The purpose of the current study was to compare the efficacy of different disinfectants against the pathogens *Saccharomyces cerevisiae*, a species of yeast, *Bacillus cereus*, a spore-forming gram-positive bacterium, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*, by determining the MIC and MBC of each disinfectant individually. *Pseudomonas aeruginosa* is a gram-negative, aerobic, rod-shaped bacterium that can infect both immunocompetent and immunocompromised hosts with various diseases [6]. *S. epidermidis* is Gram-positive, non-motile, facultatively anaerobic, and non-spore-forming cocci [7]. *B. cereus* is a gram-positive, aerobic, or facultatively anaerobic, pathogenic, spore-forming bacteria capable of creating oxygen-resistant endospores and whose spores may cling to the surface of stainless steel [8]. The findings of our investigation demonstrated that the microdilution technique effectively verified the effectiveness of the tested disinfectants. In addition, we discovered that every disinfectant we selected to validate our disinfectant technique was appropriate for surface disinfection since it decreased and controlled the spread of contamination on surfaces that were indicative of sensitive areas without causing corrosion. The aims of this study were: (1) to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the disinfectants used and (2) to study the corrosion of surfaces in contact with these disinfectants.

2. Materials and Methods

2.1 Materials

This experimental study was conducted in the Diagnostic Microbiology Lab of Goya innovative company, Karaj, Iran in 2022. Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), Sabouraud Dextrose Broth, Sabouraud Dextrose Agar and skim milk containing 15% sterile glycerol (all purchased from Merck, Germany) were used for cultivation of the bacteria, determining the MICs and MBCs, and storing the isolates. *Bacillus cereus* IBRC-M 10948, *Staphylococcus epidermidis* ATCC:3270, *Pseudomonas aeruginosa* ATCC:27853, *Saccharomyces cerevisiae* IBRC-M 30069, and *Bacillus cereus* IBRC-M 10948 were obtained from the Iran Pasteur Institute and the Iranian Biological Resource Center (IBRC) and stored in 20% skim milk with 10% glycerol at -80 °C. The strains that were used had not gone through more than five generations of propagation from the initial culture. The culture concentration was adjusted to 0.5 on the McFarland scale, and the number of bacteria present in the microbial suspension was approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL). This process was repeated for each strain. The tested chemical agents, benzalkonium chloride (BC), deconex 50 FF, and peracetic acid (PAA), were purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). Ethanol, purchased from Inoclon Company, Karaj, Iran.

2.2 Determination of MICs and MBCs of disinfectants

The microdilution method was utilized in order to ascertain the MICs and MBCs values of the disinfectants in relation to the microbiological strains. An aseptic solution of the BC compound was produced in sterile distilled water before the beginning of the experiment. To make different concentrations of disinfectants, we used 25 sterile test tubes and numbered them from one to twenty-five. After adding 1 ml of disinfectant and 1 ml of culture medium to the first tube, serial dilutions were carried out in the tubes. The tubes were incubated at 35 °C for 24 h. Following the period of incubation, a value known as the minimum inhibitory concentration (MIC) was calculated by finding the lowest concentration at which the growth of bacteria was stopped. Culture media with disinfectant alone served as the negative control, while culture media without disinfectant and inoculated with the microbial suspension was used as a positive control. Then, the test tubes were examined for their turbidity. In order to measure the MBCS of disinfectants, 100 µl of each tube, where no growth was observed after incubation time, was sub-cultured at 37 °C on the surface of TSA plate agars. For bacteria, the CFUs were counted after 24 h, and for *S. cerevisiae*, after 4-5 days. MBC was regarded as the lowest disinfectant concentration that stopped 99.9% (3-log) of bacteria from developing more than 10 CFU/mL on plate agar [9]. Given that the detection limit for this method is 10 CFU/mL, the lack of growth on TSA plates shows that the concentration is lower than this value. The studies were carried out in triplicate on different days.

2.3 Macroscopic check the corrosion of surfaces in contact with detergents

Due to the oxide layer that forms on the surface, stainless steels were created for use in both mild and severe corrosive environments. Stainless steels contain a range of impurities in the form of oxides, silicates, aluminates, and sulfides, such as 316 steel (UNS S31600). These are frequently found in mixed species, where the oxides, silicates, and aluminates are encased in sulfide shells. The cleaned surfaces were

initially brought into contact with the disinfectant for two days to test the corrosion of stainless steel and stained plastic sandwiches with dimensions of 1 × 1. After an incubation period, stained plastic sandwiches and steel corrosion were visually inspected using macroscopical techniques. This approach only treats surface defects and delivers a rough description of the condition of the structure and its deterioration [9].

3. Results and Discussion

A good cleaning and sanitization program is required to produce pharmaceutical products for controlled conditions utilized to prevent the microbiological contamination of these products [1]. On inanimate surfaces and objects, chemical disinfectants are used to eliminate contagious fungus, viruses, and bacteria, but not necessarily their spores. The tested disinfectants' MIC and MBC concentrations are shown in TABLE 1:

Table 1: Minimum inhibitory concentration (MIC, ppm) and minimum bactericidal concentration (MBC, ppm) for three disinfectants on four tested microbial strains.

Strain	Biocide		
	Deconex 50ff	BC	PAA
S.cerevisiea	0.101	0.031	0.005
	(0.404)	(0.061)	(0.009)
B.cereus	12.939	7.813	0.146
	(25.879)	(15.625)	(0.146)
P. aeroginosae	3.235	3.906	0.073
	(12.939)	(7.813)	(0.146)
S. epidermidis	1.617	0.488	0.009
	(6.470)	(1.953)	(0.009)

BC: benzalkonium chloride, PAA: peracetic acid. The values not in brackets correspond to the MIC, whilst bracketed values indicate the MBC.

3.1 Deconex 50 FF

Commercial deconex 50 FF is a blend of three different quaternary ammonium compounds based on alcohol. These substances are antimicrobials that are employed in healthcare and pharmacy facilities and are ready for use [10]. Ethanediol, pentanediol, and didecyldimethylammonium chloride (DDAC) are ingredients in the deconex 50 FF utilized in this investigation. DDAC is a quaternary ammonium compound that is a potent biocide with a rapid killing ability due to an extreme initial adhesion to cell walls. It induces internal molecular leakage, which results in cell death [11]. Based on MIC and MBC testing, the effectiveness of four commercial disinfectants was examined to assess four strains of bacteria and yeast. Truthful MBC values are typically higher than MIC, and larger doses of an antibacterial agent are typically required for bactericidal effects instead of bacteriostatic ones [10]. The *S. cerevisiae* strain had the lowest stability among the strains studied in this investigation. In contrast, the *B. cereus* strain had the highest resistance to this disinfectant due to its capacity for spore production. According to a study conducted in 2020 [12], the three-layer envelope of gram-negative bacteria is the primary cause of resistance to various antibiotics and disinfectants. Cleanroom disinfection is the primary responsibility in preventing *S. epidermidis* infection because *S. epidermidis* is a significant source of contamination of medical equipment and one of the most prevalent cleanroom isolates [13]. In a study, the effect of Deconex disinfectant against pathogens was examined. According to the study, Deconex was the most potent disinfection of the three disinfectants studied, with an MBC ranging from 4 to 32 mg/L, and *P. aeruginosa* was the most resistant bacterium and that Gram-negative bacteria were more resistant to disinfectants [14]. Deconex, Dettol, Nitrofurazone powder (2%), Silver sulfadiazine (1%), Povidone-iodine (10%), Peranacid M_1, and Sodium hypochlorite were all compared by Rahmati *et al* [15], in their 2020 study. Deconex had the highest mean diameter of inhibition zones (DIZ) among the used disinfectants, whereas nitrofurazone had the lowest.

3.2 Benzalkonium chloride (BC)

Four alkyl groups coupled to central quaternary nitrogen make up cationic biocides known as quaternary ammonium compounds. The antiseptic cationic surfactant, BC, is a quaternary ammonium compound that can break down cell proteins, lessen surface tension, inactivate enzymes, and damage the lipid bilayer membrane of microorganisms [16, 17]. BC demonstrated significant antibacterial activity in the current investigation. The results showed that *B. cereus* and *P. aeruginosa* strains were the most resistant to the other pathogens investigated, showing no turbidity at low disinfectant concentrations. It has been found that spore-forming foodborne bacteria have significant heat and chemical tolerance, including the foodborne pathogen *B. cereus* [8]. Turbidity was seen in Falcon 14 (*S. cerevisiae*), which had the lowest resistance. In a study, the resistance of *B. cereus* and other spore-forming bacteria to BC was studied [18]. It was discovered that *P. aeruginosa* was more resistant to BC than *E. coli* and *S. aureus*. Because of their high surface activity, these cationic surfactants damage yeast and bacterial cell membranes by causing cation adsorption on them.

3.3 Percidine

The effectiveness of a peracetic acid-based disinfectant against *S. cerevisiae, B. cereus, P. aeruginosa, and S. epidermidis*, was examined. Percidine commercial mixture contains acetic acid, hydrogen peroxide, PAA, and water. It is well known that PAA and H_2O_2 work synergistically to increase their bactericidal and sporicidal effects when administered significantly (P/H) compared to when either agent is used alone. Leggett et al. (2016) found that PAA, not H_2O_2, is primarily responsible for the combination's sporicidal action [19]. The oxidation of cell components also

serves as the foundation for PAA's antibacterial activity [20]. Low doses of PAA can have a number of beneficial effects, including cutting purifying costs, reducing organic matter, and reducing the need to store PAA during the summer. Low doses of PAA do not produce any harmless compounds or chemical residues [21]. The amount of MIC and MBC for *B. cereus* and *S. epidermidis* was the same, as demonstrated, and this might be because of the optimal antibacterial properties of percidine. The findings indicated that *B. cereus* had a higher level of resistance to this disinfectant than the other examined strains. Studies suggest that PAA kills spores by causing damage to the inner spore membrane [22]. Peracetic acid (PAA) was tested in a study to see if it could effectively clean dental acrylic resins that had been artificially contaminated with *Candida albicans*, *E. coli, S. aureus,* and *P. aeruginosa*. Data showed that Surface disinfection using peracetic acid effectively inhibited *C. albicans* growth on all acrylic resins [23].

3.4 Ethanol

The results of this study are shown in Table 2.

Table 2: lists the test isolates' minimum inhibitory concentration (MIC) of Ethanol.

Alcohol %	Microbial strains			
	B.cereus	*S.cerevisiea*	*P.aeroginosae*	*S.epidermidis*
60	+	-	-	+
70	-	-	-	-
80	-	-	-	-
MIC%	70	60	60	70

Alcoholic solutions have excellent antibacterial characteristics, but studies have revealed that they lack sporicidal activity [24]. According to the findings, ethanol concentrations at greater levels were superior to concentrations at lower levels for the disinfection process. According to CDC.3, *P. aeruginosa* was killed by all doses of Ethanol from 30 to 100 %(v/v) in under 10 seconds, but Ethanol's bactericidal effect rapidly decreases when diluted below 50%, and the ideal bactericidal concentration ranges from 60% to 90% [25]. In a study, the effectiveness of chlorine, chlorine dioxide, alcohol, and a quaternary ammonium compound against *B. cereus* on stainless steel, wood, glass, polyethylene, and polypropylene was examined [26]. Although all sanitizers considerably decreased the bacterial counts in the biofilm-formed groups compared to the baseline population count, chlorine had the most incredible bactericidal action against *B. cereus*. In a study, the effectiveness of Ethanol (70 % v/v) as a disinfectant against 205 bacterial isolates from six species, including Bacillus spp., *S. epidermidis*, and *E. coli*, was evaluated [27]. The results indicated that 10 minutes after disinfection, a significant reduction in microbial contamination of surfaces was observed. On sterile stainless-steel plates infected with *S. aureus, P. aeruginosa*, and Aspergillus spp., the effectiveness of a no-touch disinfection approach that aerosolizes 5 % hydrogen peroxide and 10 % ethyl alcohol was assessed. Following sanitization, air and surface sampling was conducted. The results showed that the air sanitization system had good efficacy for both bacteria and fungi in the air and on stainless-steel plates [28]. Visual Inspection for the corrosion detection on steel and plastic structures Macroscopic analysis of stainless steel and plastic sandwiches treated with the concentrations specified in the paper revealed no corrosion. Observations revealed that the potential corrosion difference between stainless steel and stained plastic sandwiches did not vary with increasing disinfectant concentration. There was no discernible change between the control and disinfectant-treated samples after two days of soaking.

4. Conclusion

MIC and MBC determinations are also utilized to select the commercial chemical agent presenting the better performance, compared with others. Data showed that the *B. cereus* showed greater resistance to all tested disinfectants whereas *Saccharomyces yeast* had the lowest resistance to detergents. These findings indicate that the same disinfectants should not be used for all types of disinfection. This investigation also showed that Deconex 50ff was the most potent disinfectant that prevented microbial development compared to other disinfectants at lower concentrations. Corrosion visual inspection showed that sandwiches made of stained plastic and stainless steel that had come into contact with detergents had not deteriorated.

Authors' Contributions

Bardia Farzamfar: Study design; Project management. Siavash Norouzi: Project management; collecting data. Yasamin Maleki: Manuscript preparation; Revising the manuscript; collecting data. Kosar Mohammadi: collecting data.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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