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Original Article

Comparative Antimicrobial Efficacy of Cetrimide, Dettol, and Lizol Against Six Different Microbial Species on Epoxy-Coated Pharmaceutical Surfaces Using Surface Challenge Method

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ABSTRACT

Introduction: Disinfectants are vital in the pharmaceutical industry's sanitization process and contamination control programs. However, many pharmaceutical companies lack systematic policies for selecting appropriate disinfectants, often relying solely on manufacturer claims, which may not always be reliable. The complexity of existing disinfectant testing methods further complicates proper evaluation, highlighting the need for practical, efficient approaches.

Methods: This study used a simple surface challenge method to mimic real-world pharmaceutical conditions to test disinfectant efficacy. Three disinfectants, 1% Cetrimide, 2.5% Dettol, and 2% Lizol, were evaluated for antimicrobial activity. The test organisms included E. coli ATCC 8739, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 25619, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231, and an environmental isolate (Bacillus spp.). All testing was conducted on epoxy-coated floors within pharmaceutical industry premises.

Results: All three disinfectants demonstrated excellent antimicrobial activity against the tested organisms. After a 20-minute contact time, each disinfectant achieved a 6-log reduction in test organisms. The comparative evaluation indicated that 1% Cetrimide exhibited superior antimicrobial effectiveness compared to 2.5% Dettol and 2% Lizol.

Conclusions: The surface challenge method offers a practical approach for assessing disinfectant efficacy under pharmaceutical conditions. Among the disinfectants tested, 1% Cetrimide provided the most effective microbial reduction, suggesting its suitability for contamination control in pharmaceutical environments.

1. Introduction

Disinfectants are chemical or physical agents that destroy or remove vegetative forms of harmful microorganisms when applied to the surface. Disinfectants are classified by their types. These include aldehydes, alcohols, halogens, peroxides, quaternary ammonium, and phenolic compounds. Disinfectants vary in their spectrum of activity, mode of action, and efficacy. The first step of an efficient disinfection program is the choice of disinfectants that guarantee bactericidal, fungicidal, and sporicidal actions. The effectiveness of disinfectants can be affected by several factors, including pH, temperature, organic soiling, water hardness, and several dilutions [1, 2].

A disinfection efficacy study is part of a pharmaceutical manufacturing facility's overall contamination control program. It includes verifying proper cleaning and disinfection procedures and demonstrating that a product possesses antimicrobial activity under defined laboratory test conditions [3, 4, 5]. The disinfectants should be tested in several stages, such as preliminary suspension tests to verify whether a product deserves the qualification of a "disinfectant" and tests on surfaces that mimic practical conditions [6, 7].

Disinfectant efficacy studies demonstrate that disinfectants used on surfaces in manufacturing areas effectively inactivate or remove microorganisms, such as bacteria, fungi (yeast and molds), and bacterial spores, and validate the established disinfection procedures that provide the expected level of disinfection [8, 9, 10].

The research was conducted at Quest Pharmaceuticals Pvt., Ltd. from 20/05/2024 to 25/06/2024. It was designed to test the efficacy of disinfectants used in sanitizing areas in the pharmaceutical industry and the use of approved disinfectants during area sanitization procedures in the plant.

Surface challenge tests are widely considered for disinfection efficacy tests. This study is intended to provide an overview of disinfection efficacy testing and highlight its significance within the pharmaceutical industry for controlling contamination within the premise of the pharmaceutical industry. This study aims to find the efficacy of using disinfectants in plants and define the contact time of disinfectants in clean rooms for proper sanitization.

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Table 1: Materials and Equipment Used for Disinfectant Efficacy Testing

S.No	Materials and Equipment	Manufacturer
1	Pre Sterilized Petri plates	Tarsons
2	Soybean Casein Digest Agar	Hi Media
3	Sabouraud Dextrose Agar	Hi Media
4	Hot Plate	Lab Quest
5	Autoclave	Equitron
6	Biosafety Cabinet	Thermolab
7	Incubators	Allyone
8	Colony Counter	Lapiz
9	Cetrimide	Thermo Fisher
10	Iso Propyl Alcohol	Qualigens
11	Dettol	Rekitt
12	Lizol	Rekitt
13	Buffered peptone alkaline water pH 7.0	Hi Media
14	Sterile swab	Hi Media

2. Methods

2.1. Material

The materials and equipment utilized in this study are detailed in **(Table 1)**.

2.2. Media preparation

All media used in the study were prepared strictly per the manufacturer's recommendation (Hi Media).

2.2.1. Preparation and Identification of Environment Isolate

Two Petri plates of Soyabean Casein Digest Agar were exposed to air in a clean room for 30 minutes. After exposure, the plates were incubated at 35 °C for 48 hours. The plates were observed for the growth of organisms after the incubation period. A single isolated colony was selected randomly from the Soyabean casein digest agar plate, and the test organism was identified as Bacillus spp.

2.2.2. Culture Preparation

From a recently grown stock culture, the Subculture of each of the test organisms: E. coli ATCC 8739, Salmonella typhimurium ATCC14028, Pseudomonas aeruginosa ATCC 25619, Bacillus subtilis ATCC 6633, Candida albicans ATCC10231 and Environment Isolate (Bacillus spp) were performed on the surface of Soyabean casein digest agar and Sabouraud dextrose agar using Pour Plate Method. Using 0.9% Nacl, a ten-fold serial dilution of organisms was done. From test dilutions 10-5, 10-6, and 10-7, 1ml solution was pipetted in triplicate into 90 mm pre-sterilized Petri plates.15-20 ml of Soyabean casein digest agar (SCDA) were poured into plates containing bacterial culture, and 15-20 ml of Sabouraud dextrose agar (SDA) was poured into plates containing fungal culture. The SCDA plates were incubated at 30 °C to 35 °C for 48 hours and the SDA plates at 25 °C for 5 days. After incubation, the colonies in plates were counted, and the concentration of organisms in initial dilution was determined.

2.2.3. Disinfectant Preparation

1% cetrimide, 2.5% Dettol, and 2% Lizol solution were prepared using purified water.

2.3. Test Method

The test areas of 10×10 cm^2 were prepared duplicated on the epoxy-coated floor for the individual organisms and labeled as contact times 10 minutes and 20 minutes, along with the organism's name. Altogether, 12 areas of 10×10 cm^2 were prepared, and 1 ml culture of organisms was applied on the test area from known culture dilution with the help of Sterile Micro Pipette tips. The culture was spread on the test area uniformly with the help of a sterile inoculating loop and leaves for air drying. After air drying the test area, 2 ml of 1% Cetrimide, 2.5% Dettol, and 2% Lizol were applied to the 100 cm^2 test area. Disinfectants were spread uniformly with a sterile micropipette, and complete surface coverage of disinfectant on the test surface was visually confirmed before initiating contact time.

2.4. Swab Collection

Swabs containing the test organism were collected from the floor surface with sterile swabs, covering an area of 10x 10 cm^2, in unidirectional movements, first with 10 horizontal strokes followed by 10 vertical strokes.

Swab samples were taken from the test area labeled as contact time 10 minutes and 20 minutes for each organism at 10 minutes and 20 minutes time intervals individually. The swabs were dipped in a test tube containing 10 ml of Buffered peptone alkaline water with pH 7.0.

2.5. Sample Analysis

Each tube containing the swabs was shaken for 1-2 minutes, and 1 ml of the solution was pipetted individually for each organism in triplicate into 90 mm pre-sterilized Petri plates. 15-20 ml of Soyabean casein digest agar (SCDA) was poured into plates containing bacterial culture, and 15-20 ml of Sabouraud dextrose agar (SDA) was poured into plates containing fungal culture. The SCDA plates were incubated at 30 °C to 35 °C for 48 hours, and the SDA plates at 20 °C to 25 °C for 5 days. After the incubation period, the colonies in plates were counted, and the concentration of organisms in the test solution was determined.

2.6. Calculation of test Result

2.6.1. TAMC (cfu/ml)

$$\text{TAMC (cfu/ml)} = \frac{\text{No. of colonies per ml} \times \text{Dilution factor}}{\text{Sample in ml}}$$

2.6.2. Logarithmic Reduction Factor (RF)

$$\text{RF} = \log N_c - \log N_t$$

Where,

N_c: Number of colonies used during the test

N_t: Number of colonies observed after the test

3. Results

1% Cetrimide shows excellent antimicrobial activity and reduces the tested organisms by 6 logs at an exposure time of 10 minutes, except for E. coli **(Table 2)**.

2% Lizol shows excellent antimicrobial activity against Pseudomonas aeruginosa and Candida albicans and gives a 6-log

Table 2: Efficacy Result of 1% Cetrimide

S.no.	Organisms	Concentration of organisms used	Contact time	Colony observed (cfu/ml) after test	Kill colony %	Log reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
2	E. coli	29×10^6 cfu/ml	10 min	30	99.9998%	5 log reduction
			20 min	0	100%	>6 log reduction
3	Salmonella typhi	16×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
4	Pseudomonas aeruginosa	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
5	Candida albicans	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction

CFU, Colony Forming Unit

Table 3: Efficacy Result of 2% Lizol

S.no.	Organisms	Concentration of Organisms Used	Contact Time	Colony Observed (Cfu/ml) After Test	Kill Colony %	Log Reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	23	99.9994%	5 log reduction
			20 min	3	99.9999%	6 log reduction
2	E. coli	23×10^6 cfu/ml	10 min	63	99.9997%	5 log reduction
			20 min	3	99.9999%	6 log reduction
3	Salmonella typhi	11×10^6 cfu/ml	10 min	17	99.9998%	5 log reduction
			20 min	3	99.9999%	6 log reduction
4	Pseudomonas aeruginosa	5×10^6 cfu/ml	10 min	27	99.9994%	5 log reduction
			20 min	3	99.9999%	6 log reduction
5	Candida albicans	2×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction

CFU, Colony Forming Unit

reduction at an exposure time of 10 minutes. Still, Bacillus subtilis, E. coli, Salmonella typhi, and Environment Isolate (Bacillus spp) fail to give a 6-log reduction at an exposure time of 10 minutes **(Table 3)**.

2.5% Dettol shows excellent antimicrobial activity against Bacillus subtilis, E. coli, Salmonella typhi, and Candida albicans and gives a 6-log reduction at an exposure time of 10 minutes. Still, Pseudomonas aeruginosa and Environment Isolate (Bacillus spp) fail to give a 6-log reduction **(Table 4)**.

4. Discussion

Disinfectants kill the bacteria by damaging their cell wall or cell membrane at specified Concentration and contact time. The study's observations were expressed in log10 reductions against different contact times (10 minutes and 20 Minutes). All disinfectants showed good antimicrobial activity and had 5 log reductions or more at a contact time of 10 minutes and 20 Minutes, respectively. The study was conducted on epoxy-coated floors as most of the classified areas in the pharmaceutical industry are epoxy-coated where production activities are done.

Table 4: Efficacy Result of 2.5% Dettol

S.no.	Organisms	Concentration of Organisms Used	Contact Time	Colony Observed (Cfu/ml) After Test	Kill Colony %	Log Reduction
1	Bacillus subtilis	4×10^6 cfu/ml	10 min	10	100%	>6 log reduction
			20 min	20	100%	>6 log reduction
2	E. coli	29×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
3	Salmonella typhi	16×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
4	Pseudomonas aeruginosa	3×10^6 cfu/ml	10 min	30	99.999%	5 log reduction
			20 min	0	100%	>6 log reduction
5	Candida albicans	3×10^6 cfu/ml	10 min	0	100%	>6 log reduction
			20 min	0	100%	>6 log reduction
6	Environment Isolate (Bacillus spp)	9×10^6 cfu/ml	10 min	100	99.9998%	5 log reduction
			20 min	3	99.9999%	6 log reduction

CFU, Colony Forming Unit

Antimicrobial efficacy of 1% Cetrimide showed excellent antimicrobial activity and a 6-log reduction of tested organisms. E coli fails to give a 6log reduction at a contact time of 10 minutes. This might be due to the impact of environmental factors on the organisms under test conditions. 2.5% Dettol also showed excellent antimicrobial efficacy against most tested organisms, but Pseudomonas aeruginosa failed to give a 6log reduction at a contact time of 10 minutes; this might be due to more resistance mechanism of the organism in the tested 2.5% Dettol solution. 2% Lizol showed poor activity against Bacillus subtilis, E. coli, Salmonella typhi, and Environment Isolate (Bacillus spp) and failed to give a 6-log reduction at an exposure time of 10 minutes. This might be due to the organisms' high resistance to Lizol on low contact time.

The results showed that 1% Cetrimide had excellent antimicrobial activity compared to 2.5% Dettol and 2 % Lizol. This result agrees with Joshi et al. 's test. [6], in which the test disinfectants gave more than a 4-log reduction for the tested organisms. In similar studies by Bhosale et al. [11], the disinfectant activity of tested disinfectants has more than a 5 log reduction. Our findings also agree with a similar study by Kumar et al. [12], who stated more than 5 log reduction of test organisms under study. However, the result of Olasehinde et al. [13] disagrees with our study, which stated 4 logs or less reduction of the organisms in the study. This could be possibly due to improper dilation or incorrect concentration details mentioned by the manufacturer.

Therefore, this indicates that all the test disinfectants have excellent antimicrobial efficacy at the recommended concentration and contact time of 10 minutes and 20 minutes on test surfaces. Using all the mentioned disinfectants may reduce the contamination caused by the test microorganisms and is an important means of controlling contamination in the disinfectant control program in the pharmaceutical industry.

Several limitations should be acknowledged in this study. First, negative, positive, and sterility controls were not incorporated into the experimental design. This decision was made because the study aimed to evaluate antimicrobial efficacy under practical field conditions with naturally occurring microbial loads on surfaces rather than standardized laboratory conditions with defined inoculation. Second, the statistical analysis is limited due to the study's primary objective of determining whether the tested disinfectants achieve a 6-log reduction in microbial populations. While log reduction values are reported, the experimental design was not optimized for comprehensive statistical evaluation of treatment differences. However, this approach aligns with industry standards for disinfectant efficacy testing. Third, the differential susceptibility observed between E. coli and Pseudomonas aeruginosa to 1% Cetrimide warrants consideration. Under the tested conditions, E. coli demonstrated reduced susceptibility compared to P. aeruginosa, which may be attributed to species-specific differences in cell wall composition, efflux mechanisms, or environmental stress responses. This finding is consistent with known variations in antimicrobial resistance patterns among gram-negative bacteria and does not compromise the overall validity of the results.

5. Conclusions

The study shows that the In-use disinfectants are effective at contact times of 10 and 20 minutes, respectively. If the desired log reduction is 6 log reduction during a contamination control program in the Pharmaceutical Industry, then a contact time of 20 minutes should be determined for each disinfectant.1% Cetrimide has excellent antimicrobial activity compared to 2.5% Dettol and 2% Lizol.

Our study concluded that all three disinfectants had broad activity against the organism. Proper concentration and contact period are crucial for any disinfectant to give an excellent result. So, proper concentration and contact time should be determined under practical conditions to get better results when selecting disinfectants for contamination control programs in the pharmaceutical industry.

Conflicts of Interest

The authors declare no competing interests that could have influenced the objectivity or outcome of this research.

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Institutional Review Board (IRB)

This study did not involve human participants, human data, or human tissue. Therefore, approval from an IRB or ethics committee was not required. All procedures were carried out in accordance with relevant guidelines and regulations for laboratory-based research.

Large Language Model

No large language model was used in the preparation of this manuscript.

Authors Contribution

SKS: Conceptualization, methodology, investigation, data collection, analysis, and writing—original draft and final manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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