

Comparative Efficacy of Freshly Prepared and Stored Disinfectants in Working Dilutions by in Use Test

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Abstract: Disinfectants are used extensively in the homes, healthcare settings, laboratories and industries for a variety of topical and hard surface application particularly as an essential component of infection control practice with a purpose of preventing infections. Disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy. The study was aimed to evaluate the efficacy of some locally available disinfectants and also study the stability and long term effectiveness of such disinfectants at their working concentrations. A total of six disinfectants/ antiseptics specimens were collected from various areas in hospitals like clinical departments including Out Patient Department (OPD), Indoor Patient Department (IPD) and Intensive care unit of YMCH. A 10 μ l of the 0.5 McFarland bacterial culture (ATCC strains of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) was suspended into the 5ml of disinfectant solution at working dilution, after immediate preparation and after 7 days and inoculated on nutrient agar. Differences in the number of colony forming units were noted on both plates with and without disinfectant to know microbicidal efficiency (ME) of the disinfectant. Out of all disinfectants/ Antiseptics tested Glutarex, Rapid multi-enzyme cleaner, Avagard and Dettol were most effective against all organisms and yielded no growth on both nutrient agar plates. On the other hand Savlon and fresh day disinfectants were not effective even in freshly prepared working concentrations, and had decreased efficacy after 7 days of storage. The test disinfectants used in this study have been confirmed to be very effective as it has demonstrated that they have both antibacterial and antifungal efficacy. Recommended concentrations based on Suspension test apply only to freshly prepared solutions but the solutions are likely to be kept for more than 24 hours in a health care setting hence it is advisable to test the efficacy of working dilutions of disinfectants after storage of more than 24 hrs.

Keywords: Disinfectant, working dilutions, quantitation test, colony forming unit

INTRODUCTION

Infectious disease and its physical, physiological and economical impact remains a significant problem in today's society. Through research, we have learnt that by limiting the number of infectious agents to which people are exposed, the chances of disease transmission can be reduced. One important control measure to help prevent the spread of infectious diseases is through disinfection and to maintain asepsis as far as possible [1].

Disinfectants are substances that are applied to inanimate surfaces and objects to destroy harmful microorganisms, although they may not kill bacteria spores. They are categorized by their spectrum of anti microbial activity. Disinfectants are used extensively in the homes, healthcare settings, laboratories and industries for a variety of topical and hard surface application particularly as an essential component of

infection control practice with a purpose of preventing infections[2]. Although there has been an interest in improving the sterilization and disinfection procedures to reduce the infection risk for hospitalized patients and health care workers as disinfectants resistant bacteria strains have arisen. This may be due to lack of standardization of some factors like as criteria for use of chemical agents, specification in the label of available products and scarcity of well trained personnel. The prevalence of disinfectant-resistant hospital bacteria have increased significantly in the world.

Disinfectants used in hospitals and laboratories must be tested periodically to ascertain its potency and efficacy. As certain disinfectants lose potency on standing and addition of organic matter, their efficacy must be tested. While certain methods help in selecting the right dilution of disinfectant for use others test the efficacy of disinfectant already in use. Some methods

compare the performance with that of phenol whereas other methods simply state if the disinfectant is effective or not. There are several methods of testing disinfectants, with their own advantages and disadvantages. All these tests can be allocated to one of the following disinfectant [3].

AIM AND OBJECTIVES OF THE STUDY

The following study was aimed to evaluate the efficacy of some locally available disinfectants and also study the stability and long term effectiveness of such disinfectants at their working concentrations.

MATERIALS AND METHODS

The disinfectants and antiseptics being used in various departments of the hospital were subjected to the study. The study was carried out during January 2017 to March 2017. A total of six disinfectants/antiseptics specimens were collected from various clinical departments including Out Patient Department (OPD), Indoor Patient Department (IPD) and Intensive care unit of YMCH. The disinfectants used in this study along with their detailed composition are given in table 1.

Table 1: Disinfectants used in study

Name of disinfectant	Composition
Dettol	Dichloroxylenol 1.5% Essential oils 3%
Savlon	Chlorhexidine gluconate solution 0.3% v/v Cetrimide I.P 0.6 w/v
Glutarex™	Gluteraldehyde 2 % w/v Inert ingredients 98%
Rapid multi-enzyme cleaner (Surgical, dental, medical instruments)	Ethylene glycol, surfactant, Methoxymethylethoxy propanol, Sodium borate dehydrate c10 – c16 alkyl derivatives and glycerin
Fresh day (Floor disinfectant)	Benzalkonium chloride 2% w/v Other ingredients : Amphoteric detergent, preservation perfume ,DM water
Avagard (Hand rub)	2 propanol I.P 45% w/w 1 propanol 30% w/w

METHODOLOGY[4]

Sterilization of Glass Wares

All glass wares such as test tubes and beakers were sterilized using hot air oven at a temperature of 160°C for 1 hour prior to use.

Media Preparation

Nutrient agar (Lab M Ltd) was used for sub culturing of *Escherichia coli* and *Staphylococcus aureus*, while Potato dextrose agar (Lab M Ltd) was used for *Candida albicans*. They were all cultured first in Buffered peptone water which is a pre-enrichment broth before subculture onto the solid media. All the media were prepared according to manufacturer's instruction.

Quantitative Suspension test

A 10µl of the 0.5 Mc Farland bacterial culture (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) was suspended into the 5ml of disinfectant solution at working dilution and after exposure for 1hr, sub-cultured on dry nutrient agar plate to verify whether this inoculum is killed or not. On another Nutrient agar plate 10µl of same bacterial suspension was directly inoculated. Both the plates were incubated at 37°C overnight. The number of surviving organisms in disinfectant solution is counted and compared to the original inoculum size. By subtracting the colony forming units (CFU) of the

former from the CFU of the latter, the microbicidal effect (ME) was obtained.

Test for stability and long-term effectiveness

Recommended concentrations based on Suspension test apply only to freshly prepared solutions but the solutions are likely to be kept for more than 24 hours in a health care setting. The effectiveness of these concentrations was confirmed by a supplementary test for stability of unused solution to prevent multiplication of bacteria. *P. aeruginosa* is used as test organism. Sufficient disinfectant solution was prepared for two tests. One portion was inoculated immediately and tested for growth (Suspension test). The other portion was kept at room temperature for seven days and then inoculated with a freshly prepared suspension of test organism.

RESULTS

A total of six disinfectants /antiseptics used in Yenepoya hospital in various areas were tested from between January 2017 and March 2017. The organisms used for testing were *Pseudomonas*(ATTC27853), *Escherichia coli* (ATCC25922), *staphylococcus* (ATCC25923), *Acinetobacter* (ATCC19606) and *Candida* (ATCC10231)

The disinfectants/ antiseptics used were Dettol, Savlon, Glutarex™, Rapid multi-enzyme cleaner, Fresh Day (Lysol), and Avagard (2Propanol IP).

Out of all disinfectants/Antiseptics tested Glutarex, Rapid multi-enzyme cleaner, Avagard and Dettol were most effective against all organisms and yielded no growth on both nutrient agar plates (37°C

incubation in freshly prepared and 7 days stored reagents). On the other hand Savlon and fresh day disinfectants were not effective even in freshly prepared working concentrations, and had decreased efficacy after 7 days of storage. In this study it was also seen that Avagard was equally effective against all test strains and had not yielded any growth but was totally ineffective against *Pseudomonas*.

Table 2: Microbicidal effect of disinfectants used in study

Test Organism	Glutarex		Rapid multi-enzyme cleaner		Dettol		Avagard		Savlon		Fresh day	
	F	S	F	S	F	S	F	S	F	S	F	S
<i>Pseudomonas</i>	0	0	0	1	0	2	10	10	5	6	10	10
<i>Escherichia coli</i>	0	0	0	0	2	3	0	0	2	3	3	4
<i>Staphylococcus</i>	0	0	0	0	0	0	0	0	5	6	9	10
<i>Acinetobacter</i>	0	0	0	0	0	0	0	0	10	10	10	10
<i>Candida</i>	0	0	0	0	0	0	0	0	3	4	0	0

Note: All Values in table indicate CFU/ml of disinfectant

F: Freshly prepared disinfectant at working dilution

S: Disinfectant at working dilution stored for 7 days

DISCUSSION

Cleaning is related to the clearance of foreign material from a surface or equipment, allowing the removal of some organic material and microorganisms by detergents. However, this process does not kill bacteria, which, under favourable conditions, can redeposit elsewhere and disease[5]. Consequently, cleaning must always precede disinfection and sterilization in order to eliminate infectious microorganisms. A significant amount of microorganisms is destroyed by the disinfection process, which involves the use of chemical agents such as quaternary ammonium compounds, aldehydes, alcohols and halogens. The control of hospital infection must involve both disinfection and sterilization processes and sometimes the use of aerosols to clean the air.

The efficacy activities of the test disinfectants against *Pseudomonas*, *Acinetobacter*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* was investigated in this study. It was found that majority of the Glutarex, Rapid multi-enzyme cleaner, Dettol, and Avagard were best in freshly prepared and even in stored solutions after preparation. Although phenolic agents exhibit high toxicity and low biodegradability, they are still in use in developing countries because of their low cost. In our study Dettol showed excellent microbicidal and fungicidal property and hence can be a cost effective alternative in non invasive situations. Aldehyde-containing disinfectant, “Glutarex” and Rapid multi-enzyme cleaner were found to be a very effective antimicrobial agent on almost all the tested organisms in both fresh and stored solutions. In this study, we also wanted to test the disinfectants against

heavy organic waste, which was simulated by immersing culture plates in disinfectants and monitored by in-use test.

CONCLUSION

The test disinfectants used in this study have been confirmed to be very effective as it has demonstrated that they have both antibacterial and antifungal efficacy but their rates of efficiency varies due to the differences in their chemical composition and mechanism of action, such cases can be observed mainly in Fresh day and savlon which were not effective as disinfectants. Recommended concentrations based on Suspension test apply only to freshly prepared solutions but the solutions are likely to be kept for more than 24 hours in a health care setting hence it is advisable to test the efficacy of working dilutions of disinfectants after storage of more than 24 hrs.

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