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Original Article

Microbiology Section

Evaluation and Comparison of Microbicidal Activity of Commonly Used Disinfectants: A Laboratory Based Study

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ABSTRACT

Introduction: As per World Health Organisation (WHO), around 1.4 million people per year are affected by hospital acquired infections. Disinfection of hospital environment and equipment plays a major role in preventing these infections. Disinfectants used in hospital and laboratories must be tested periodically to ascertain their efficacy.

Aim: The study aimed to evaluate the Microbicidal Effect (ME) of some commonly available disinfectants against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* and to compare fresh versus long-term effectiveness of such disinfectants at their working concentrations.

Materials and Methods: A total of seven commonly used disinfectants namely Dettol, Savlon, Cidex, Phenyl, Unilon alcoholic hand rub, Sterilium and Sodium Hypochlorite (Chlorox) were taken and working dilutions were made as per manufacturer's guidelines. "Quantitative suspension test"was carried out on these disinfectants to check their ME when fresh and after storage for seven days. Statistical analysis was done using Microsoft excel 2010 and SPSS version 2020.

Results: Cidex and Sodium Hypochlorite came out to be highly effective with ME of 7 and 6, respectively when used fresh followed by Phenyl, Sterilium, Unilon alcoholic Hand rub, Savlon and Dettol. All the disinfectants showed markedly decreased ME on long-term storage for seven days as compared to fresh preparation (p-value <0.05).

Conclusion: To conclude, a variety of disinfectants with different mechanisms of action are available in the market. All disinfectants tend to have decreased ME on standing. Therefore, it is advisable to test the efficacy of working dilutions of disinfectants on regular basis to ensure good disinfection at all times.

Keywords: Colony forming unit, Quantitative suspension test, Working dilution

INTRODUCTION

Disinfectants are chemical agents which inhibit or destroy microorganisms on inanimate surfaces and objects whereas antisepsis achieves so on living tissue [1]. Disinfectants are widely used in health care settings such as laboratory, hospital and health care industries as important component of infection control practice. Disinfectants which are generally used need to be regularly tested to determine their potency and effectiveness. Different classes of disinfectants are available based on their effectiveness against vegetative bacteria, tubercle bacilli, fungal spores, enveloped and non enveloped viruses and bacterial spores [2].

Adequate disinfection and sterilisation procedures are a must for control of hospital-acquired infections, as failure can result in many hospital-acquired infections thus leading to increased cost, morbidity and mortality. Centre for Disease Control estimates that on any given day, 1 in 31 hospital patients have hospital-acquired infection. WHO estimates these infections to occur among 7-12% of the hospitalised patients globally, with more than 1.4 million people suffering from infectious complications acquired in the hospital at any time [3,4]. In particular, disinfection is an essential part of infection control practices and aids in the prevention of nosocomial infections.

Bacterial contamination of disinfectant solutions has been observed when prepared by unskilled personnel, using unsterilised containers and kept for use over prolonged period. Other factors contributing to high levels of contamination were using tap water for dilution of disinfectants, inadequate maintenance of stock solution bottles and overstay of the diluted disinfectants in the wards or ICUs [5].

Thus, it is important to check the disinfectant efficacy or ME before it is brought in use. The standard tests to check disinfection efficacy include Suspension Test, Capacity Test (Kelsey and Sykes, 1969), and Carrier Test. Suspension Test can further be Qualitative like Rideal-Walker phenol Coefficient (RWC) test, Chick-Martin Test and Quantitative such as Quantitative Suspension Test [6-11].

In Quantitative Suspension Test, the number of surviving organisms after treating with disinfectant is counted and compared with the original inoculum size [12,13]. The test appears practical and easy to use. No study from Northern India has been carried out in this regard. Therefore, this study was planned to evaluate and compare the ME of some locally available disinfectants in hospital against commonly isolated microorganisms and to observe long-term effectiveness of such disinfectants at their working concentrations.

MATERIALS AND METHODS

Study Design and Settings

It was a laboratory based observational study which was carried out at Department of Microbiology of a Tertiary Care Institute located in a remote area of Nuh (Mewat) in Haryana from November 2019 to January 2020. The approval was obtained from the Institutional Ethics Committee wide letter no SHKM/IEC/2019/145 dated 24th October 2019. The Institute handles referral from peripheral hospitals.

Sterilisation of Glass Wares

All glass wares like test tubes and beakers were sterilised using hot air oven at a temperature of 160°C for one hour prior to use.

Media Preparation

Nutrient agar was prepared according to manufacturer's instruction. All the test organisms namely, *Pseudomonas aeruginosa* (ATTC27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), and *Candida albicans* were incubated in peptone water for six hours before subculture onto the solid media.

Methodology

A total of seven disinfectants commonly used in the hospital namely Dettol, Savlon, Cidex, Phenyl, Unilon alcoholic hand rub, Sterilium and Chlorox were taken and working dilutions were made as per manufacturer's guidelines.

Quantitative Suspension Test

Quantitative Suspension Test is in-vitro quantitative test to check the efficacy of disinfectant [12]. A 10 µL of the 0.5 Mc Farland bacterial/fungal culture (*Pseudomonas aeruginosa* (ATTC27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), and *Candida albicans*.) was suspended into the 5 mL of disinfectant solution at working dilution and after a contact period of one hour, subcultured on nutrient agar plate to see whether microorganism is killed or not. On another Nutrient agar plate 10 µL of same bacterial suspension without disinfectant was directly inoculated. Both the plates were incubated at 37°C overnight. The number of surviving organisms on subculture plate with biocide (B) was counted in Colony Forming Units and compared to the growth on directly inoculated plate showing number of surviving organism on plate without biocide (A). ME was obtained by subtracting log of (B) from log of (A) where A is number of viable organisms before treating with biocide and B is number of viable organisms after treating with biocide. Therefore:

ME=Log (A)-Log (B).

On the basis of ME the disinfectants were categorised in highly effective when log 10 reduction value was more than 5, effective when log 10 reduction value of 5, less effective with log 10 reduction value between 1 and 5 and ineffective with log 10 reduction value of 1 or less [12,13].

Test for Long-Term Effectiveness

The working dilution of disinfectant calculated based on Suspension test apply only to freshly prepared solutions but in a health care facility these solutions are likely to be kept for more than 24 hours. To see this Author again checked for the effectiveness of disinfectants by a repeated Quantitative Suspension Test after keeping the prepared disinfectants at room temperature for seven days which could be a practical scenario in many hospitals.

STATISTICAL ANALYSIS

The log reduction values were calculated and ME was interpreted. Comparison of ME of disinfectants when fresh and stored was done using Paired t-test. A p-value less than 0.05 was considered significant. Statistical analysis was done using Microsoft excel 2010 and SPSS version 2020.

RESULTS

A total of seven disinfectants/antiseptics were tested from November 2019 to January 2020. The organisms used for testing were *Pseudomonas aeruginosa* (ATTC27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), and *Candida albicans*.

The disinfectants/antiseptics used were Dettol, Savlon, Cidex, Phenyl, Unilon alcoholic hand rub, Sterilium and Chlorox. The chemical composition of each is explained in [Table/Fig-1].

The ME of all the tested disinfectants is depicted in [Table/Fig-2]. On the basis of ME the disinfectants were categorised in highly effective (Log reduction value >5), effective (Log reduction value=5), less effective (Log reduction value between 1-4) and ineffective (Log reduction value <5).

Therefore, Cidex and Chlorox came out to be highly effective with log reduction value of 7 and 6, respectively. Phenyl was effective for *S. aureus* but highly effective for *E coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Alcohol based disinfectants Unilon and Sterillium was effective with log reduction values of 5. Savlon and Dettol were less effective as there log reduction value ranged between 1 to 4.

S. No.	Name of disinfectant	Composition
1	Dettol	Dichloroxylenol 1.5%
2	Savlon	Chlorhexidine 0.3% Cetrimide 0.6%
3	Phenyl (Floor disinfectant)	Carbolic acid, Cresol 2%
4	Unilon (Hand rub)	Ethyl alcohol 70%
5	Cidex	Glutaraldehyde 2%
6	Sterilium	Isopropyl Alcohol 75%
7	Chlorox	Sodium hypochlorite 1%

[Table/Fig-1]: Disinfectants used in study with their chemical composition.

All the disinfectants were less effective to ineffective on storage for seven days as their log reduction value ranged between 1 and 0. These findings are depicted in [Table/Fig-2,3].

When ME of freshly prepared disinfectants was compared with the ME of disinfectants kept for seven days, it was found that ME was decreased many fold in all disinfectants, this difference being significant (p-value <0.05) thereby emphasising regular determination of ME [Table/Fig-3].

DISCUSSION

Antiseptics and disinfectants are chemical agents that inhibit or destroy microorganisms on living tissues and on inanimate surfaces and objects. Disinfection is defined as a process which eliminates many or all pathogenic microorganisms but not bacterial spores, on non living surface whereas, Antisepsis achieves so on living tissue. Many factors determine the efficacy of disinfectants such as cleaning of the object beforehand; presence of organic load; nature and load of microbial contamination; appropriateness of concentration and contact period of germicide used. Also, physical factors such as temperature, pH, humidity and type of objects to be disinfected affect the disinfection process. Periodic testing of disinfectants being used in hospitals and laboratories is a must as many disinfectants tend to lose their effectiveness on standing [5].

Many health care facilities in India lack standardised protocol for testing efficacy of disinfectants. Health care workers are often clueless about how to choose an appropriate disinfectant and so majority follows information provided by manufacturers which may be inadequate and misleading.

In this study, quantitative suspension test was performed to evaluate the ME of seven chemical disinfectants at defined contact period, in the absence of interfering substances. Concerning the ME of the tested disinfectants, the results indicate that only the aldehyde-based disinfectant (Cidex), chlorine-based (Chlorox) and Phenyl had an excellent killing activity (highly effective ME >5). Sterilium, and Alcohol rub showed good killing activity (effective with ME =5). Savlon had ME of 4 whereas Dettol was the least effective with ME of 2. Similar findings were observed by Sheraba NS et al., [12]. However, all the disinfectants tested showed decreased ME of either 1 or 0 on standing and storage.

The findings of this study also correlated with other studies performed on other disinfectants with same composition [14]. In this study, it was found that Savlon and Sterilium were more effective in freshly prepared solution contrary to another study which was conducted in Mangalore which showed that these were less effective. Sateesh K et al., showed that Savlon was not effective in fresh as well as stored solution which was contrary to the present study [15]. In this study, Cidex showed highly effective ME against all microorganisms in fresh concentration. Singh M et al., also quoted that aldehyde formulations are the best disinfectants for disinfection of heavy contamination [16]. A study done by Misbah R et al., in Mysore stated that Glutaraldehyde was found to be 11% more effective than isopropyl alcohol and under anaerobic condition glutaraldehyde

Microorganism	Dettol		Savlon		Phenyl		Unilon		Cidex		Sterillium		Chlorox	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
S.aureus	++	-	++	+	+	-	+	-	+++	+	++	-	+++	+
E. coli	+	-	++	-	+++	+	++	+	+++	+	++	+	+++	+
Pseudomonas	+	-	++	-	+++	-	++	-	+++	+	++	+	+++	+
Candida albicans	+	-	++	-	+++	+	++	+	+++	+	++	+	+++	+

[Table/Fig-2]: Microbicidal Effect (ME) of disinfectants used in study: F: Freshly prepared disinfectant at working dilution S: Disinfectant at working dilution stored for seven days. Highly effective (+++) = Log10 reductions of value >5. Effective (++) = Log10 reductions of value = 5. Less effective (+) = Log10 reductions of values 1-4. Ineffective: (-) = Log10 reductions of 1.

Microorganism	Dettol		Savlon		Phenyl		Unilon		Cidex		Sterillium		Chlorox	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
S.aureus	2.0	0	4.09	1	5	0	4.0	0	7.03	2.0	5.01	0	6.01	2.0
E. coli	2.0	0	4.0	0	6	1	5.0	1	7.09	1	5.03	1	6.0	1.0
Pseudomonas	1.09	0	3.01	0	6	0	5.0	0	7.0	1	5.0	1	6.01	1.0
Candida albicans	2.0	0	3.0	0	6	1	5.0	1	7.01	2.0	5.0	1	6.03	2.0
Mean (95% CI)	1.7 (1.04-2.4)		3.27 (2.5-4.04)		5.25 (4.4-6.04)		4.25 (3.4-5.04)		6.03 (5.9-6.09)		4.26 (3.4-5.05)		5.01 (4.9-5.03)	
p-value	0.004		0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

[Table/Fig-3]: Comparison of Log Reduction values of disinfectants obtained in the study.

S. No.	Author	Journal with year	Results	Results of present study
1	Sheraba NS et al., [12]	African Journal of Microbiology Research, 2014	Alcohol and chlorine based compounds and gluteraldehyde showed bactericidal and fungicidal activity.	Similar results were observed in this study as aldehyde and Hypochlorite showed strong bactericidal and fungicidal activity.
2	Indeever NK et al., [14]	International Journal of Bioassay, 2014	Bacilloid which contains Glutaraldehyde activity on all the organisms having concentration as high as 108 cfu/mL showed 7.09-8.01 log reduction	Similar results were observed in this study as aldehyde showed strong bactericidal and fungicidal activity.
3	Sateesh K et al., [15]	Scholars Academic Journal of Biosciences, 2017	Glutarex, and Dettol were most effective against all organisms. However, Savlon was not effective even in freshly prepared and stored working concentrations.	Results were contrary to the study as Savlon was more effective than Dettol specially when used fresh.
4	Singh M et al., [16]	Indian Journal of Critical Care Medicine, 2012	Newer quaternary ammonium compounds and aldehyde formulations were found to be the best disinfectants for disinfection of heavy contamination	Similar results were observed in present study as aldehyde showed strong bactericidal and fungicidal activity. We did not check Quaternary Ammonium compounds.
5	Misbah R et al., [17]	Indian Journal of Microbiology Research, 2019	Glutaraldehyde was found more efficient than isopropyl alcohol.	Similar results were observed in this study as aldehyde showed strong bactericidal and fungicidal activity than alcohol.
6	Massola PG et al., [18]	Brasilian Journal of Pharmaceutical Sciences, 2019	Isopropyl alcohol was found to be more effective disinfectant compared with Dettol and Savlon.	In present study Isopropyl alcohol was more effective than Savlon and Dettol.
7	Fukusaki S [19]	Biocontrol Science, 2006	Sodium Hypochlorite had broad antimicrobial spectrum and rapid bactericidal action	Similar results were observed in present study as Sodium hypochlorite shows strong bactericidal and fungicidal activity.
8	Rutala WA and Weber DJ [20]	American journal of Infection Control, 2016	Glutaraldehyde exhibit high bactericidal, fungicidal and virucidal activity.	Similar results were observed in this study as aldehyde showed strong bactericidal and fungicidal activity.
9	Bhosle NK [21]	International Journal of Current Microbiology and Applied Sciences, 2017	Comparative evaluation of the disinfectants concluded that Hospal-OT (Gultaraldehyde) had the maximum disinfectant activity whereas Savlon and Isopropyl Alcohol had the lowest.	These results were similar to this study as Glutaraldehyde showed more disinfectant activity than Isopropyl Alcohol but showed effective disinfection activity when compared with 70% Alcohol, Savlon and Dettol.

[Table/Fig-4]: Comparison of present study with previous studies [12,14-21].

was only 4% more effective than isopropyl alcohol [17]. A study done by Massola PG et al., concluded that glutaraldehyde exhibit high bactericidal, fungicidal and virucidal activity and can be used as high level disinfectant but it demands safe work environment and a training programme to ensure workers safety and standards. In this study, Isopropyl alcohol was found to be an effective disinfectant compared with Dettol and Savlon [18]. The comparative analysis of the present results with previous studies has been depicted in [Table/Fig-4] [12,14-21].

Present study has found that Sodium hypochlorite is having broad activity against the pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The highly effective disinfectant activity of Sodium Hypochlorite against bacteria, viruses and fungi has been well documented in another study [19,20]. In another study conducted in Mangalore, it was stated that 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 hours [21]. The similar trends have been observed in this study. Almost all the disinfectants have shown lower ME on standing.

The relatively poor performance of Dettol and Alcohol hand rub emphasise the need for routine potency and efficacy testing of these disinfectants commonly used in hospital. Adequate concentration and sufficient contact period is a must for efficient action of these disinfectants. Strict policy should be formulated for disinfectant selection and their use in order to curb the use of low quality and unauthorised disinfectants from local manufacturers.

Limitation(s)

There are few limitations to this study. Firstly, it was a laboratory based study without taking in to account the presence of organic matter which persists on instruments inspite of thorough cleaning in hospital scenario. Secondly, this study compares only two scenario

of disinfectants freshly prepared versus stored for seven days, there is a possibility of gradual loss of efficacy over days which we could not record.

CONCLUSION(S)

To conclude, variety of disinfectants with different mechanisms of action are available in the market. Every health care institute should check the efficacy before recommending their use. Though several methods have been developed for disinfectant testing most of them are not feasible because of their complex procedures. Thus, in this study Authors have employed a simple quantitative suspension method for testing the efficacy of the disinfectants. It is advisable to test the efficacy of working dilutions of disinfectants on regular basis to ensure good disinfection at all times.

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