Health Facility Based Efficacy Assessment of In-use Disinfectants

Shraddha Gajbhiye, Rashmi Mahalle
Department of Microbiology, NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Nagpur

Original Research Article

ABSTRACT

Disinfection in hospital practice is mainly achieved either by surface disinfection or immersing the contaminated objects in the disinfectant solution. This study was undertaken for assessing the efficacy of commonly used disinfectants in a tertiary care teaching hospital against Multi Drug Resistant *Pseudomonas aeruginosa* isolate, and to evaluate the different methods of testing of disinfectants like Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test. In this study, samples of disinfectants and those in use, namely, Lysol, Sodium Hypochlorite, Glutaraldehyde and Bacillocid were collected from different sites in the hospital. The standard tests to check efficacy included Rideal-Walker Phenol Coefficient Test, Chick –Martin Test, Kelsey -Sykes Test, Surface Disinfection Test, In-Use Test. The tests were carried out by standard procedures. It was evident in the present study that Bacillocid had good efficacy in its working concentration.

KEY WORDS: bacillocid, disinfectants, efficacy, *pseudomonas aeruginosa*

INTRODUCTION:

Disinfectants are used extensively in healthcare settings, playing an important role in the control of infections. Testing the efficacy of disinfectants is a very important component in hospital infection control, but largely overlooked in many hospitals[1]. Antiseptics are agents that destroy or inhibit the growth of microorganisms in or on living tissue while disinfectants are similar but are used on inanimate objects or surface[2]. These agents such as alcohols, phenols, iodine and chlorine were used extensively in hospitals for infection control and prevention of nosocomial infections[3]. An ideal disinfectant, to overcome the antimicrobial resistant pathogens, should have a broad spectrum of antimicrobial activity[4] and the efficacy of these agents may be affected by pH, detergent base, temperature, organic matter, ionic and type of surfactants[5]. Disinfection in hospital practice is mainly achieved either by surface disinfection (eg. disinfection of surfaces of the tables, trolleys, instruments, walls and floors, etc.) or immersing the contaminated objects in the disinfectant solution. Many hospitals are still using phenolic disinfectants, while their use is being discouraged throughout advanced countries[6].

Hospital acquired infections are largely caused by multidrug resistant organisms (MDR) including *Pseudomonas aeruginosa.* These infections can be overcome by proper sterilization and disinfection practices. Various disinfectants are used in the hospitals and healthcare settings; however, their appropriate working concentration is pertinent to achieve disinfection. Hence this study.

Corresponding Author:
Dr Rashmi Mahalle,
Senior Resident
Department of Microbiology,
NKP Salve Institute of Medical Sciences
\and Lata Mangeshkar Hospital,
Nagpur (Maharashtra) India
Phone No.: 8108750960
E-mail: rashmidhote@yahoo.co.in

MATERIAL AND METHODS:

This observational study was conducted in the Microbiology Laboratory of NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital at Nagpur over a period of 3 months after approval of Institutional Ethics Committee. We tested four disinfectants commonly used in this hospital, namely- Lysol, Bacillocid, Sodium Hypochlorite and Glutaraldehyde by standard methods which were Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test. The organism used for testing was MDR *P. aeruginosa* strain which was also used as a control for the testing methods. No inclusion or exclusion criteria were envisaged (Table 1).

Disinfectant samples were collected in their working concentrations from various Wards,

Table 1: Disinfectants used for testing with their compositions and working concentrations.

Sr. No	Name of disinfectant	Composition	Working Concentration
1.	Bacillocid	Ethylenedioxydimethanol and glutaraldehyde	1% solution
2.	Sodium hypochlorite	Sodium hypochlorite solution	1% solution
3.	Cidex (Glutaraldehyde)	Activated glutaraldehyde Solution	2.2-2.7% solution
4.	Lysol	p-chloro-o-benzyl phenol, ethanol, isopropyl alcohol, potassium hydroxide and hydrogen peroxide (H2O2)	0.5% solution

Intensive Care Units, Operation Theatres, Labor Rooms and OPDs where they are frequently used for disinfection purposes. All these tests for each disinfectant and observed their efficacy. For Rideal-Walker Test (for phenolic disinfectants only)[7], calculated as ratio of 'highest dilution of Lysol that killed in 10 min but not in 5 min' and that of 'phenol that killed in 10 min but not in 5 min', a 24 hour broth culture of *P. aeruginosa* was prepared by inoculating in nutrient broth and incubating at 37°C overnight. Phenol was diluted using distilled water. Five dilutions ranging from 1:95, 1:100, 1:105, 1:110 and 1:115 were prepared and 5 ml of these dilutions were taken separately in five different test tubes and labeled according to their corresponding dilution. In each test tube, 0.2 ml of *P.aeruginosa* broth was added and the test tube was shaken thoroughly. From these test tubes of phenol dilution and culture mixture, subcultures were made into 5 ml of nutrient broth at time intervals of 2.5, 5, 7.5 and 10 minutes for each dilution and labeled accordingly. These test tubes were incubated at 37°C for a period of 72 hours. The tubes were then observed for presence or absence of turbidity. The whole procedure was repeated with lysol using dilutions 1:100, 1:200, 1:250, 1:300 and 1:350 and results were compared with that of phenol. The Rideal-Walker coefficient was calculated by dividing the highest dilutionof lysol that killed in 10 min and not in 5min by highest dilution of phenolthat killed in 10 min but not in 5 min. To perform Chick-Martin Test (for phenolic disinfectants only)[8], a small quantity of about 3 ml of autoclaved yeast suspension was prepared and dissolved in 100 ml of distilled water to give a 3% v/v suspension. To 48 ml of this yeast suspension, 2 ml of 24 hour broth culture of *P. aeruginosa* was added. Lysol was diluted serially from 1:100, 1:200, 1:250,1:300 up to 1:350 and phenol was diluted from 1:95, 1:100, 1:105, 1:110 and 1:115. A 2.5 ml of Lysol dilution was mixed separately with 2.5 ml of the culture-yeast suspension. Lysol was made to act in the presence of yeast suspension to simulate the presence of organic matter. After a contact time of 30 minutes, a standard loop full of the lysol- *P. aeruginosa*- yeast suspension was transferred in duplicate to 10 ml of nutrient broth and incubated at 37°C for 48 hours; thereafter the presence or absence of growth was recorded. The Chick-Martin coefficient was calculated by dividing the concentration of phenol by the concentration of lysol at which similar presence or absence of growth was recorded.

Kelsey-Sykes Test[9,10] was conducted to determine concentrations of disinfectant that will be effective in clean and dirty conditions. The disinfectant was challenged by three successive additions of bacterial suspension during the course of the test. The duration of the test took over 30 minutes to perform[11,12]. In 'clean condition', a 24 hour broth culture of *P. aeruginosa* was prepared and was added to 3 ml of each disinfectant (Bacillocid, Sodium hypochlorite and Glutaraldehyde) respectively having concentration of 1% at time intervals of 0 minutes, 10 minutes and 20 minutes. After a contact time of 8 to 10 minutes, 0.2 ml of the disinfectant- *P.aeruginosa* mixture was transferred to 9 ml of sterile peptone broth containing Tween 20 in three sets of five replicates at time intervals of 8 minutes, 18 minutes and 28 minutes and labeled accordingly. All inoculated peptone broth tubes were incubated at 37°C for 48 hours after which the tubes were examined for growth (turbidity) or no growth. This procedure was repeated for all disinfectants at concentrations of 0.5% and 0.25% using the same time intervals of contact. In 'dirty condition', the procedure was same as for clean condition along with addition of autoclaved yeast cells to 3 ml of each of the disinfectants under test at concentrations of 1%, 0.5% and 0.25% with same time interval of contact.

Two types of surfaces were chosen- smooth and rough for Surface Disinfection Test[13,14] and the efficacy of the disinfectants on these surfaces was evaluated. For 'smooth surface', a 24 hour broth culture of *P. aeruginosa* was prepared and its turbidity was adjusted to 0.5 McFarland standard which is equal to 1.5×10^8 colonies per ml in normal saline. Four glass

slides of dimensions 7 cm x 3 cm were autoclaved out of which three slides were used for testing of each disinfectant (Bacillocid, Sodium hypochlorite and Glutaral-dehyde) and one slide was taken as a control and was labeled accordingly. Further, 0.25 ml of microbial suspension of *P. aeruginosa* was evenly spread over each of the labeled slides with a micropipette and was allowed to dry for 1 to 2 hours. To the three labeled slides, their corresponding disinfectants were applied by a sterile cotton gauge soaked in 5 ml of the respective disinfectant. One slide was left without disinfectant which was used as the control showing only the growth of the test organism i.e. *P. aeruginosa.* After a contact time of 10 to 15 minutes, all the four slides were swabbed using sterile swab sticks and each swab stick was vortexed in four tubes containing 5 ml of neutralizing broth. Serial dilutions of ranging from 1:10, 1:100 and 1:1000 of this mixture were made for each disinfectant and for the control. Five drops of 0.2 ml of each of the dilutions were dropped on Mueller Hinton agar plates and labeled specifically. This was performed for all the disinfectants as well as for the control. These agar plates were incubated for 48 hours for bacterial growth at 37°C and for 7 days at 22°C. After incubation, the number of colonies of microorganisms was counted on control and test slides, total counts were calculated and log reduction factor was calculated. For 'rough surface', while using same procedure, ceramic tiles of dimensions 5 cm x 5 cm were taken in place of glass slides. After incubation, the number of colonies of microorganisms was counted and total counts were calculated. For analysis of surface disinfection test, an average of multiple observations was taken and $\log_{10}$ reduction factor was calculated by using the following formula: Log_{10}Reduction Factor (RF) =

$$\text{Log}_{10}\text{Prevalue} - \text{Log}_{10}\text{Postvalue}.$$

To conduct 'In Use Test', a 1 ml sample of the disinfectant in its working concentration was added to 9 ml of diluent which was taken to be normal saline. Ten drops, each of 0.02 ml volume of the diluted sample are placed on each of the two nutrient agar plates. One is incubated at 37°C for three days and the other at room temperature for seven days. This procedure was performed for all the three disinfectants namely Bacillocid, sodium hypochlorite and glutaraldehyde. Five or more colonies on either plates indicated contamination.

All the above mentioned tests for evaluating efficacy of disinfectants were done in controlled and sterile conditions using the recommended working concentrations of each disinfectant. Accordingly, for each test, the results were calculated and statistically analyzed using standard statistical tests.

RESULTS:

At 1:100 and 1:200 dilutions of Lysol, growth was recorded at 2.5 minutes but not at 5 minutes and above for both dilutions Rideal Walker Test (Table 2). At dilution of 1:250 of Lysol, growth was observed at 2.5 and 5 minutes' contact time, but not at 7.5 and 10 minutes' contact time. At 1:95 dilution, growth was recorded at 2.5 minutes but not at 5 minutes and above. At dilution of 1:100 of Phenol, growth was observed at 2.5 and 5 minutes' contact time, but not at 7.5 and 10 minutes' contact time. Thus, giving a Rideal-Walker coefficient of 2.5. Chick-Martin coefficient, the ratio of 'concentration of phenol in which growth was seen' and 'concentration of Lysol in which growth was seen', was found to be 0.95/0.4= 2.3 by Chick Martin Test (Table 3). "+" denotes growth in the recovery medium and "-" denotes no growth in the recovery medium.

Table 2: Determination of Rideal-Walker coefficient for Lysol and Phenol using *Pseudomonas aeruginosa*as test organism.

Disinfectant	Dilution of Disinfectant	Contact time with culture (in minutes)			
		2.5	5	7.5	10
Lysol	1:100	+	-	-	-
	1:200	+	-	-	-
	1:250	+	-	-	-
	1:300	+	+	+	+
	1:350	+	+	+	-
Phenol	1:95	+	-	-	-
	1:100	+	+	-	-
	1:105	+	+	-	-
	1:110	+	+	+	-
	1:115	+	+	+	+

"+" denotes growth in the recovery medium and "-"denotes no growth in the recovery medium.

Rideal-Walker coefficient = Highest dilution of Lysol that show growth in 5 min but not in10 min / Highest dilution of Phenol that show growth in 5 min but not in 10 min

The Rideal-Walker coefficient was found to be 250/100 = 2.5

Table 3: Determination of Chick-Martin coefficient for Lysol and Phenol.

Disinfectant	Concentration (%)	Subcultures	
		1	2
Lysol	1	-	-
	0.5	-	-
	0.4	+	+
	0.33	+	+
	0.28	+	+
Phenol	1.05	-	-
	1	-	-
	0.95	+	+
	0.90	+	+
	0.87	+	+

"+" denotes growth in the recovery medium and "-" denotes no growth in the recovery medium.
Chick-Martin coefficient =
Concentration of phenol in which growth was obtained
Concentration of Lysol in which growth was obtained

The Chick-Martin coefficient was found to be 0.95/0.4= 2.3.

Table 4: Results of Kelsey-Sykes Test

A. Clean Condition			
Disinfectant	Growth in contact time after subculture		
	8 min	18 min	28 min
Bacillocid	---++	----+	-----
Sodium Hypochlorite	-----	---++	--+++
Glutaraldehyde	----+	---++	-++++
B. Dirty Condition			
Disinfectant	Growth in contact time after subculture		
	8 min	18 min	28 min
Bacillocid	-----	-----	-----
Sodium Hypochlorite	----+	---++	---++
Glutaraldehyde	---++	---++	--+++

At the working concentration of the disinfectant used in both clean and dirty conditions, the results of the growth obtained after subculture showed in Kelsey-Sykes Test (Table 4) that Bacillocid had good antimicrobial activity challenged at time intervals of 8 minutes, 18 minutes and 28 minutes as compared tothat of Sodium hypochlorite and Glutaraldehyde which showed reduced antimicrobial activity as increasing bacterial growth was obtained at increasing time intervals of 18 to 28 minutes. The 'Surface Disinfection Test' done on smooth and rough surfaces in Surface Disinfection Test: (Table 5 and Table 6) showed that Bacillocid was effective on both the surfaces. On statistical analysis, it was seen that p value was 0.8371 using the formula $Log_{10}RF$ *(Reduction Factor)* $= Log_{10}PreV$ *(Pre value)* $- Log_{10}$ *Postv (Post value),* which was not statistically significant. Efficacy of the disinfectants was estimated in In- Use Test: (Table 7) on the principle that five or more colonies on either of the subculture plates indicated contamination. Bacillocid had good efficacy at both room temperature and at 37°C in its working concentration.

DISCUSSION:

The efficacy of four disinfectants was compared by different standard methods like Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test.

The Rideal-Walker coefficient in the present study study was 2.5. In a study by Elias *et al.* in 2013, they studied efficacy of disinfectants such as Izal and Dettol with phenol, and got a Rideal-Walker coefficient of 2.5[10]. The present study is comparable with the findings of Elias *et al* of 2013. Phenolic disinfectants that are more effective than phenol have a coefficient more than 1. Those that are less effective have a coefficient less than 1.

In the study, Chick-Martin test was performed where yeast cells were used and *P. aeruginosa* which acted as a test organism. We found that the Chick-Martin coefficient was 2.3. However, in a study by Elias *et al.* in 2013, they found the Chick-Martin coefficient for Izal and Dettol when compared to phenol to be 2.

Kelsey-Sykes test showed that Bacillocid achieved 0% culture positivity after disinfection or 100% high level disinfection compared to Glutaraldehyde and Sodium hypochlorite. The culture positivity in clean and dirty conditions was determined. The findings of the study are comparable with a study by Malkit Singh *et al(2012)* who found bacillocid to be a very effective antimicrobial agent on both the test conditions [13].

In the present study, an attempt was made to evaluate the efficacy of the commonly used disinfectants in a healthcare setup by different standard methods. Awodele *et al 2007* from Nigeria did a study using similar organisms and methylated spirit, sodium hypochlorite, savlon, kerosene as disinfectants and revealed that savlon was a 100% effective microbicide[15]. Miles R Majcher *et al* 2008 from Canada studied sporicidal activity of sodium hypochlorite, accelerate hydrogen peroxide, chlorine

Table 5: Results of surface disinfection test on smooth surface

Concentration	Disinfectant	Log10 Reduction Factor	Efficacy
1:10	Glutaraldehyde	0.6353	Less effective
	Sodium Hypochlorite	0.6540	Less effective
	Bacillocid	0.9489	Effective
1:100	Glutaraldehyde	0.0366	Less effective
	Sodium hypochlorite	0.0757	Less effective
	Bacillocid	0.0910	Effective
1:1000	Glutaraldehyde	0.0191	Less effective
	Sodium hypochlorite	0.0617	Less effective
	Bacillocid	0.0793	Effective

Table 6: Results of Surface disinfection test on rough surface

Concentration	Disinfectant	Log10 Reduction Factor	Efficacy
1:10	Glutaraldehyde	0.9617	Less effective
	Sodium hypochlorite	0.8353	Less effective
	Bacillocid	1.0136	Effective
1:100	Glutaraldehyde	0.0275	Less effective
	Sodium hypochlorite	0.0139	Less effective
	Bacillocid	0.6504	Effective
1:1000	Glutaraldehyde	0.0234	Less effective
	Sodiumh ypochlorite	0.0101	Less effective
	Bacillocid	0.0495	Effective

Table 7: Results of In-Use test.

Disinfectant	Subcultures (No. of colonies)		Efficacy
	1 (Room temp.)	2 (At 37°C)	
Control	>5 colonies	>5 colonies	-
Glutaraldehyde	>5 colonies	>5 colonies	Not effective
Sodium hypochlorite	>5 colonies	>5 colonies	Not effective
Bacillocid	3 to 4 colonies	No growth	Effective

dioxide, peracetic acid and found peracetic acid acting fastest followed by hypochlorite and accelerated hydrogen peroxide[16]. In the study, when microbicidal activity was studied for four different disinfectants by standard methods, bacillocid was shown to have good antimicrobial activity at a given working concentration and specific contact time.

The effective use of disinfectants constitutes an important factor in preventing hospital acquired infections. The organism tested was known to be a common contaminant and colonizer of patients, Intensive Care Units, Operation Theatres, laboratory surfaces, etc. In the study, *P. aeruginosa* was the most common pathogen in the hospital setup. Hence, we used this as a test organism. Among all the tests employed for testing efficacy of disinfectants, we found that Bacillocid was more effective than the other three disinfectants studied.

There were times when no disinfection policies were in place for the use. The situation has changed markedly in the recent era and many hospitals do have such policies, but implementation is unsatisfactory. The study suggests the need for strict vigilance by the authorities over the local products. Also, there is a need to test the quality of disinfectants routinely supplied to the laboratory or hospital to ensure proper control of infections by using the right disinfectant in right concentration for a right contact time. In all the tests performed for evaluating the efficacy of disinfectants. It was seen that bacillocid was more effective than sodium hypochlorite and glutaraldehyde when tested under different conditions and in the working concentration in which they were used in the hospitals.

In Rideal-Walker test, phenolic agents were tested for their efficacy. Although phenolic agents exhibit high toxicity and low biodegradability, those are still in use in developing countries because of their low cost. Phenolic agents showed poor activity as microbicides. Phenolic agents cannot be used in neonatal and pediatric ICU as they cause eye irritation, contact dermatitis/ urticarial and depigmentation of the skin.[17,18] In this study, phenolics showed poor activity, therefore, better and safer disinfectants are required to replace them. Capacity test of Kelsey-Sykes evaluates the efficacy of disinfectant in clean and dirty conditions. The disinfectants were subjected to a triple challenge test along with the test organism, each consisting of five subcultures. The disinfectant that showed minimal or no growth in the subcultures at specific contact time intervals proved to be more effective than the others. Bacillocid proved to be an effective disinfection agent in comparison with that of glutaraldehyde and sodium hypochlorite. In surface disinfection test, p value was statistically not significant. Bacillocid was found to be an effective antimicrobial agent on the tested organism and on both types of surfaces. In-use test helped to evaluate the disinfectant efficacy used in its working concentration. In the study, Bacillocid passed the test and showed high antimicrobial activity.

CONCLUSION:

Although significant progress has been made with bacterial investigations, the mode of action of

antiseptics and disinfectants still remains to be deciphered. It will also make for efficient use of these agents clinically with the potential for design of newer, more effective compounds and products. An ideal disinfectant, being easily available, non-toxic, non-corrosive, easy to use and cost effective, is difficult to find. Hence, there is need for formulation, implementation and supervision of a comprehensive disinfection policy in the country.

LIMITATIONS OF THE STUDY:

The tests for anti-mycobacterial and antiviral effects of any of the test disinfectants were not conducted. In addition, each test performed here for efficacy testing of disinfectants has its own limitations. Those are cumbersome and complex and Efficacy of locally available disinfectants largely depends on manufacturers' instructions.

REFERENCES:

1. Reybrouck G. Milestones in the testing of surface disinfectants: from Robert Koch to CEN TC 216. GMS Krankenhhyg. Interdisziplinar.2007;2(1).
2. Mckenry LM, Salerno E. Pharmacology in nursing. 21st Edn., Mosby. 2001. pp.1198-1208.
3. MacDonnell G, Russell D. Antiseptics and disinfectants: activity, action and resistance. Clin Microbiol Rev. 1999;12(1):147-179.
4. Mandell Z, Nikaido H, Pool K. Role of mexA-mex B-opr min antibiotic efflux in Pseudomonas aeruginosa. Antimicrob. Agents. Chemother. 1995;39.1984-1953.
5. Schorer M, Eisele M. Accumulation of inorganic and organic pollutants by biofilms in the aquatic environment. Water Air Soil Pollut. 1997; 99:651-659.
6. BSG Guidelines for decontamination of equipment for gastrointestinal Endoscopy. BSG Working party Report. 2003. Available from: http://www.bsg.uk/pdf_word_docs/disinfection.doc. [Last accessed] Accessed on 01.04.2018.
7. Rideal S, Walker JT. The standardization of disinfectants. J R Sanit Inst.1903;24:424-41.
8. Garrod LP. A study of the Chick-Martin test for disinfectants. J Hyg (Lond.) 1934;34(3):322-32.
9. Kelsey JC, Sykes G. A new test for the assessment of disinfectants with particular reference to their use in hospitals. Pharm J. 1969;202:607-609.
10. Elias EA, Samuel O[b], Emmanuel N, Abrahama O. Evaluation of efficacy of disinfectants using standard methods in healthcare facilities in Kogi state, Northcentral Nigeria. Asian J Biomed Pharmac Scien. 2013;03(27);34-38.
11. Reybrouck G. The testing of disinfectants. International Biodeterioration & Biodegradation. 1998;41,269-272.
12. Gardner JF, Peel MM. Introduction to sterilization and disinfection control. 2nd Edn.; Melbourne: Churchill Livingstone;1991.pp.267.
13. Singh M, Sharma R, Gupta PK, Rana JK, Sharma M, Taneja N. Comparative efficacy evaluation of disinfectants routinely used in hospital practice: India. Indian J Crit Med. 2012;16(3):123-129.
14. Kawamura-Sato K, Wachino J, Kondo T, I to H, Arakawa Y. Correlation between reduced susceptibility to disinfectants and multi drug resistance among clinical isolates of Acinetobacter spp. J Antimicrob Chemother. 2010; 65:1973-83.
15. Awodele O, Emeka PM, Agbamucle HC, and Akintonwa A. The antimicrobial activities of some commonly used disinfectants on Bacillus subtilis, Pseudomonas aeruginosa, and Candida albicans. Afr J Biotech. 2007; 6: 987-990.
16. Majcher MR, Bernard KA, and Sattar SA. Identification by quantitative carrier test of surrogate spore forming bacteria to assess sporocidal chemicals for use against Bacillus anthracis.Appl Environ Microbiol. 2008;74: 676-681.
17. Rutula WA. APIC guidelines for selection and use of disinfectants. Am J Infect Control. 1996; 24:313-42.
18. Pierson J. Choosing a disinfectant for hospital environment. Indoor Environment Connections. 2009. Available from www.ieconnection.com /pdfs/newsletter/2009/IEC-04-2009.pdf. Accessed on 30.11.2018.

Cite this article as: Gajbhiye S & Mahalle R: Health Facility Based Efficacy Assessment of In-use Disinfectants . PJSR ;2019:12(1):32-37.
Source of Support : Nil, Conflict of Interest: None declared.