Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2016/493

Original Article

Evaluation of Hospital Supply and Locally Available Disinfectants by In-use Test in Bacteriology Laboratory of Tertiary Care Hospital

Srijita Ghosh[1], Rupali Dey[2], Jayanta Bikash Dey[3], Sayoni Datta[1], Angshuman Jana[4]

[1]Post Graduate Student, Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India, [2]Associate Professor, Department of Microbiology, Burdwan Medical College, Burdwan, West Bengal, India, [3]Professor and Head, Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India, [4]Assistant Professor, Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India

Abstract

Introduction: Hospital-acquired infection (HAI) is an infection that is contracted in the hospital environment or any other clinical settings where infection is spread to the susceptible patients from health-care staff, contaminated equipment, and environment. The use of disinfectants is important in preventing HAI. Rationalization of the use of disinfectants in hospitals is desirable for the purpose of proper quality control measurement. Because of potency loss on standing and presence of organic matter, disinfectants used in hospital laboratory must be tested periodically. Although the various methods of testing disinfectants have the same final purpose of measuring the antimicrobial activity, some of them help in selecting right dilution of disinfectants for use and other evaluate the efficacy of disinfectants which are already in use. For quality control in clinical bacteriology laboratory "in-use" test is recommended.

Purpose: The aim of this study was to analyze the efficacy and costs of the three disinfectant materials-conventional liquid hospital supplied phenol (1%), locally acquired phenol crystal (2 m%), conventional hypochlorite solution (1%), and pitting them against another conventional and standard disinfectant of 2% hospital supplied liquid phenol.

Materials and Methods: All the disinfectants at selected proposed dilutions were tested for bactericidal efficacy by "in-use" test. This test was used to detect the number of living organisms in a vessel of disinfectant solutions which were in actual use. It was performed on disinfectants in discard jars using a standard protocol.

Results: The study shows 1% conventional hypochlorite solution and 2% local phenol crystals are equally efficient bactericidal with that of the 2% conventional liquid phenol. Hypochlorite solution is most costly where phenol crystal is cheap and available locally.

Conclusion: Instead of expensive aldehyde containing commercially available agents, conventional liquid phenol, and locally available phenol crystal with their comparable low cost and similar efficacy can be used.

Key words: Bacteriology, Disinfectants, Hypochlorous acid, In-use test, Phenol, Quality control

INTRODUCTION

Nosocomial infections, also called "hospital-acquired infections" (HAI), are infections acquired during hospital care which are not present or incubating at admission. Infections occurring more than 48 h after admission are usually considered nosocomial. They are based on clinical and biological criteria and include approximately 50 potential infection sites such as urinary tract infections, surgical site infections, nosocomial pneumonia, nosocomial bacteremia, and others.[1] Nosocomial/hospital acquired/healthcare associated infection occurs worldwide at the rate of 5-10%.[2] The use of disinfectants is important in hospital infection control as failure can result in many such HAI leading to increased cost, mortality, morbidity.[2]

Access this article online

www.ijss-sn.com

Month of Submission : 07-2016
Month of Peer Review : 08-2016
Month of Acceptance : 09-2016
Month of Publishing : 09-2016

Corresponding Author: Dr. Srijita Ghosh, 71/A, Dr. Nilmoni Sarkar Street, Anurodh Apartment, Baranagar, Kolkata - 700 090, West Bengal, India. Phone: +91-8013843039/9433192949. E-mail: ghosh.srijita@gmail.com

Disinfectants can be used either as surface disinfectants or by immersing the contaminated objects in the solution.[3] The activity of disinfectants as germicides against micro-organisms depends on intrinsic qualities of the organism like number and location of microorganisms, innate resistance of microorganisms along with external physical environmental and chemical factors such as temperature, contact period, pH, potency and concentration of disinfectant, bioburden, organic soil and hardness of water used for dilution, biofilm.[3,4] Efficiency and potency of disinfectants depend on each of these factors. There is least awareness among the healthcare providers about choosing an appropriate disinfectant, especially in small health care settings of developing countries. Usually, an agent with wide-range of antimicrobial activity is selected based on the literature provided by the manufacturers.[3]

While certain methods help in selecting right dilution of disinfectants for use, others test the efficacy of disinfectant already in use. For evaluation of their activity, various tests are available like minimum inhibitory concentration (which is the lowest concentration of the disinfectant that inhibits the growth of a known strain of bacteria), Rideal-Walker test,[4] Chick-Martin and Garrod tests (based on the phenol coefficient of disinfectants),[5] capacity-use-dilution test (Kelsey and Sykes, 1969),[6] modified by Kelsey and Maurer in-use test,1974 (measure the efficacy of disinfectants already in use for a particular period and condition),[7] stability test and various other microbial time kill assay.[6] Some other tests such as suspension test (qualitative and quantitative), practical tests, and surface killing test are also to be mentioned.[8] As all these standard tests cannot be performed by the laboratories belonging to small hospitals, one has to be solely dependent on the literature provided by the manufacturer regarding the efficiency of the disinfectants which are usually broad-spectrum antimicrobial agent suitable for various applications.[3]

In view of the above, the following short study was planned with an aim to evaluate and to compare the practically achieved bactericidal efficacy and cost effectiveness of hospital supplied disinfectants with some locally available disinfectant product in the bacteriology laboratory of a tertiary care rural hospital. The efficacy was tested by performing Kelsey and Maurer's in-use test.

MATERIALS AND METHODS

In the absence of any universally agreed test methods and to prevent malpractice by the manufacturers Kelsey and Maurer's "in-use" test was chosen to establish the efficacy of disinfectants at the in-use concentration against a significant bacterial challenge at an ambient temperature.[9]

This study was conducted at bacteriology laboratory of Bankura Sammilani Medical College Hospital, Bankura as a laboratory-based experimental study over 3 months. Effectiveness of two conventionally used hospital supplied disinfectants (phenol in two different concentrations and 1% hypochlorite solution) and one locally available disinfectant reagent were studied using this test.

The hospital supplied conventional disinfectants were phenol (80% W/V) manufactured by Indian Drug House (West Bengal, India) taken in two different concentrations of 1% and 2% and hypochlorite solution (4%) manufactured by Stanbio Reagent Pvt. Ltd. (Kolkata, India) used in a concentration of 1%. Locally purchased commercially available phenol/carbolic acid crystal manufactured by New Bengal Drug House (Kolkata, India) was used for comparison at a concentration of 2% (Figure 1).

For the biomedical wastes from bacteriology and tuberculosis laboratory, the recommended effective concentration of phenol for spillage or cleaning is 2%.[9] Phenol from hospital supply was used in this study in two concentrations, the recommended 2% and also 1% solution (as certain references have mentioned this to be bactericidal by leaking the amino acids from the bacterial cell.).[10] Furthermore, 2% solution was made from locally acquired phenol crystals. Hospital supplied phenol was also taken in 1% concentration for comparison as it acts as bactericidal. Hospital supplied conventional 1% hypochlorite solution was also chosen as it is appropriate for spillage and where contaminants are present.[9,18]

Considering 2% liquid phenol from hospital supply as the gold standard, bactericidal efficacy and cost-effectiveness

Figure 1: (a) Hospital supplied liquid phenol. (b) Hospital supplied hypochlorite solution. (c) Locally purchased phenol crystal. (d) SET 1: Liquid phenol 2% versus liquid phenol 1%. (e) SET 2: Liquid phenol 2% versus locally acquired phenol crystal 2%. (f) SET 3: Liquid phenol 2% versus hypochlorite solution (1%)

of the other three solutions were tested and compared. These were hospital supplied liquid carbolic acid (1%) having market price of Rs. 130 of 500 ml (80% w/v), locally acquired phenol crystal (made up to 2 m%) with the cost of Rs. 30/100 g, hospital supplied hypochlorite solution (1%) having cost Rs. 95/500 ml of 4% solution (Table 1).

Hypochlorite solution decays rapidly and readily gets inactivated by organic material and light and also it is corrosive to metal at high concentration (more than 0.05%). Therefore, this test was performed with freshly prepared disinfectant using distilled water in dark glass jars each containing 500 ml of disinfectant solutions in the specific concentrations mentioned. These solutions were left overnight as recommended in these jars after dropping bacteriological contaminated materials in them throughout the working hours.[10] The next day, 1:10 dilution of the contents of the jars was made in nutrient broth so that the tested disinfectants became neutralised.[9] Each of the nutrient agar plates (product number M001., HiMedia Laboratories (P) Ltd. Mumbai, India) was marked at 10 different sites and with a "50-dropper" pipette, and 10 small drops each of 0.02 ml were then transferred in those 10 different areas of two well dried nutrient agar plates. For each set, one plate was then incubated at 37°C for 3 days while the other was held at room temperature for 7 days. The number of drops that yielded growth was counted after incubation. If growth was more than five drops on either plate, it represented failure of disinfectant.[8,9] Such a result was considered as approximately 1000 living organisms 1 ml in the tested sample of disinfectant.[21] In the study, each disinfectant solution was paired with the gold standard, that is 2% hospital supply phenol and each pair was tested daily in the bacteriology laboratory for 14 days. Therefore, three such pairs were tested over 6 weeks in the first cycle. The whole process was then repeated in the next 6 weeks. Hence, two such cycles of the study were completed in 3 months (Figure 1).

For performing the test, that is, to prepare 500 ml of each of the disinfectant solutions 28 times (14 times each for two cycles) it costed Rs. 532 for 1% hypochlorite solution (market price Rs. 95/500 ml of 4% hypochlorite solution), Rs. 91 and Rs. 45.5 for 2% and 1% conventional hospital supplied liquid phenol, respectively, as this liquid phenol had market price Rs. 130/500 ml of 80% (w/v) solution. The cost of making 500 ml of 2% phenol from locally purchased phenol crystal was Rs. 84 (market price Rs. 30/100 g) (Table 1).

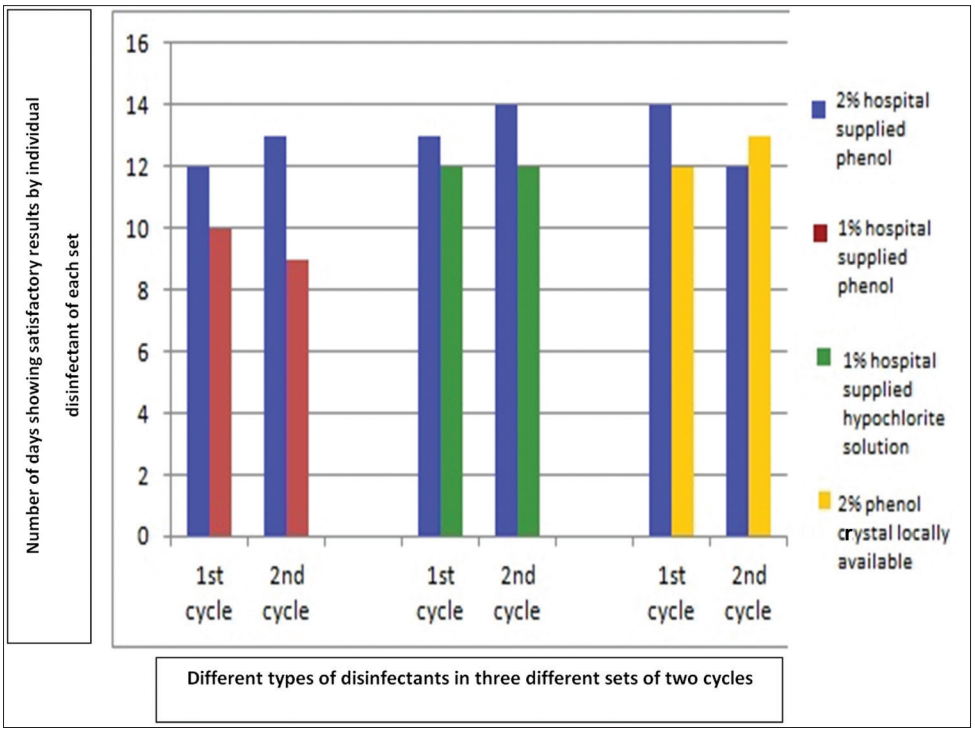
RESULTS

Hospital supplied liquid phenol (2%) that is the gold standard in this study consistently showed growth not more than five colonies per plate for at least 12 days out of the 14 days cycle in all the tests. Hospital supplied liquid phenol (1%) showed satisfactory result that is growth of not more than five colonies per plate for 10 out of 14 days and nine out of 14 days in the consecutive two cycles, respectively. Hypochlorite solution (1%) showed growth of not more than five colonies per plate for 12 out of 14 days in both the cycles. Whereas, 2% solution made from locally available phenol crystal showed a satisfactory result of not more than five colonies per plate for 12 out of 14 days and 13 out of 14 days for the two cycles, respectively (Table 2 and Graph 1). The colonies on examination showed growth of *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa* (Figure 2). 2% liquid phenol which is recommended for use in tuberculosis and general bacteriological laboratory was chosen as the gold standard to validate this study.[9] In terms of efficacy, hypochlorite solution (1%) showed satisfactory result. Therefore, it can substitute the 2% hospital supplied liquid phenol. Efficacy of 2% locally available phenol crystal is comparable to the 2% liquid phenol. On the other hand, 1% liquid phenol shows comparatively less bactericidal effect than

Figure 2: (a) Growth in ≤5 drops/plate. (b) Growth in >5 drops/plate

Table 1: Cost of disinfectants

Disinfectants	Market price	Total costs to prepare 500 ml of such tested solution 28 times	Proportion (in terms of cost)
1% hypochlorite solution	Rs. 95/500 ml of 4% hypochlorite solution	Rs. 532	5.85
2% hospital supplied liquid phenol	Rs. 130/500 ml of 80% (w/v) solution	Rs. 91	1.0
1% hospital supplied liquid phenol	Rs. 130/500 ml of solution of 80% (w/v)	Rs. 45.5	0.5
2% locally purchased phenol crystal	Rs. 30/100 g	Rs. 84	0.92



Graph 1: Overall comparative efficacy of three disinfectants in four different concentrations by in-use test method, considering 2% phenol liquid as the standard one

Table 2: Effectiveness of disinfectant

Disinfectants	1st cycle satisfactory result (days)	2nd cycle satisfactory result (days)
In 1st set		
Hospital supplied phenol (2%)	12	13
Hospital supplied phenol (1%)	10	9
In 2nd set		
Hospital supplied phenol (2%)	13	14
Hospital supplied hypochlorite solution (1%)	12	12
In 3rd set		
Hospital supplied phenol (2%)	14	12
Locally available phenol crystal (2%)	12	13

that of the standard 2% phenol Graph 1. As 1% phenol had poor bactericidal effect, it was considered to be unsuitable for the use in bacteriology and tuberculosis laboratory and cost effectiveness was not considered. 1% hypochlorite shows the same efficacy with that of the 2% liquid phenol buth as higher cost whereas cost of 2% phenol made up from the locally available crystal is comparable or little less than the hospital supplied liquid phenol. In comparison to 2% hospital supplied liquid phenol (standard) and 1% hypochlorite proportion of cost is 5.85, whereas it is 0.92 with 2% phenol from locally purchased phenol crystal Table 1.

DISCUSSION

Because of increasing resistance to antimicrobials and even disinfectants as evident in some recent international scientific research studies,[11-15] it is essential to perform efficacy testing of disinfectants in a regular manner in health care facilities. A study in Summerfield Hospital at Birmingham in 1972 showed satisfactory assessment of the contamination of disinfectants by the "in-use" test, along with membrane filtration technique.[22] Another study in Belgium reported the field test or the in-use test as one of the important tests for disinfectant.[23] Some earlier literature study revealed that there was no WHO recommended clear number of days before discarding disinfectants in the in-use method.[24] Some of the researchers showed antimicrobial activities of disinfectants were concentration dependent. They confirmed that if appropriate concentrations are not used even in the in-use testing, there will be contamination of disinfectants by various organisms like *P. aeruginosa*, *S. aureus*, and *Proteus* spp.[22,25-27] A recent study in Nigeria reported the degree of failure of a disinfectant is highest in constant use at beyond 2 weeks of the use. This finding clearly mentioned the need to periodically check the effectiveness of in-use disinfectant solutions for the purpose of early detection of the threat of disinfectant failure.[24,28] Our study results reveal that phenol must be used at a concentration of 2% otherwise its efficacy will be reduced. Alternatively, 1% hypochlorite solution can be used though it is not cost-

effective. Moreover, some of the previous studies highlighted its ineffectiveness against organic materials.[19,20] According to the logistic management, activity-based costing technique, which is a widely accepted and utilized method of production costing was used to calculate cost effectiveness. This balances two basic targets that are quality of service and low cost.[18] Our study result showed, 2% phenol, made from phenol crystal can be good alternative both in terms of efficacy and cost-effectiveness. Therefore, it can easily be used in remote health care setups where central supply may be erratic or those having limited resources**.** Proper biomedical waste disposal is an important aspect for control and prevention of HAI. As this in-use test shows reproducible result, it can be easily applied in the peripheral laboratory set ups for quality control purpose where other standard tests cannot be performed so easily.[2] Other germicides healthcare institutions should also be thoroughly evaluated for their efficacy and cost-effectiveness.[16]

Limitation of this study is that we tested only for the bactericidal effect of few disinfectants, but the considerable data generated from this type of study can be utilized for making hospital infection control policy.

CONCLUSION

Although the utility of high-level disinfection and sterilization mandates effective clearing, no single real-time test exists to employ in a clinical setup to verify proper disinfectant.[17] Our study concludes that the most simple and acceptable method of in-use test can be performed in hospital laboratory for the biomedical wastes (category 3) to confirm the chosen disinfectant which has been effective under specific conditions along with its duration of use.

Along with the testing method, this short study also generated suitable data regarding bactericidal efficacy for the different type's disinfectants which can be utilized for hospital infection control policy. Therefore, in this era of expensive commercially available aldehyde containing disinfectants such as Des Net, Hi-giene, Clea-N-sept, Bacillocid special, and nonaldehyde containing newer hospital disinfectants such as Virkon, Novacide, Silvicide,[3] locally available phenol crystal (used domestically to avoid snake) with its least cost, availability and good bactericidal efficacy can replace others to equip discard jars especially in rural laboratory setup if hospital supplied phenol is not available.

Therefore, this study is relevant regarding the selection of the appropriate disinfectants in the bacteriology laboratories with their effective concentration and period of use, cost and availability. Along with this, the "in-use" technique is proved to be one of the simplest methods of testing the disinfectants, which is already in use in bacteriology laboratory for the purpose of maintain good quality control in biomedical waste disposal.

ACKNOWLEDGMENT

I thank the faculties, staff and my colleagues of the Department of Microbiology, B.S.M.C.H., Bankura.

REFERENCES

1. World Health Organization. Department of Communicable Disease, Surveillance and Response. Prevention of Hospital-Acquired Infection: A Practical Guide. WHO/CDS/CSR/EPH/2002.12. Geneva: WHO; 2002.
2. Chakraborty B, Pal NK, Maiti PK, Patra SK, Ray R. Action of newer disinfectants on multidrug resistant bacteria. J Evol Med Dent Sci 2014;3:2797-813.
3. 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 Care Med 2012;16:123-9.
4. Rideal S, Walker JT. The standardisation of disinfectants. J R Sanit Inst 1903;24:424-41.
5. Garrod LP. A study of the chick-martin test for disinfectants. J Hyg (Lond) 1934;34:322-32.
6. Kelsey JC, Sykes G. A new test for the assessment of disinfectants with particular reference to their use in hospitals. Pharm J 1969;202:607-9.
7. Kelsey JC, Maurer IM. An improved Kelsey Sykes test for disinfectants. Pharm J 1974;213:528-30.
8. Rao PN. Testing of disinfectants. Available from: http://www.microrao.com/micronotes/pg/testing_of_disinfectants.pdf.
9. Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. New Delhi: Elsevier; 2008.
10. Gale EF, Taylor ES. The assimilation of amino-acids by bacteria; the action of tyrocidin and some detergent substances in releasing amino-acids from the internal environment of *Streptococcus faecalis*. J Gen Microbiol 1947;1:77-84.
11. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Comparative biocidal activity of peracetic acid, benzalkonium chloride and ortho-phthalaldehyde on 77 bacterial strains. J Hosp Infect 2011;78:208-13.
12. Stewart PS, Rayner J, Roe F, Rees WM. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J Appl Microbiol 2001;91:525-32.
13. Rajamohan G, Srinivasan VB, Gebreyes WA. Molecular and functional characterization of a novel efflux pump, AmvA, mediating antimicrobial and disinfectant resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:1919-25.
14. Lorena NS, Duarte RS, Pitombo MB. Rapidly growing mycobacteria infection after videosurgical procedures - The glutaraldehyde hypothesis. Rev Col Bras Cir 2009;36:266-7.
15. Batra R, Cooper BS, Whiteley C, Patel AK, Wyncoll D, Edgeworth JD. Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant *Staphylococcus aureus* in an intensive care unit. Clin Infect Dis 2010;50:210-7.
16. Vij A, Kant S, Gupta S. Analytic evaluation of cleaning agents and disinfectants in use for housekeeping practices at tertiary care hospital. J Acad Hosp Adm 2001;13:1-6. Available from: http://www.indmedica./journals.php?journalid=6&issueid=19&articleid=154&action=article.
17. Naik R. Disinfection and Testing for Disinfection [Presentation] Andhra Medical College. 18th February; 2013.
18. Bokor Z. Integrating logistics cost calculation into production costing. Acta Polytech Hung 2012;9:163-81.
19. Bloomfield SF, Miller EA. A comparison of hypochlorite and phenolic disinfectants for disinfection of clean and soiled surfaces and blood

spillages. J Hosp Infect 1989;13:231-9.
20. Chitnis V, Chitnis DS, Patil S, Chitnis S. Hypochlorite (1%) is inefficient in decontaminating blood containing hypodermic needles. Indian J Med Microbiol 2002;20:215-8. Available from: http://www.ijmm. org/text.asp?2002/20/4/215/6960. [Last cited on 2015 Jun 23].
21. Cruickshank R, Duguid JD, Maramar BR, Swain RH. Medical Microbiology. 12th ed., Vol. II. London: Churchill Livingstone; 1975.
22. Prince J, Ayliffe GA. In-use testing of disinfectants in hospitals. J Clin Pathol 1972;25:586-9.
23. Reybrouck G. The testing of disinfectants. Int Biodeterior Biodegradation 1998;41:269-72.
24. Akabueze EC, Obi SC, Nwankwo EO. Evaluation of disinfectants by the in-use technique in healthcare facilities in Kogi State North central Nigeria. Nig J Microbiol 2015;28:2833-7.
25. Atoyebi OA, Niemogha MT, Odugbemi T, Oyewunmi OK. Bacterial pathogen isolated after surgical hand scrub at Lagos University Teaching Hospital. J Nig Infect Control Assoc 1999;2:19-23.
26. Okesola P, Abiola O, Olola O, Aderonke F. The efficacy of the commonly used hospital disinfectants on *Pseudomonas aeruginosa*. Int Res J Microbiol 2011;2:226-9.
27. Awodele O, Emeka P, Agbamuche H, Akintowa A. The antimicrobial activities of some commonly used disinfectants on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. Afr J Biotechnol 2007;6:887-990.
28. Oie S, Kamiya A. Microbial contamination of antiseptics and disinfectants. Am J Infect Control 1996;24:389-95.

How to cite this article: Ghosh S, Dey R, Dey JB, Datta S, Jana A. Evaluation of Hospital Supply and Locally Available Disinfectants by In-use Test in Bacteriology Laboratory of Tertiary Care Hospital. Int J Sci Stud 2016;4(6):93-98.

Source of Support: Nil, **Conflict of Interest:** None declared.