RESEARCH ARTICLE

Efficacy study of some antiseptics and disinfectants

Raut Gargi[1], Pimpliskar Mukesh R[2] , Vanmali HS and Jadhav Rahul[1]

[1]Vidyavardhini's Zoology Research Laboratory, E. S. A. College of Science, Vasai Road. 401 202.Dist: Palghar, MS, India
[2]K. M. Es, G. M. Momin Womens College, Bhiwandi, Thana road Dist- Thana 421 302, MS, India
Corresponding Authors E.mail:- jadhav2010@rediffmail.com | mukesh227@yahoo.co.in

Manuscript details:

Received: 18.08.2017
Revised 18.09.2017
Accepted: 14.11.2017
Published : 05.12.2017

Editor:
Dr. Arvind Chavhan

Cite this article as:
Raut Gargi, Pimpliskar Mukesh R, Vanmali HS and Jadhav Rahul (2017) Efficacy Study of some Antiseptics and Disinfectants; *International J. of Life Sciences*, 5 (4): 593-598.

ABSTRACT

Antiseptics and Disinfectants are widely used in hospitals and other health care centers to control the growth of microbes on both living tissues and inanimate objects. Different pathogens responded different antiseptics and disinfectants. The phenol coefficient was also done to check comparative account with antiseptic and disinfectant with reference to time of killing the pathogens. Antibacterial effects of the antiseptics and disinfectants were also concentration dependent.

Keywords: Efficacy, Antiseptic, disinfectants, bacterial contamination, infection.

INTRODUCTION

Day by day importance of sanitation and thereby use of antiseptics and disinfectants increased in routine life of mankind. Antiseptics and disinfectants are used extensively in hospitals and other health care centers to control the growth of microbes on both living tissues and inanimate objects. They are essential parts of infection control practices and aid in the prevention of nosocomial infections (Larson and Morton, 1991). But a common problem is the selection of disinfectants and antiseptics because different pathogens vary in their response to different antiseptics or disinfectants (Russell, 1996).

Over the last few years alcohol-based hand disinfectants have become widely available within health care, providing an alternative means of achieving good hand de-contamination. In the hospital setting their advantage over soap and water is that they can be applied in transit to the next patient or task and therefore may help improve compliance with hand decontamination. Within the community setting they provide a suitable alternative to hand washing, particularly where there may be inadequate hand washing facilities (Pratt *et al*., 2001). It is well known that hand hygiene is a crucial factor in the control of health care-acquired infections (HCAIs) (Boyce and Pittet, 2002). This is because hands may readily become contaminated with transient micro-organisms during the delivery of health care. Transient flora such as *Staphylococcus aureus* are microorganisms colonizing the superficial outer layers of the skin, and may be readily removed by hand washing (NDAC, 2005). Equally, where hand hygiene is

poor these micro-organisms may be transmitted from the hands of one patient to another. Hands contaminated by the hospital environ-ment can also contribute to HCAIs (Boyce and Pittet, 2002).

Recently, the FDA has divided into healthcare antiseptics, food handler antiseptics and consumer antiseptics. It has also been decided that all antiseptic products that include antimicrobial labeling, i.e. kills the germs that cause body odor, are drugs and are required to demonstrate (NDAC, 2005).

Recently, in vitro and in vivo studies have tested the reduction of transient bacteria. *In vitro* studies observe the number and movement of organisms as well as the potential for the development of resistance (Jackson, 2005). *In vivo* test methods look at other aspects, such as patient-to-patient contamination, and whether or not there is adequate bacterial reduction through tests that mimic actual use. Hands are contaminated, washed, and then the number of flora is noted. Within all antiseptic products, there is an active chemical agent (called a biocide) responsible for the destruction of microorganisms. These active ingredients include alcohol, iodine, triclosan, chlorohexidine gluconate, benzalkonium chloride, triclocarban, and para-chloro-meta-xylenol, and triclosan (NDAC, 2005). Leave-on and washes contain alcohol, benzalkonium chloride, and benzethonium chloride. Benzalkonium chloride used as disinfectant on some important foodborne pathogens (NDAC, 2005). Yet, although all of these biocides may be used by manufacturers, only two active ingredients have been recognized as safe and effective by the TFM. These active ingredients are 60-95% alcohol and 5-10% povidone-iodine (NDAC, 2005; Jackson, 2005).

The real concern is that biocides may stop working altogether. Researchers and the FDA suggest that biocides be monitored in the future, so that if a strong resistance occurs, decisions can immediately be made on whether this substance is more of a risk rather than a benefit. In an FDA literary search, they found that other studies examining bacterial resistance (besides Sheldon's research) revealed a reduced susceptibility to biocides as well (Gerald and Russell, 1999). Present study is an effort to check the claim of some of the branded product available in market as best suited antiseptic and disinfectants to avoid the cross contam-ination in healthcare or even at house hold sanitation.

MATERIALS AND METHODS

Disinfectants and Antiseptics used

Dettol, Savlon, Hydrogen peroxide, Lifebuoy sanitizer and Phenol were obtained from Boisar Market,Palghar, Maharashtra State, India.

Source of Microorganisms

Cultures of the test organisms *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi,* and *Streptococcus pyogen* were collected from Sanjeevani hospital, Virar, Maharashtra State, India.

Test Organism Suspension

Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and ino-culating in a Muller and Hinton agar slants. The tubes of the sub cultured organisms were incubated at 37^{o} C for 24 hours.

Inoculation of the Test Organisms

Using sterile loop, 24hour old culture of each of the test organisms was collected. The loops full of the different bacterial culture were swirled into different test tubes containing 10ml of sterile saline water. The content of each of the tubes was properly homogeni-zed before the inoculation. Sterile swab sticks were dipped into each of the bacterial solution and were used to inoculate the solidified Muller and Hinton agar plates ensuring that the plates were completely covered for uniform growth.

Preparation of the Disinfectants

The disinfectants were poured into different sterile test tubes and these became the stock solutions. Disti-lled water used as diluent. The concentrations of disin-fectants prepare by following table (1) and table (2):

Paper Disc Diffusion method

This involves a heavy inoculation of an agar plate with the test organism. Sterile filter paper discs were impr-egnated with the different antiseptics or disinfectants and equally spaced on the inoculated plate. Following incubation, the agar plate was examined for zones inhibition (areas of no growth) surrounding the discs. A zone of inhibition is indicative of microbial activity against the organism. Absence of zone of inhibition indicates that the antiseptic or disinfectant was ineffective against the test organisms (Tortora, *et.al*., 2004).

Table 1: Preparation of concentration for Dettol, Savlon, Lifebuoy. (Standard concentration 100%)

Concentration of Anticeptics and Disinfectants	Stock Solution (Ml)	Diluent (Distilled Water) (Ml)	Total Volume (Ml)
1%	0.05	4.95	5
2%	0.1	4.9	5
3%	0.15	4.85	5
4%	0.20	4.80	5
5%	0.25	4.75	5
6%	0.30	4.70	5
7%	0.35	4.65	5
8%	0.40	4.6	5
9%	0.45	4.55	5
10%	0.50	4.5	5

Table 2: Preparation of concentration for Hydrogen peroxide (standard concentration)

Concentration	Stock Solution (Ml)	Diluent (Distilled Water) (Ml)	Total Volume (Ml)
1%	1	4	5
2%	2	3	5
3%	3	2	5
4%	4	1	5
5%	5	0	5

Impregnation of the Discs

The sterile filter paper discs were impregnated with 0.1ml each of the dilutions of the disinfectant using different sterile pipettes.

Inoculation of Impregnated Disc

Using sterile forceps, the different discs impregnated with different dilution of the disinfectants were placed on each of the plates inoculated with the test organisms. The forceps were used to press down each of the disc gently against the agar surface so as to ensure good contact. The plates were incubated in an inverted position at 37 ^{0}c for 24 hours. The zones of inhibition were observed, and then measured accurately.

Method for Determining the Phenol Coefficient of the Disinfectants

The phenol coefficient of the disinfectants was determined using standard microbiological method. Different dilutions of the phenol stock solution were made (i.e.1:80,1: 90 and 1:1000) in sterile test tubes. 0.1ml each of the suspension of the test organisms was introduced into each of the dilutions and mixed properly.

0.1ml was inoculated into tubes of (2ml each) sterile nutrient broth after 5 minutes, 10 minutes and 15 minutes, for each of the dilutions.

The same procedure was repeated for each of the test disinfectants using dilutions 1:400, 1:450 and 1:500. The tubes were incubated for 24 hours at 37^{0C} and then observed for growth (turbidity).

Phenol coefficient for each of the test disinfectants was calculated using the formula:

RESULTS AND DISCUSSION

The result obtained in this study of the zone diameter of inhibition of the disinfectants on the various test microorganisms is presented in Table 3 (undiluted) and fig.1 (diluted) Table 4 is the result of phenol coefficients of the various disinfectants used.

The undiluted and diluted concentrations of the disinfectants showed varying zones of inhibition on the test microorganisms. The undiluted showed higher zones of inhibition than the diluted disinfectants. The zones of inhibition of the undiluted ranged from 10mm

(Lifebuoy) to 23mm (Savlon) on *Escherichia coli;* 8mm (Lifebuoy) to 23mm (Savlon) on *Staphylococcus aureus* whereas on *Salmonella typhi* it ranged from 9mm (Lifebuoy) *to*15mm (Savlon, Dettol). The higher the dilution factor the lower the zones of inhibition on the test microorganisms. The zones of inhibition of the undiluted on the *Streptococcus pyogen* is 0mm (Lifebuoy) to 30mm (Dettol) when compared to the different dilutions of the disinfectants.

In this study, the phenol coefficient obtained with each of the disinfectants on *Escherichia coli* ranged from 0 *(Lifebuoy)* to 9 *(Dettol), on Staphylococcus aureus it* ranged from 0 (Lifebuoy) to 8 (Dettol), on *Salmonella typhi* it ranged from 0 (Lifebuoy) to 10 (Dettol) also on *Streptococcus pyogen* the phenol coefficient ranged from 0 (Lifebuoy) to 10 (Dettol).

From Table 3, the disinfectant that is most effective is that which the ratio of the phenol to disinfectant is > 1. Dettol is the most effective of the other disinfectants on *E. coli* (9), followed by Hydrogen peroxide (5), then Savlon (2). On S. aureus Dettol and Savlon is the most effective (8), followed by Hydrogen peroxide (5). Dettol is the most effective on *Salmonella typhi* (10), followed *by* Savlon (9), then Hydrogen peroxide (2). On *Streptococcus pyogen* Dettol and Savlon is the most effective (10), followed by Hydrogen peroxide (5). Lifebuoy is less effective on all the four test microorganisms than phenol being < 1.

Table 3: Anti-bacterial sensitivity of the disinfectants. (Undiluted)

Test microorganism	Disinfectants	Diameter zone of inhibition (mm)
Escherichia coli	Dettol	20
	Savlon	23
	Hydrogen peroxide	12
	Lifebuoy	10
Staphylococcus aureus	Dettol	22
	Savlon	23
	Hydrogen peroxide	13
	Lifebuoy	8
Salmonella typhi	Dettol	15
	Savlon	15
	Hydrogen peroxide	10
	Lifebuoy	9
Streptococcus pyogen	Dettol	30
	Savlon	22
	Hydrogen peroxide	10
	Lifebuoy	0

Table 4: Phenol coefficients of the disinfectants against the test.

Test microorganism	**Disinfectants**	**Phenol coefficient comparison with phenol**
Escherichia coli	Dettol	9
	Savlon	2
	Hydrogen peroxide	5
	Lifebuoy	0
Staphylococcus aureus	Dettol	8
	Savlon	8
	Hydrogen peroxide	5
	Lifebuoy	0
Salmonella typhi	Dettol	10
	Savlon	9
	Hydrogen peroxide	2
	Lifebuoy	0
Streptococcus pyogen	Dettol	10
	Savlon	10
	Hydrogen peroxide	5
	Lifebuoy	0

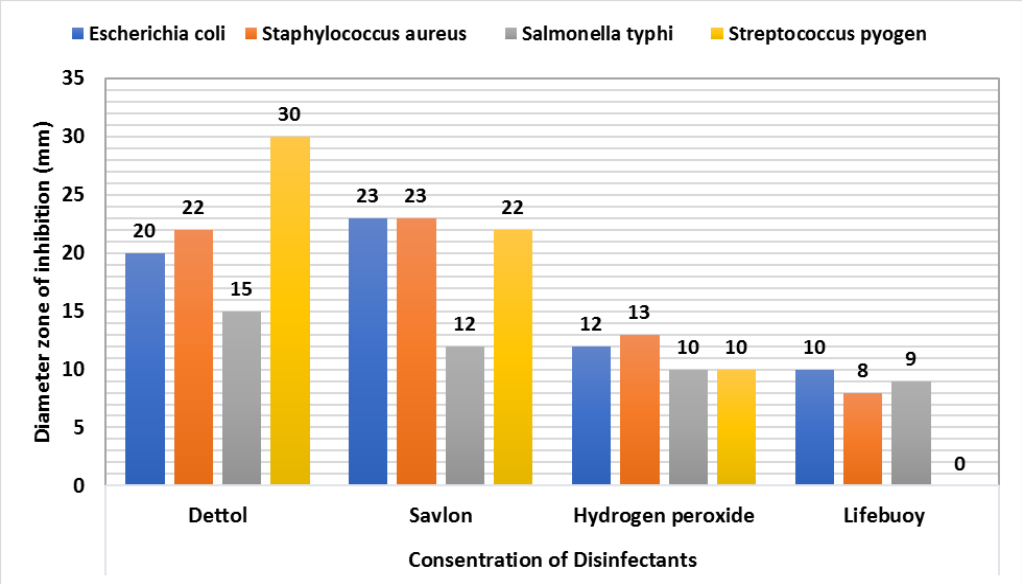

Fig.1: Anti-bacterial sensitivity test of the disinfectants against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus pyogen.*

Figure 2: Zone of inhibition of disinfectant and antiseptics

DISCUSSION

The results show that different types of microorganisms vary in their response to different types of antiseptics and disinfectants. The different concentration of Lifebuoy recorded no antimicrobial activity against all the pathogens. Dettol recorded the highest zone of inhibition on S*treptococcus pyogen* (20 mm). The dilutions of different disinfectants showed remarkable zones of inhibitions when compared with the undiluted concentration. The test microorganisms differ in their susceptibilities to the disinfectants. Dettol had broad spectrum activity as it inhibited the growth of gram positive (*Staph aureus; Streptococcus pyogen*) and gram negative (*E. coli*). The antimicrobial activity was more on the gram positives; Savlon and Hydrogen peroxide showed broad spectrum activity on both gram positive and gram-negative test microorganisms.

The different dilutions of the disinfectants gave different zones of inhibition. No zones of Inhibition were recorded with Lifebuoy in all the concentrations used on the test microorganisms. The outcome of this study proves Dettol to be the strongest antimicrobial agent irrespective of the dilutions when compared with the other disinfectants used in this study. Also, the report of Olowe, (2004) and Olasehinde *et. al*., (2008) showed that Dettol is a strong antimicrobial agent. It also showed that the dilutions of the other disinfectants exhibited remarkable growths of the test microorganisms, (that is the form in which these disinfectants are used). The antimicrobial effects of these disinfectants against the test microorganisms showed the biocide effects of these disinfectants against these microorganisms that are associated with water. *Escherichia coli* is an indication of water contamination of faecal origin which is the cause of many diseases. *Staphylococcus aureus* is well known to

cause wound infection, and *Streptococcus sp.* is well implicated to cause sour throat (Rutala and Weber, 2001). *Sallmonela typhi is also well* known to cause typhoid fever. The use of these disinfectants may be means to reduce cases of acquired diseases caused by the test microorganisms. An ideal disinfectant should have a broad antimicrobial spectrum, should be non-irritating, less toxic, noncorrosive and inexpensive (Willey *et.al,* 2008).

Although phenolic agents exhibit high toxicity and low biodegradability, they are still in use in developing countries because of their low cost. They are considered a health risk by the Environmental Protect Agency (EPA), and cannot be used in neonatal, pediatric ICU or on any infant contact surface. Eye irritation, contact dermatitis/utricaria and depigmentation of the skin have been linked to phenol residue contact (Pierson, 2009).

However, antiseptic compounds are still active against bacterial strains isolated from surgical wound infection despite increasing antibiotic resistance (Giacometti *et. al*., 2002). It will be necessary to always evaluate new disinfectants before their application in the hospitals and also check same periodically in-use to ensure efficacy.

CONCLUSION

The potency of disinfectants is very important to enhance efficacy of these disinfectants towards the controlling microbial population which includes prevention of disease transmission and infection. It also prevents deterioration and spoiling of materials which could also lead to microbial infection. Determination of antimicrobial effectiveness of disinfectants is essential to achieve total disinfection of pathogens. The use of good disinfectants should be encouraged to reduce cases of nosocomial infection by most microorganisms.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Boyce JM, Pittet D (2002) Guideline for hand hygiene in health-care settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Centers for Disease Control and Prevention. *Morbidity and Mortality Weekly Report* (MMWR); 51: 16, 1–45.

Gerald McDonnell1, and Russell A. Denver.(1999) Antiseptics and Disinfectants: Activity, Action and Resistance, *Clin. Microbiol. Rev*. (1): 147–179. PMCID: PMC88911

Giacometti A, Cirioni O, Greganti G, Fineo A, Ghiselli R, Del Prete MS, et al. (2002) Antiseptic compounds still active against bacterial strains isolated from surgical wound infections despite increasing antibiotic resistance. *Eur. J. Clin. Microbiol. Infect Dis*.; 21(7):553-556.

Jackson (2005) Focus: Antimicrobial Resistance: Topical Antiseptics in Healthcare. *Clinical Laboratory Science: J. Amer. Soc. Med. Tech.* 18 (3): 160-169.

Larson EL and Morton HE (1991) Alcohols. In: Philadelphia, Pa: Lea Febiger; p. 191–203.

NDAC (2005) Briefing Document: Effectiveness Testing Criteria for Healthcare Antiseptic Drug Products. The Healthcare Topical Antiseptic Review Team. February 24. 1-15.

NDAC (2005) Briefing Document: Benefits and Hazards of Consumer Antiseptic Drug Products. The Healthcare Topical Antiseptic Review Team. September 22. 1-17.

Olasehinde GI, Akinyanju JA, Ajayi AA (2008) Comparative antimicrobial activity of Commercial Disinfectants with naphtholics. *Res. J. Microbiology,* 3(4):262-268.

Olowe OA, Olayemi AB, Eniola KIT, Adeyeba O.A. (2004) Antibacterial activity of some selected Disinfectants regularly used in hospitals. *African J. of clinical and experimental microbiology*; 5(1):126-130. ISSN 1595-689X, AJCEM/2002111/2412.

Patel S (2004) The efficacy of alcohol-based hand disinfectants products. *Nursing Times*: *100: 23, 32-34.*

Pierson J (2009) Choosing a disinfectant for hospital environment. Indoor Environment connection.;10. Available from: www.ieconnections.com/pdfs/ newsletter /2009/IEC-04-2009.pdf.

Pratt RJ, Pellowe C, Loveday HP, Robinson N, Smith GW, Barrett S, Davey P, Harper P, Loveday C, McDougall C, Mulhall A, Privett S, Smales C, Taylor L, Weller B, Wilcox M (2001) The epic project: developing national evidence-based guidelines for preventing healthcare associated infections. Phase 1: guidelines for preventing hospital acquired infections. Department of Health. *Journal of Hospital Infection;* 47 (suppl): *S3-82.*

Russell AD (1996) Activity of biocides against mycobacteria. *J. Appl. Bacteriol. Symp.* Suppl. 81; 87–101.

Rutala WA, Weber DJ (2001) Surface disinfection: Should we do it? *J. Hosp. Infect*., 48: S64-8.

Tortora GJ, BR Funke, Case CJ (2004) Microbiology: An Introduction, 8th ed., New York: Pearson Education and Dorling Kindersley Pvt, Ltd, pp. 224-225,.

Willey JM, Sherwood LM and CJ (2008) Woolverton, Prescott, Harley, and Klen's Microbiology, 7th ed., New York: McGraw-Hill Higher Education, pp. 158-165, 2008.