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[Research article]

Evaluate the Disinfectant Activity of Some Commercial Preparations by Rideal -Walker Test.

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ABSTRACT

The aim of this study was to evaluate and compare practically achieved disinfection efficacy of some locally available disinfectants on surfaces and infectious microbiological hospital waste. Three disinfectants were tested at concentrations recommended by manufacturers on rough and smooth surfaces that were contaminated experimentally by locally circulating isolates of methicillin-resistant *Staphylococcus aureus*, multi drug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* strains, standard isolate of *Salmonella typhi* and *Candida albicans*. Reduction in microbial counts before and after surface disinfection was expressed as log reduction. A very heavy microbial waste load was simulated by immersing culture plates with heavy microbial growth in disinfectants. Daily, a sample of disinfectant was taken and subjected to ***Rideal-walker*** test.

Keywords: Disinfectant, evaluation, hospital practice.

INTRODUCTION

Disinfection is the process of destruction or removal of pathogenic microorganisms and the object is said to be disinfected. The control of micro organisms extends into many diverse areas such as pharmaceuticals, medicine, hospital environment, food processing, and every day household. Whereas the destruction or removal of all forms of life is called sterilization, disinfection is the process of destruction or removal of pathogenic micro organisms and the object is said to be disinfected.

Ancient Egyptians used antibacterial oils, spices and balsams as disinfectants. Chlorine compounds were referred to as powerful disinfecting agents in 1827 in Lancet. Compounds including chlorine gas, silver nitrate, phenol, zinc compounds and iodine were used as disinfectants in the first half of the 19th century (1, 2). Solution of chlorinated lime was used for hand disinfection in Vienna in 1861. Pasteur and Lister also contributed in the field; ultimately Koch (1881) tested the action of different disinfectants on pure cultures.

The term disinfection is generally used for a process in which micro organisms present on non living or inanimate objects and surfaces are killed using chemical substances (3,10). The process does not necessary free the surfaces from the bacterial spores. The commonly used disinfectants belongs to the categories namely phenol and its derivatives, compounds of heavy metals, mercury compounds organic chemicals, soaps, synthetic detergents and alcohols (8, 9). The damage to the micro organisms

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occurs by denaturation or coagulation of cell components. It may also occur by non specific combination of the disinfectants with the cell compounds like cell wall, proteins, nucleic acids etc. The rate and extent of disinfectant action of any substance generally depends on many factors including, time of expose, temperature, pH, concentration, surface tension, etc.

Based on their rate of reaction and potency disinfectants have been arbitrarily classified in to 3 groups namely strong, week and moderate (4,5). The effectiveness of disinfectants is highly variable. An ideal disinfectant should be effective against all type of microorganisms. In other words, it should have a broad spectrum of activity at low concentrations. The efficacy of disinfectants can be determined by mixing them with known microorganisms under controlled experimental conditions and then inferring the extent of damage to the microbial cultures from the observations (11,12).

MATERIALS AND METHODS

The following materials were used in the present study:

APPARATUS

- Sterilized conical flask.
- Test tubes
- Sterile pipettes
- Cotton plugs
- Petri plates
- Boiling tubes.
- Measuring cylinder.

MATERIALS

Table 1: List of Chemicals used in present study

S. No	Name of the chemicals	Name of the company	Batch number
1	Beef extract	Finar chemical limited	19105950
2	Peptone	Finar chemical limited	19105364
3	Sodium chloride	Finar chemical limited	n007j10
4	Distilled water	Accent diagnostic center	14

EQUIPMENTS

Table 2: list of equipments used in the present study

S. No	Name of the equipment	Maker name
1	Hot air oven	Bio-techniques India. ISO 9001:2000
2	Incubator	Kadavil electro mechanical(kemi)
3	Laminar air flow	Kadavil electro mechanical(kemi)
4	Autoclave	Kadavil electro mechanical(kemi)
5	Electronic weighing balance	Dhona 200D

Commercial disinfectants used in present study

Table 3: List of Commercial disinfectants used in present study

S. No	Commercial Name of chemical disinfectants	Description
1	Domex	Active ingredients: Bezolkonium chloride solution 2%w/v, non ionic pine oil, parachoro meta cresol, water, perfume Hindustan unilever limited Mfg: 12/12 Exp: 18 months from mfg Caution: keep out of reach of children Storage: store upright in a cool place
2	Lyzol	Active ingredients: Bezolkonium chloride i.p 4%w/v, tartrazine yellow, preservative, perfume, water Rickettbenckiser India limited Mfg: 10/12 Exp: 09/13 Caution: do not mix with any other household cleaner or acid Storage: store upright in a cool place
3	Dettol	Active ingredients: Chloroxylenoli.p 4.8%w/v, terpenoli.p 9%w/v, alcohol absolute denature 13.1%v/v Rickettbenckiser India limited Mfg: 11/12 Exp: 4/15 Storage: store upright in a cool place

RIDEAL-WALKER METHOD

Procedure:

Dilutions of the test disinfectant and phenol are first prepared and quantities each of 5ml are measured of four chosen dilutions in each of sterile tubes. Place the tubes alongside the 24 hours broth culture in a rack in a water bath at 17.50 + 0.50;(1) fifth tube contains 5ml of one of the standard phenol dilutions (usually 1 in 105 dilutions). At 30 seconds intervals, the tubes are inoculated with 0.2 ml of the culture by means of a pipette and shake the tubes gently after each inoculation(6). Thirty seconds after the inoculation of the fifth tubes i.e. 2½ minutes after inoculating the first tube, remove the first tube from the rack, shake it gently transfer one standard loop full to a 5ml tube or R-W broth and return the tube to the rack make sure that the amount removed a loop full droplet and not just a film within the loop)(7,8). The procedure was Repeat 30 seconds intervals with each reaction tube of the disinfectant in turn until the whole cycle has been repeated four times, i.e. from each reaction tube subcultures have been made after intervals of 2.5, 5. 7.5 And 10 minutes. Incubate the broth tubes at 37^0C for not less than 48 hours and not more than 72 hours and record the presence of absence growth in each tube.

$$\text{Phenol coefficient} = \frac{\text{Dilution of disinfectant which kills in 7.5 mints but not in 5mints}}{\text{Dilution of phenol which kills in 7.5 mints but not in 5mints.}}$$

RESULTS

1) DETTOL

TRAIL 1.a

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	_	_
Test Dilutions				
1:95	_	_	_	-
1:105	_	_	_	_
1:115	+	+	+	_
1:125	+	+	_	_
1:135	+	_	_	_

Phenol coefficient = $\frac{\text{Dilution of disinfectant which kills in 7.5 but not in 5mins}}{\text{Dilution of phenol which kills in 7.5 but not in 5mins}}$

= 125÷95

= 1.315.

TRAIL 1.b

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	_	_
Test Dilutions				
1:95	_	_	_	_
1:105	_	_	_	_
1:115	+	_	_	_
1:125	+	+	_	_
1:135	+	+	+	_

Phenol coefficient = $\frac{\text{Dilution of disinfectant which kills in 7.5 but not in 5mins}}{\text{Dilution of phenol which kills in 7.5 but not in 5mins}}$

= 125÷95

= 1.315.

Average value of Dettol = 1.315+1.315/2

= 1.315.

2) LYZOL

TRAIL 2.a

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	__	_
Test Dilutions				
1:95	_	_	_	_
1:105	+	_	_	_
1:115	_	_	_	_
1:125	+	+	_	_
1:135	+	+	_	_

Phenol coefficient = $\frac{\text{Dilution of disinfectant which kills in 7.5 but not in 5mins}}{\text{Dilution of phenol which kills in 7.5 but not in 5mins}}$

= 125+135÷2

= 130

= 130÷95

= 1.368.

TRAIL 2.b

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	_	_
Test Dilutions				
1:95	_	_	_	_
1:105	_	_	_	_
1:115	_	_	_	_
1:125	+	+	_	_
1:135	+	+	_	_

Phenol coefficient = $\frac{\text{Dilution of disinfectant which kills in 7.5 but not in 5mins}}{\text{Dilution of phenol which kills in 7.5 but not in 5mins}}$

= 125+135÷2

= 130÷95

= 1.368.

Average value of lyzol = 1.368+1.368/2

=1.368.

3) DOMEX:

TRAIL 3.a

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	__	_
Test Dilutions				
1:95	_	_	_	-
1:105	_	_	_	_
1:115	+	_	_	_
1:125	+	+	_	_
1:135	+	+	+	_

Phenol coefficient = Dilution of disinfectant which kills in 7.5 but not in 5mints / Dilution of phenol which kills in 7.5 but not in 5mints

= 125÷95

= 1.315.

TRAIL 3.b

CONCENTRATION	TIME(MINS)			
	2.5	5	7.5	10
Standard phenol				
1:95	+	+	_	_
Test Dilutions				
1:95	_	_	_	_
1:105	+	+	_	_
1:115	+	_	_	_
1:125	+	+	+	_
1:135	+	+	_	_

Phenol coefficient = Dilution of disinfectant which kills in 7.5 but not in 5mins / Dilution of phenol which kills in 7.5 but not in 5mins

= 135+105÷2

= 240÷95

= 1.263.

Average values of domex = 1.368+1.263/2 =1.135.

DISCUSSION

In present investigation an attempt was made to evaluate the disinfectant of commercial preparations namely domex, Dettol, lyzol, white phenol by following standard procedure employing Staphylococcus aureus as test organism as indicated in tables it was found that lyzol shows good disinfectant activity among other the over value phenol coefficient was found to be highest in case of lyzol. As indicate in 2a, 2b &2c the phenol coefficient of LYZOL was observed as 1.368. As indicate in 3a, 3b &3c the phenol coefficient of DOMEX was observed as 1.135. As indicate in 1a, 1b &1c the phenol coefficient of DETTOL was observed as 1.332.

The order of disinfectant activity of the selected chemical disinfectants is as follows:
LYZOL > DOMEX > DETTOL.

CONCLUSION

The present investigations of 3 different commercial chemical disinfectants were evaluated for their disinfectant actually by following standard procedure.Among 3 different commercial chemical preparations Lysol has sharan good disinfectant activity against the test organism Staphylococcus aureus.

The older of disinfectant activity of the selected chemical disinfectants is as follows:
LYZOL > DOMEX > DETTOL.

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