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Efficiency of Different Disinfectants: Ethanol, Bleach, Dettol and Hydrogen Peroxide against *Staphylococcus Epidermidis* and *Staphylococcus Aureus*

Shruti A Satashia[1], Hemanshi H Kanpariya[2]
[1, 2]*Microbiology department, Atmanand Saraswati Science College, Surat, Gujarat, India*

Abstract: ***Various chemical agents are used in clinical laboratories and hospitals for disinfection. Among them ethanol, bleach, H_2O_2, Dettol are most widely used for this purpose. This study was aimed to check the efficiency of above mentioned disinfectant at various concentrations on two bacterial strain S.aureus & S.epidemidis using Kirby bauer well diffusion method. Different concentrations of ethanol (60%, 75%, 95% v/v), H_2O_2 (10%, 15%, 30% v/v), Dettol (50%, 75%, 100 % v/v), bleach (5%, 15%, 30% v/v) were tested. Results indicate 75% v/v ethanol found to be effective against both bacterial strains whereas other two concentration of ethanol has shown very less effect on the growth of microorganism. Among three selected concentration of H_2O_2 15% v/v was the most effective to inhibit the growth of test organisms. Effectiveness of bleach and Dettol found to be gradually increasing with the concentrations taken.***

Keywords: Disinfectants, Efficiency of Different Disinfectants, Dettol, Hydrogen peroxide, Ethanol, Bleach

I. INTRODUCTION

Disinfectants and Antiseptics are widely used as agents for killing or eliminate bacteria especially in microbiological laboratory, hospitals, other humans and animals care centers [1]. Many scientists have worked on development of various physical and chemical methods to control the microbial growth. Disinfection usually refers to the destruction of vegetative (non-endospore forming) pathogens example bacteria by using a disinfectant to treat an inert surface or substances [2]. Many chemical agents are now available commercially as disinfectants and antiseptics, these preparations could be halogen compounds, phenols, alcohols, peroxides, quaternary ammonium compounds, chlorohexidine and sodium hypochlorite[3,4].The most commonly used disinfectant in microbiology laboratory are Ethanol, Dettol, Chlorohexidin and soap [5].Ethanol, as a dehydrating agent causes cell membrane damage, denature the protein and cell lyses [6]. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination [7], but this effect is reversible [8].Because of the lack of sporicidal activity, and alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Bleach with a main constituent of Sodium hypochlorite effect by oxidizing of the cell of microorganism of attacking essential cell component including protein, lipid and DNA [9]. Hydrogen peroxide is commercially available in a variety of concentrations ranging from 3 to 90% [1]. H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores [10].Higher concentrations of H_2O_2 (10 to 30%) and longer contact times are required for sporicidal activity. H_2O_2 acts as an oxidant by producing hydroxyl free radicals (•OH) which attack essential cell components, including lipids, proteins, and DNA. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted [11].

The antimicrobial properties of the disinfectant agent against some of the pathogenic bacteria have been reported. Now-a-days, scientist and researchers are much concerned about improved cleaning and disinfection of environmental surface in healthcare facilities. Experts generally agree on a number of areas, including the fact that careful cleaning and/or disinfection of environmental surfaces, daily and at time of patient discharge, are essential elements of effective infection prevention programs. When disinfectants are used, they must be used appropriately to achieve the desired effects. Moreover, microorganisms are continuously acquiring resistance to new disinfectant and antiseptic [12] Therefore, it is necessary to evaluate the effectiveness of disinfectant or antiseptic against a specific pathogen so appropriate agent can be easily selected [13].

II. MATERIALS AND METHODS

A. Bacterial strains

The strains used in this study are gram positive bacteria obtained from American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC BAA 1026) and *Staphylococcus epidermidis* (ATCC 35984)

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B. Media

Nutrient agar was procured from Himedia (Mumbai, India). Media was sterilized at 121°C 15 psi pressure for 15 minutes in autoclave.

C. Disinfectants

Four different types of disinfectants as showed in table 1 were used to test susceptibility of the bacteria.

Name of disinfectant	source
Ethanol (Ethyl alcohol)	Finar Chemicals, Ahmedabad
Bleach (Sodium hypochlorite)	Finar Chemicals, Ahmedabad
Hydrogen peroxide(H_2O_2)	Research Lab fine chem, Mumbai
Dettol (Chloroxylenol)	Finar Chemicals, Ahmedabad

Three different concentration of each disinfectant; Ethanol (60%, 75%, 95% v/v), H_2O_2 (10%, 15%, 30% v/v), Dettol (50%, 75%, 100 **%**), Bleach (5%, 15%, 30%) were tested against *S.aureus* and *S.epidermidis.*

D. Preparation Of Different Concentration Of Disinfectants

The method of Committee on Research Standard (CRS) [14] And DHQP 2009[15] was adopted for preparation of different concentration of all four disinfectants.

Formula for preparation of different concentration of disinfectants is as follow.

Original concentration in Percent (%) = RV/O

Where,

R = Required concentration
V = Required volume of water
O = Original concentration

E. Antimicrobial Susceptibility Testing (Using Kirby Bauer Diffusion Assay Well Method)

Pure *Staphylococcus aureus* and *Staphylococcus epidermidis* were inoculated & incubated for 24 hours at 37°C to obtain freshly grown culture on sterile nutrient agar plate. Suspension of each bacterial strain of McFarland standard 0.5 was prepared. 16 sterile nutrient agar plates were collected and divided into four equal sectors using marker.

8 plates were labelled with one bacterial strain & disinfectant then replication of each plate was done. Three sectors in each plate were labelled with different concentrations of disinfectants and fourth sector as “control”. 8 Sterile melted and cooled (45 °c) nutrient agar were seeded with 1.0 ml of *Staphylococcus aureus* culture and 8 Sterile melted and cooled (45 °c) nutrient agar were seeded with 1.0 ml of *Staphylococcus epidermidis* culture. These were poured on previously labelled nutrient agar plate respectively. For the test plates sterile cup borer (diameter of 6mm) was used to bore the wells in each sectors. The well of “control” sector of each plate was pipetted with sterile distill water. 0.5 ml of each concentration of disinfectant was pipetted inside the well of three labelled sectors of each plate. Repeat the same for all the disinfectants.

For the diffusion of disinfectant, the plates were kept in refrigerator for 30 mintues. Then plates were incubated at 37°C for 24 hours. After incubation plates were examined for zone of inhibition and results were recorded. [16]

III.RESULTS AND DISCUSSION

1) *Controls:* control sectors showed lawn growth around the well and no clear zone was observed.
2) *Test Sectors:* efficiency of different disinfectants varied on concentrations. The result showed that all the disinfectants inhibited the growth of test organisms in their different concentration by showing different diameters of zone of inhibition around each well. Diameters were measured using ruler in millimetre. Results are shown in following table.

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TABLE I

Results of diameter of zone of inhibition of tested disinfectants on test organisms

Name of Disinfectant	Concentration (%v/v) or (% w/v)	Diameter of zone of inhibition (mm)	
		S.aureus	*S.epidermidis*
Ethanol	60	2	3
	75	18	16
	95	6	5
Bleach	5	6	8
	15	14	16
	30	19	21
H_2O_2	10	5	7
	15	17	16
	30	8	6
Dettol	50	6	8
	75	12	15
	100	16	18

Graphical representation of tested disinfectant on test organisms

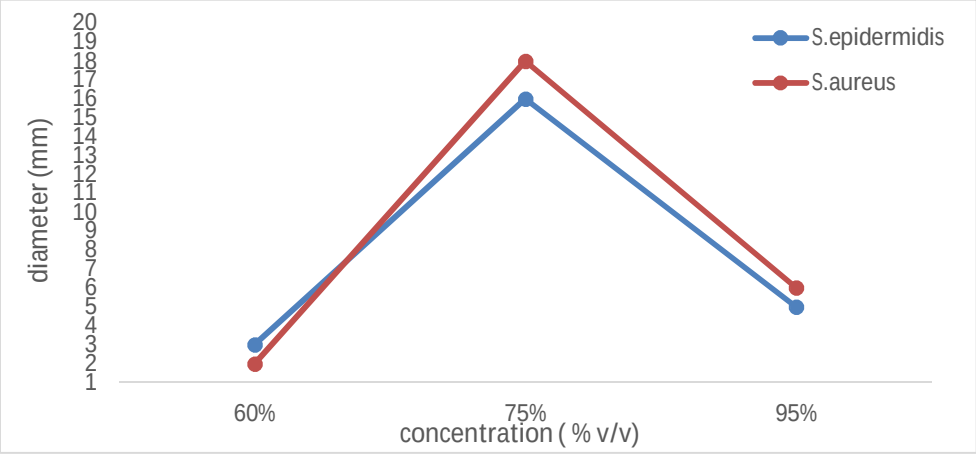


Fig. 1 Effect of ethanol on test organisms

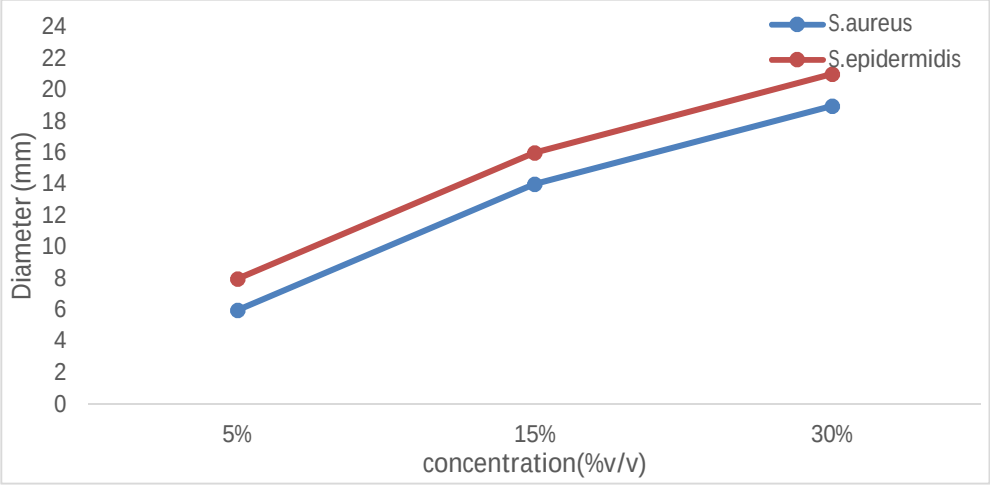


Fig.2 Effect of bleach on test organisms

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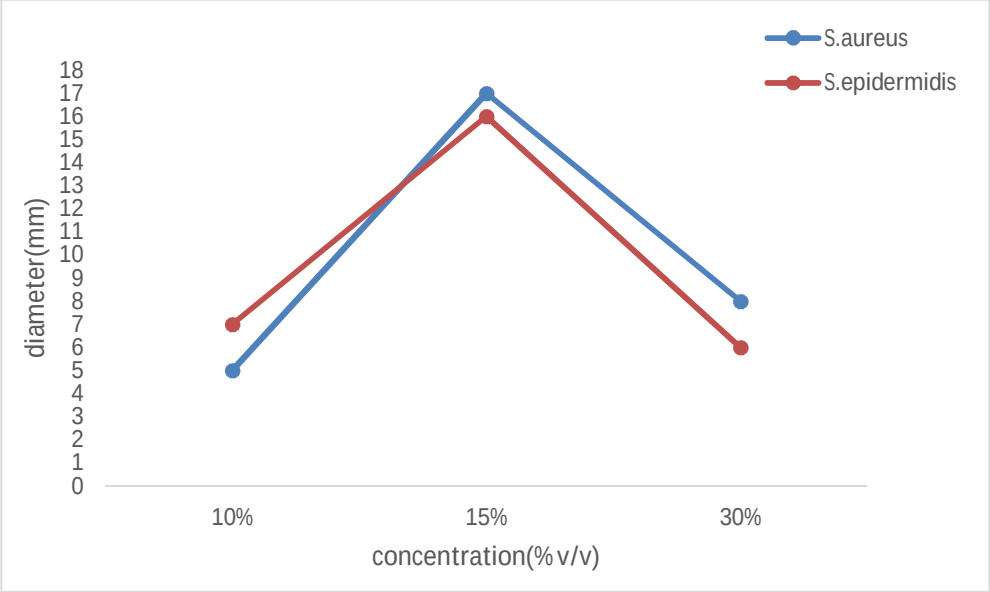


Fig.3 Effect of H_2O_2 on test organisms

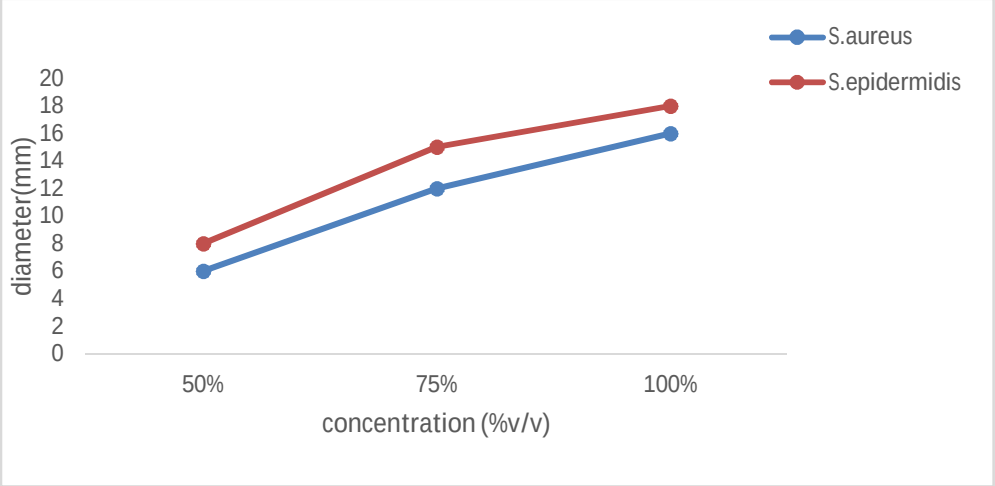


Fig: 4: effect of Dettol on test organisms

The goal of disinfection is to reduce the risk of endemic and epidemic nosocomial infections in patients. A great number of disinfectants are used in the healthcare setting when used in appropriated concentrations and are
recommended for patient-care items and instruments . From the different diameter of zones of inhibition of the four disinfectants under study, it was discovered that all the disinfectants inhibited the growth of the test organisms at different concentrations. Ethanol at 75% showed highest activity, whereas 60% and 95% showed least activity on both the test organisms. Ethanol is rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria (gram +ve and gram-ve), but their cidal activities drop sharply when diluted below 60% concentration and optimum bactericidal concentration in the range of 60% - 90% solution in water**,** volume/volume [17].On the other hand bleach showed highest activity at 30%, moderate activity at 15% and least activity at 5%.in the similar way to ethanol. Moreover, from the results, it is indicated that bleach is the most effective of all the tested disinfectant. It is found that oxidation reactions will occur when bleach is dissolved in water, which can destroy organisms fold structure leading to sterilization [18].

H_2O_2 showed highest activity at 15% and lowest activity at 10% and 30%. H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores [10]. However, on the contrary dettol showed highest activity at 100% moderate at 75% and least activity at 50%. Past studies showed that Chloroxylenol is rapidly lethal to gram positive and gram negative bacteria in dried state [19].

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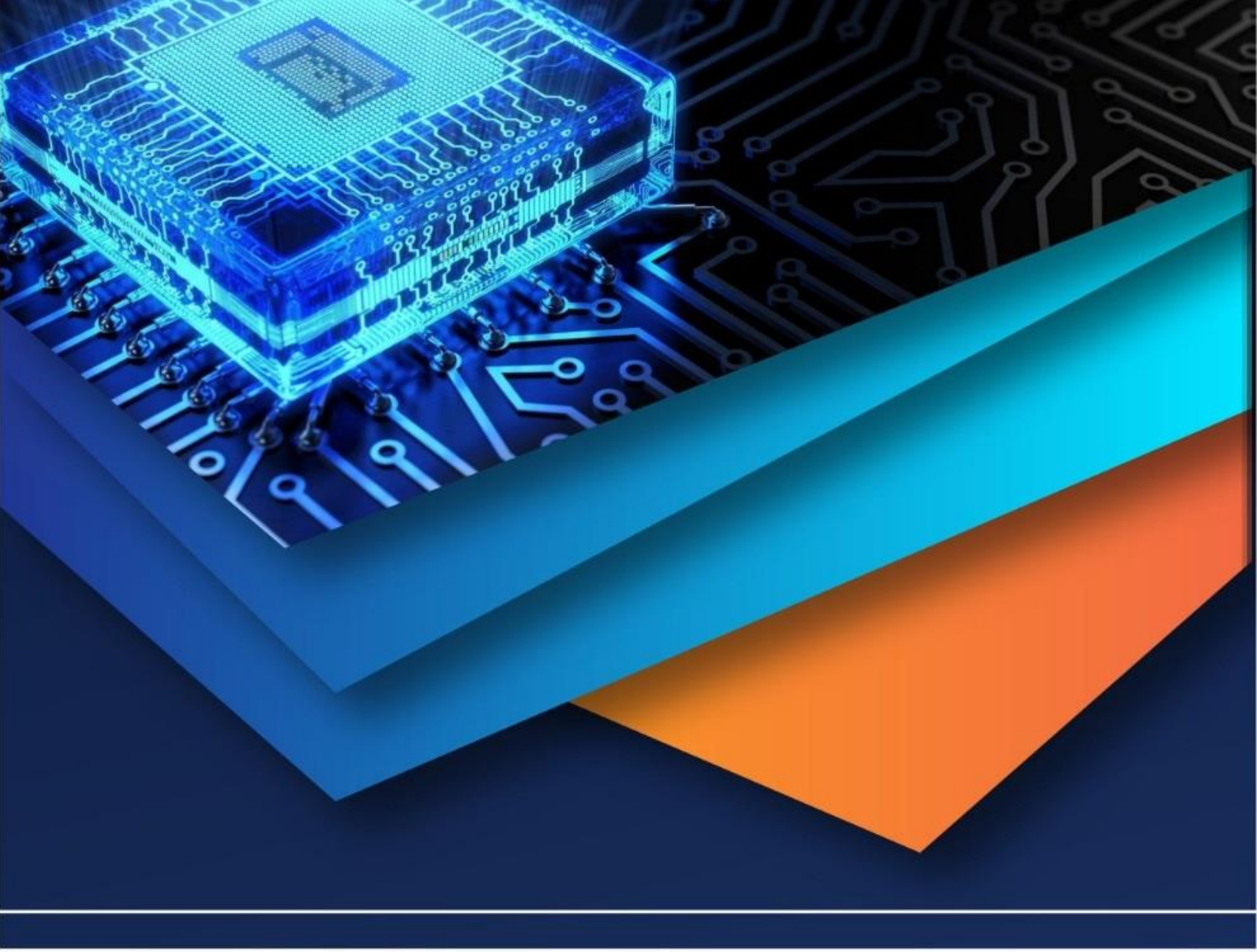
IV. CONCLUSIONS

The study was designed to determine the effectiveness of disinfectants against two test organisms *S.aureus* and *S.epidermidis.* Amongst different concentrations checked ethanol (60%, 75%, 95% v/v), H_2O_2 (10%, 15%, 30% v/v), Dettol (50%, 75%, 100 % v/v), bleach (5%, 15%, 30% v/v) it can be concluded that 75% v/v ethanol was effective against both bacterial strain whereas other two concentration of ethanol has shown very less effect on the growth of microorganism. Among three selected concentration of H_2O_2 15% v/v was the most effective to inhibit the growth of test organisms. Effectiveness of bleach and Dettol found to be gradually increasing with the concentrations taken. Bleach was the most effective of all four disinfectants tested.

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