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COMPARATIVE EFFICACY OF DISINFECTANT AGAINST ROUTINE LAB BACTERIAL CONTAMINANTS

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ABSTRACT

During present study lab bacteria were isolated in Nutrient agar medium using **expose plate method:** to air & identified by colonial morphology, Gram's staining & biochemical tests. Disinfectant sensitivity was determined by Kirby- Bauer's method whereas phenol coefficient test (PCT) was carried out to compare the antimicrobial activity of chemical compound to that of phenol under experimental condition so as to determine the disinfectant efficacy. The lab contaminants identified as ***Pseudomonas, Bacillus,* & *Micrococcus*.** Disinfectant sensitivity was assessed in terms of zone of inhibition (ZOI). *Pseudomonas Species* showed following pattern, Lysol>Savlon>Dettol>Betadine>Phenol while Alcohol showed nil response. *Micrococcus Spp*.- Lysol>Savlon>Dettol>Alcohol>Phenol while Betadine showed no inhibition. *Bacillus spp*.- Savlon>Dettol>Lysol> Phenol> Alcohol> Betadine. The efficacy of disinfectant assessed by testing them against standard culture *S. aureus* appear as Lysol>Savlon>Dettol>Betadine>Alcohol & same pattern was found in all the three bacteria.

KEYWARDS: Disinfectants, Kirby-Bauer method, *Micrococcus, Pseudomonas, Bacillus,* Phenol coefficient test.

INTRODUCTION

Decontamination is any activity that reduces the microbial contamination of material or surfaces to prevent inadvertent infection. The appropriateness of decontamination procedure depends on our goal. Disinfection refers to the elimination of virtually all pathogenic organisms on inanimate objects and surfaces thereby reducing the level of microbial contamination to an acceptable safe level. Sterilization refers to the destruction of all

microbial life, including bacterial endospores. There are so many disinfectants available which are cost effective and eradicate nearly all the contaminants, some of them are Savlon, Dettol, Lysol, Phenol, Alcohol, Betadine. But due to environmental changes & mutation in the genome structure of microorganism they are becoming more & more resistant to present day disinfectants & there is need to seek after newer disinfectants. Sterility is the hallmark of successful work in the microbiology lab. To achieve this it is mandatory that we use sterile technique. "**Sterilization is the process where all living microorganism including bacterial spore are killed**. Sagripati et al., 1996 has demonstrated the comparative sporicidal effects of liquid chemical agents. The scientific application of disinfectant is limited at the most past 150 years. Soldiers returning from battle were required to disinfect their equipment & clothing with heat. In 500 AD Indian physician "Shusruta" instructed fumigation of operating room with disinfecting vapors before every operations. In 865 AD ethanol was discovered by Rasis & was first used in 1773 is Paris hospital for dressing wounds. In 1839, iodine was first used in wound by Davis. In 1916 Phenol was discovered & were reported for commercial use by Reichenbach. Hammond et al (1987), Bloomsfield et al (1996) Alquarashi et al (1996) & an article given in www articles directorym.net (2008) it was clearly stated that **disinfectant are making bacterial more resistant to antibiotics**. Keeping this in mind the present investigation carried out on comparative efficacy of disinfectants against lab bacteria

MATERIAL & METHODS

- **Sampling Site:** Microbiology laboratory of Kanya Gurukul Girls Campus Gurukul Kangri University, Haridwar (U.K.) INDIA
- **Disinifectants Used**

1- Lysol:-0.6% w/w sod.hypochloride with non-ionic & anionic surfactants sod. Hydroxide, perfume & water.

2- Savlon:- Strong cetrimide solution (1.5%v/v)

3- 3-Dettol:-Chloroxylenol (4.8%w/v), Terpineol (90% v/v), Absolute alcohal (Denatured 13.1%v/v)

4- 4-Betadine:- (Povidone-iodine) Povidone-iodine IP(7.5%w/v), (0.75% w/v available iodine)

5- 5-Ethyl Alcohol:- (70% v/v)

6- 6-Phenol:- (5% w/v)

Control: Sterile distilled water impregnated filter paper discs.

Isolation of Lab Contaminants

By expose plate method, plates containing Nutrient Agar Medium expose to air at different places (for 1minute duration) in the laboratory and Incubated at 37±2°C for 24 hours.

Identification

Carried out by biochemical characterization which referring by Cappuccino & Sherman (1999) & Bergey's Manul [7]. Biochemical test i.e., Gram's staining, Catalase test, IMVIC test, H_2S Production, NO_3 Production, carbohydrate fermentation, Litmus milk test, Starch hydrolysis, Gelatin hydrolysis, Urease (Table-1)

Efficacy Test- Phenol Coefficient Test [8]

The test compared the microbial activity of chemical compound to that of phenol under standardized experimental conditions (table 3). Equal quantities of a series of pure phenol were placed in to sterilized test tube. A standardized quantity of a pure culture of the test microorganism was added to each of the tubes. Subcultures of the test microorganism were made from each dilution of the test chemical in to sterile nutrient broth media at intervals of 5, 10, 15 minutes after introduction of the organisms. All the subcultures are incubated at 37^0C for 48 hours and examine for the presence of absence of growth. And phenol coefficient determined by the using following formula.

$$\text{Phenol coefficient ratio} = \frac{\text{Highest dilution of chemical being tested that destroyed the microorganisms in 10min not in 5 min}}{\text{Highest dilution of phenol that destroyed the microorganisms in 10min not in 5 min}}$$

Sensitivity Patterns- Disc Diffusion Assay

Sensitivity test was performed by Disc diffusion Test by Kirby & Bauer (1966) to check the comparative sensitivity of chemical disinfectant against test organism.(Table-2).

Observation

Table 1:- Biochemical Characteristics of bacterial lab contaminants

Biochemical Characters	Isolates Psuedomonas	Micrococcus	Bacillus
Agar slant culture	Thin white growth with media turning green.	Moist abundant orangish colonies.	Abundant off white waxy growth
Gram stain	Negative rod	Positive cocci	Positive rod
H_2S production	Alkaline reaction	Alkaline reaction	Alkaline reaction
NO_3 reduction	-	±	+
Indole production	-	-	-
MR reaction	-	-	±
VP reaction	-	-	-
Citrate reduction	+	-	-
Urease reduction	-	-	-
Catalase test	+	+	+
Oxidase test	+	+	+
Gelatin liquefaction	+	+	+
Lipid hydrolysis	-	-	+
Starch hydrolysis	-	-	+
Fermentation			
Lactose	-	-	-
Dextose	-	-	A
Sucrose	A	-	A

Table 2:- Zone of inhibition obtained against isolates by different disinfectants [9]

S. No.	Disinfectant	Psuedomonas	Micrococcus	Bacillus
1.	Alcohol	NZI	18±2.25mm	10±1.25mm
2.	Phenol	4±0.5mm	16±2mm	12±1.5mm
3.	Lysol	26±3.25mm	56±7mm	28±3.5mm
4.	Dettol	10±1.25mm	36±4.5mm	30±3.75mm
5.	Savlon	12±1.5mm	40±5mm	34±4.75mm
6.	Betadine	10±1.25mm	NZI	8±1mm

X-Mean diameter of zone of inhibition of disinfectants, S.E.-Standard Error, NZI-No Zone of Inhibition

Table 3: Comparative account of phenol coefficient ratios (PCR) of different disinfectants [8]

S. No.	Disinfectant	Phenol coefficient ratio			
		S.aureas	Psuedomonas	Bacillus	Micrococcus
1.	Alcohol	0.8	0.9	0.8	0.8
2.	Lysol	5	6.6	5	5
3.	Dettol	3.75	3.75	4.4	3.75
4.	Savlon	4.4	2.8	5	4.4
5.	Betadine	1.1	1.25	1	1.1

RESULTS

Isolation of Lab contamiants

During present study bacteria isolated as lab contaminant were ***Bacillus***, ***S. aureus,*** ***Pseudomonas***, ***Micrococcus***. All these bacteria were identified on the basis of biochemical test given in table 1 & Bergey's mannul. **To check the efficacy of chemical sterilizants against the isolated bacteria by disc diffusion test** first the zone of inhibition of different disinfectant on lab contaminant were observed by **Kirby Bauer method (1966).** In sensitivity testing the ZOI of disinfectant were *(A)* ***Pseudomonas-*** Lysol> Savolon> Dettol > alcohol > Phenol While Alcolol Showed no ZOI (B) **Micrococcus-** Lysol> Savolon > Dettol>Alcohol>Phenol. Whereas betadine showed no ZOI (C) ***Bacillus***- Lysol=Savlon>Dettol>Phenol>Alcohol>Betadine. Efficacy of disinfectant showed following pattern when tested against standard culture of ***S.aureus*** Lysol> savlon>Dettol>Betadine>Alcohol (table-2) **Phenol coefficient ratio** of different disinfectants(Table-3) campared with the antimicrobial activity of a chemical compound to that of Phenol under standard experimental condition. A phenol cofficient is **not smaller than 1 indicates** that this agent is less effective than phenol. A Phenol coefficient is **not greater than 1 indicates** that this agent is less effective than phenol. Hence the PCR pattern of efficacy of different disinfectant were as follows **(Table-3)** ***S.aureus***- Lysol>Savlon>Dettol>Betadine>Alcohol>as the lysol was 5 times more efficient than to phenol. ***Pseudomonas spp***. – same pattern lysol 6.6 times more effective as compared phenol. ***Bacillus spp.*** Lysol> Savlon > Dettol>Betadine >Alcohol. Lysol & savlon both were 5 times more effective as compared to phenol where as Alcolol 0.8 times less effective than phenol & betadine equally effective as compared to phenol. *Micrococcus* spp. Lysol>Savlon>Dettol>Betadine>Alcohol. Lysol was 5 times more active as compared to phenol. Comparative analysis of phenol coefficients ratio of the 3 isolates with standard culture of *S.aureus* Lysol was the most effective disinfectant against all the three isolates followed by Savlon & Dettol.

DISCUSSION

Chemical used as sterillization agents are called **chemical sterilants** whereas **disinfection** is the process of elimination of most pathogenic organism including bacterial spores on animate objects. The presence of some genes in stains of MRSA to be responsible for resistant towards hospital disinfectant. White comparing the result the two isolates ***Pseudomnas Spp***. & ***Micrococcus Spp.*** showed sensitivity to Lysol, Dettol & Savlon and **Pseudomonas spp.**

showed sensitivity to betadine & ***Micrococcus spp.*** to alcohol. Mcclure et al (1992) & Bloomsfield et al (1996) have demonstrated *invitro* evaluation is done & chlorohexidene against ***Pseudomonas spp***. **Bacillus spp.** showed susceptibility to all the six disinfectants Viz. Lysol, Phenol, Dettol, Savlon, alcohol & Betadine. Walsh et al., (1997) demonstrated effect of testing methods on the activity of high level antibacterial disinfectant.

CONCLUSION

Lysol was the disinfectant of choice as it was effective against all the isolate *Pseudomons, Micrococcus* and *Bacillus spp.* followed by Savlon & Dettol. Due to environmental changes & mutation in the genome structure of the microorganism they were becoming more & more resistant to present day disinfectants & there is need to search for newer disinfectants. Hence from the present investigation it is concluded that the comparative efficacy assessment of disinfectant are essential against surrounding microbial population so as to identify the best disinfectant against existing microflora to be irradicated & to develop new disinfectants for resistant microorganism. Hence it is concluded that comparative efficacy assessment of disinfectants are essential against surrounding microbial population so as to identify the best disinfectant against existing microflora to be eradicated & to develop new disinfectant against resistant microbial population.

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