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RESEARCH ARTICLE

TIME DEPENDENT EVALUATION OF EFFICACY ON INHIBITION OF BACTERIAL AND FUNGAL GROWTH OF THREE DISINFECTANTS BY IMMERSION AND SPRAYING METHOD ON POLYVINYL SILOXANE IMPRESSION MATERIAL - AN IN-VITRO STUDY

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0.2% Peracetic Acid, Polyvinylsiloxane Disinfection, Impression Disinfection,2 % Glutaraldehyde,70 %Isopropyl Alcohol, Immersion and Spraying

Abstract

Aim: To determine the efficacy of three disinfectants-2% Glutaraldehyde,0.2% Peracetic acid,70%Isopropyl alcohol with time(3 minutes and 7 minutes) on Staphylococcus Sp. and Candida Sp. on polyvinyl siloxane impression material.
Settings and Design: This was an in vitro comparative study.
Materials and Methods:A standard inoculum of 0.5 McFarlandwas applied on the prepared polyvinyl siloxane disc surface.Discs were disinfected by immersion and spraying method for 3 minutes and 7 minutes.After that discs were collected in nutrient broth,vortexed and a small volume of the broth was centrifuged.It was thentransferred to test tube containing neutralizing agents of the corresponding disinfectants.0.1 ml from the test tube was transferred to culture plate and incubated to study thesurvivality of Staphylococcus Sp. and Candida Sp.
Statistical Analysis Used: One way ANOVAtest was performed to check the mean reduction of microorganism in each group after disinfection by immersion and spraying.Inter group comparison of disinfection efficacy within certain time(3 minutes or 7 minutes) span was done by Tukey's post hoc test.
Results: Percentage(%) of mean reduction of staphylococcus and candida's growth obtained from this studywere 99.99% by immersion and spraying method within 3 minutes and 7 minutes.
Significance: Both immersion and spraying methods had similar reduction efficacy(99.99%) when compared with control.

Introduction:-

The primary duty of the healthcare professionals is to provide the appropriate methods of cleaning, disinfection and sterilization for various surfaces and instruments and materials to avoid the spread of pathogens.Disinfection is thedestruction of all pathogenic organisms on inanimate objects except bacterial sporeswhile sterilization is the complete elimination of all microorganisms including spores.The nature of the disinfection protocol varies according to the product used.On this basis disinfectant can be divided into three categories namely low level, intermediate and high-level disinfection[1].Glutaraldehyde is a high-level disinfectant.It is available in neutral, alkaline and acidic forms.It is a broad-spectrum chemical agent with fast killing capability which is also called chemo sterilizer.Recommended concentration for dental equipment sterilization of glutaraldehyde is 2 % and it's an

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alkylating agent for proteins which mainly affects amines, amides and sulfhydryl groups.Isopropyl alcohol provides intermediate level disinfection.It is non-oxidizing and mainly used within the range of 60-90%.It solubilize the lipid content of cell membrane and precipitate the protein. Isopropyl alcohol is normally used as antiseptic[2].Now a days peracetic acid is considered a feasible alternative because it is composed of acetic acid and hydrogen peroxide and its byproducts are biocompatible substances.Peracetic acid is utilized in several healthcare areas as an effective high-level disinfectant.This material is biocompatible, biodegradable, presents a fast action even in low concentrations, acts in the presence of organic matter,may sterilize contaminated materials in short periods and has a low corrosive potential reducing the risk of equipment damage[3].

The aim of the study was to determine the disinfection efficacy of three disinfectants-2% Glutaraldehyde,0.2% Peracetic acid,70% Isopropyl alcohol with time(3 minutes and 7 minutes) on Staphylococcus Sp. and Candida Sp. on polyvinyl siloxane impression materialconsidering the null hypothesis of this study that 2% glutaraldehyde,0.2% per acetic acid or 70%isopropyl alcohol was not effective in inhibiting the growth of Staphylococcus Sp. and Candida Sp.significantly within a predetermined period of time.

Materials And Methods:-

Specimen Prepatration

The study was approved by the Institutional Ethical Committee.150 polyvinylsiloxane disc samples of 15 mm in diameter and 3 mm of thickness were prepared by commercially available light-body hydrophilized PVS impression material (DPIPhotosil Light Body)[4,5].The impression material was molded into disk-shaped specimens in metal molds (15 mm diameter and 3 mm height)(Fig-1).The molds were arranged on a glass plate covered with a polyethylene (PE) sheet and loaded to excess with impression material.A glass plate was placed on the fixed tray of a hydraulic press.Another glass plate covered with a PE sheet was pressed slowly onto the impression material, and a uniform load was applied to the entire assembly between the fixed and movable trays for the time recommended by the manufacturer(Fig-2).After polymerization, the specimens were carefully separated from the molds.Excess material on specimen was removed with BP blade.The specimens were given the shape of a disc with dimeter of 15 mm and thickness of 3 mm.(Fig-3)

Microbiologicalsample Preparation

To prevent cross contamination, surface of the disc sample is sterilized with Ultraviolet Ray in the safety cabinet.(Fig-4).Mueller Hinton Agar,Saboraud's dextrose agar(SDA) was prepared and mediums were poured into different sterile plates.The respective sterile plates were then inoculated with clinical isolates of Staphylococcus species and(MHA)(Fig-5-i),Candida species(SDA)(Fig-5-ii).Culture plates were then incubated in a bacteriological incubator at 37^0C for 24 hours for Staphylococcus species and 48 hours for Candida species.

Thus reservoirs of respective plates were prepared and colonies were suspended in McFarland 0.5 turbidity standard and was prepared according to the method recommended by the National Committee for Clinical Laboratory standards (NCCLS, 1999), the standard was prepared by adding 0.5 mL of 1.175% w/v Barium Chloride and 99.5 mL of 15% w/v Sulphuric acid.This was mixed well and then aliquots were made into test tubes identical to the ones used in preparing inoculum suspensions of the test organisms (Fig-6-i).The accuracy of the density of the standard was verified using a spectrophotometer (Fig-6-ii).

Evaluation Of Disinfection Efficacy

Previously prepared standard concentrations (0.5 Mcfarland) of Staphylococcus Sp. and Candida Sp. were used for making inoculums in test tubes containing normal saline each having microorganism of 1.5×10^8 CFU/ml.An inoculums of 0.1 ml was applied using sterile pipette (Fixapette,Tarsons) (Fig-7) on the surface of the polyvinylsiloxane disc kept on the sterile petridish.Inoculated discs were made to dry in a class 2 laminar flow (BIOBASE INTERNATIONAL) for 20 minutes.Two blank discs with inoculum and without disinfectant acted as positive control(3 min & 7 min)) and one blank disc without inoculum and disinfectant acted as negative control in each round of study.For Staphylococcus group,a total of 10 discs after inoculation were not disinfected and acted as positive control.Rest discs(60) in each group were divided into 3 groups that were disinfected with 3 disinfectants by immersion or spaying for 3 and 7 minutes.For estimation of bacterial specimen of control,serial dilution method was applied and colony forming unit within 30-300 was considered.Disinfected discs were kept aseptically in 5 ml nutrient broth in centrifuge tubes and vortexed for 1 minute, after which 1 ml aliquots were removed and kept in 1.5 ml Eppendorf tubes and centrifuged at 3000 rpm for 5 minutes (REMI R-8C)(Fig-8) for recovery of bacterial cells.Followingcentrifugation,pellets(1 ml) were resuspended in 4 ml nutrient broth that was supplemented with

sodium bisulfite for neutralization of glutaraldehyde[6,7],0.5% sodium thiosulfate for neutralization of peracetic acid[8], polysorbate 80 for neutralization isopropyl alcohol[9]of remaining disinfectant and were platedon Mueller Hinton Agar (HIMEDIA) for Staph aureus and Sabouraud Dextrose Agar (SDA) (TM MEDIA) for Candida albicans.Followinginoculation,media were kept in incubator (ESCO INDUSTRIES) at 37 degrees centigrade for 24 hours.Incubation colonies on media if any,were counted under light microscope and recorded.The number of colonies were then multiplied by 50 to calculate number of bacterial and fungal CFU/ml[10,11].The data was collected and transferred to a table.On the basis of this collected data statistical analysis was made.

Schedule Of Data Collection

Data was collected after incubation of inoculated microbial strain at the definite intervals of 24hours for Staphylococcus species and 48 hours for Candida species.

Statistical.Methods

Colony-forming unit (CFU) is a measure of viable bacterial or fungal cells. In direct microscopic counts (cell counting using haemocytometer) where all cells, dead and living, are counted, CFU measures only viable cells. The CFU/ml was calculated using the formula:

CFU/ml = (no. of colonies x dilution factor) /volume transferred (1)

For performing the statistical analysis,data were entered into a Microsoft excel spreadsheet and then analysed by using SPSS software (IBM Corp 2013; Version 22.0; Armonk, NY). ANOVA one way test was performed to check the mean reduction of microorganism in each group after disinfection by immersion and spraying.(Fig-12,13)Inter group comparison of disinfection efficacy within certain time(3 minutes or 7 minutes) span was done by Tukey's post hoc test.(Fig-14)

Result:-

The results of the study showed that by both immersion and spraying method, there was 99.99% of reduction of Staphylococcusand Candida species within 3 minutes and 7 minutes.In terms of reduction in colony count Glutaraldehyde for 7 minutes of immersion showed highest reduction efficacy[mean log reduction 2.39(Fig-11) for Staphylococcus and 2.32(Fig-9) for Candida species] among all the groups.

In case of both Candida species and Staphylococcus species,comparison of reduction of species with control was statistaicallysignificant,but intergroup comparison was not significant(Fig-14)

Discussion:-

The Centers for Disease Control and Prevention (CDC),in its infectioncontrol guidelines, mentioned that dental impressions are potential sources of cross contamination and should behandled in a manner that prevents exposure of practitioners, patients, and the environment to the pathogens.

Before checking the efficacy of 3 disinfectants, the surface of the polyvinyl siloxane discs were sterilized with ultraviolet rays so that there won't be presence of any other microorganism on the disc surface which could hamper the growth of desired microorganism on the culture plate. Godbole et al. in 2014, found that the UV chamber could be safely used for the polyvinyl siloxane impressionmaterial[12].Nimonkar Set al. in 2019,concluded that UV sterilization of polyvinyl siloxane disc sample for 20 minutes did not cause any significant dimensional changes[13].

In the present study, it is found that the averages of colony counts of the disinfectant treated groups show a decline in value for all the organisms studied.This proves the general efficacy of all the disinfecting protocols to reduce microorganism counts from the impressions recorded.A 3 log 10 or 99.9% reduction in colony forming unit count has been taken as standard for determination of efficacy of tested disinfectants as recommended by Drennon DG et al[14].Application of 2% glutaraldehyde significantly lower retained Staphylococcus species on polyvinylsiloxane disc surface ($p<0.00001$).Hence,it can be assumed from this finding that eradication of Staphylococcus species from impression surface might be easier with 2% glutaraldehyde.The result of the study is also similar with the study done by Azevedo MJ et al. (2021) ,where using glutaraldehyde based disinfectant(MD520) reduced microbial load by more than 99.9%[15].Ganavadiya R et al.(2014) in their study evaluated total viable count after disinfection with 2% glutaraldehyde, 6% hydrogen peroxide (H_2O_2), and 99.9% ethyl alcohol with distilled water as a negative control and autoclaving as a positive control.It was found that maximum reduction in microbial load was seen with H_2O_2 followed by glutaraldehyde, ethyl alcohol, and distilled water[16].Result of this study is also almost similar

with the study done by Bustos J et al.(2010) who investigated the effect of 0.05% NaOCl and glutaraldehyde after 5 and 10 min on silicone impressions. Silicone specimens immersed in 2% glutaraldhyde for 10 minutes was able to completely eliminate the bacteria absorbed in the impressions[17].The duration of immersing the polyvinyl siloxane was restricted to 7 minutes because of the ADA recommendations where soaking impression materials in disinfectant solutions was recommended to be <30 min [18].Muller-Bolla Met al.(2005) stated that the soaking method is applied in 63% of alginate impressions and in 73% of silicone impressions in European schools of dentistry.Furthermore, the approximate time of disinfection was 10.3 ± 6.3 min[19] .Results of this study are also similar with study done by Sukhija U et al.(2010),where alginate impression material was used for antimicrobial evaluation of disinfectants.The intergroup comparison of efficacy of Glutaraldehyde with Peracetic Acid showed that the difference is statistically non-significant with p value of 0.49.Comparing Glutaraldehyde with Isopropyl Alcohol,the results obtained were highly significant(p value is 0.006).When Peracetic acid was compared with Isopropyl Alcohol for microbial growth values, the difference was statistically significant with p value 0.0004[20].

Al-Jabrah et al. (2007) in his study compared the efficacy of 4 disinfectants(Dimenol(Isopropyl alcohol based),Perform-ID,MD520(Glutaraldehyde based),and Haz-tabs)applied by spraying method for 10 minutes on 3 impression materials(Alginate,Polyether,Polyvinylsiloxane).The results showed that no growth(100% reduction) was observed from any sample of impression materials treated with any disinfectant regimens under investigation[21].Complete elimination of microorganism(both bacteria and fungus) occurred after 5 minutes of immersion in 96% isopropyl alcohol in the study reported by Ahmed HM et al.(2020)[22].Demajo JK et al.(2016) in his study concluded that spraying glutaraldehyde-based disinfectant(MD 520) was effective in eliminating all microbial forms for both alginate and silicone without modifying the dimensional stability[23].Chino Tet al.(2017) investigated the bactericidal fast-acting effects of peracetic acid,a high-level disinfectant,against Staphylococcus aureus and Pseudomonas aeruginosa biofilms in tubing.Peracetic acid showed the most rapid killing of all 24 strains (within 1 min).In the same study glutaraldehyde took 5 min for 100% bactericidal activity for Staphylococcus aureus and Pseudomonas aeruginosa[24].

Samra Retal.(2010) in their study used Korsolex [Glutaraldehyde 2%] in 1:19 dilution to check disinfection efficacy for a polymicrobial specimen.(Streptococcus viridans,Diphtheroids,Streptococcus,Candida albicans,Pseudomonas aerugenosa and Staphyloccus albus).Average disinfectioneffectiveness of glutaraldehyde was found to be ranged from 64 to 72% while that of sodium hypochlorite ranged from 92 to 96% for almost all the groups[25].

Results are not similar with the study done by Ghahramanloo A et al.(2009),whereDeconex(alcoholic based disinfectant) could eradicate only 70.4% of microorganisms[26].The main reason for this difference would be that they used irreversible hydrochloride,which has more porosities and cause deep penetration of microorganism into this impression material and can define the lesser capacity of disinfectant agent in eradicating microorganisms.

Fig-1

Fig-2

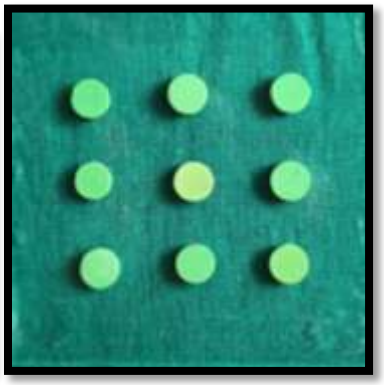

Fig-3

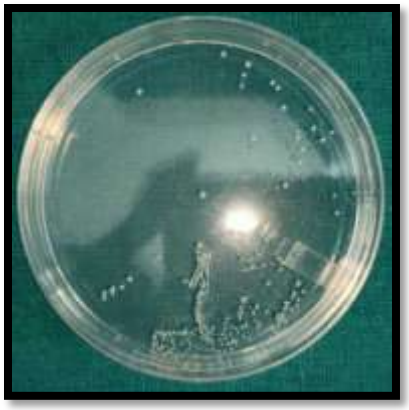

Fig-4

Fig-5-i

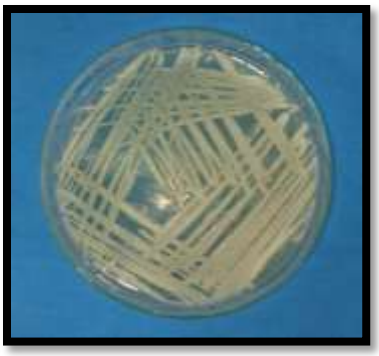

Fig-5-ii

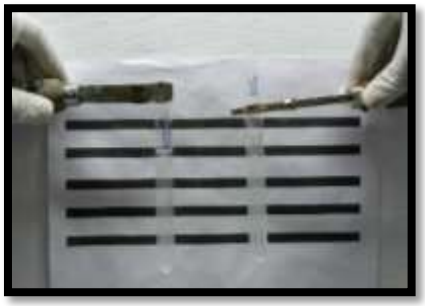

Fig-6-i

Fig-6-ii

Fig-7

Fig-8

Fig 9:- Bar diagram showing mean log value of recovered cell density (CFU/ML) of Candida sp. at 7 min.

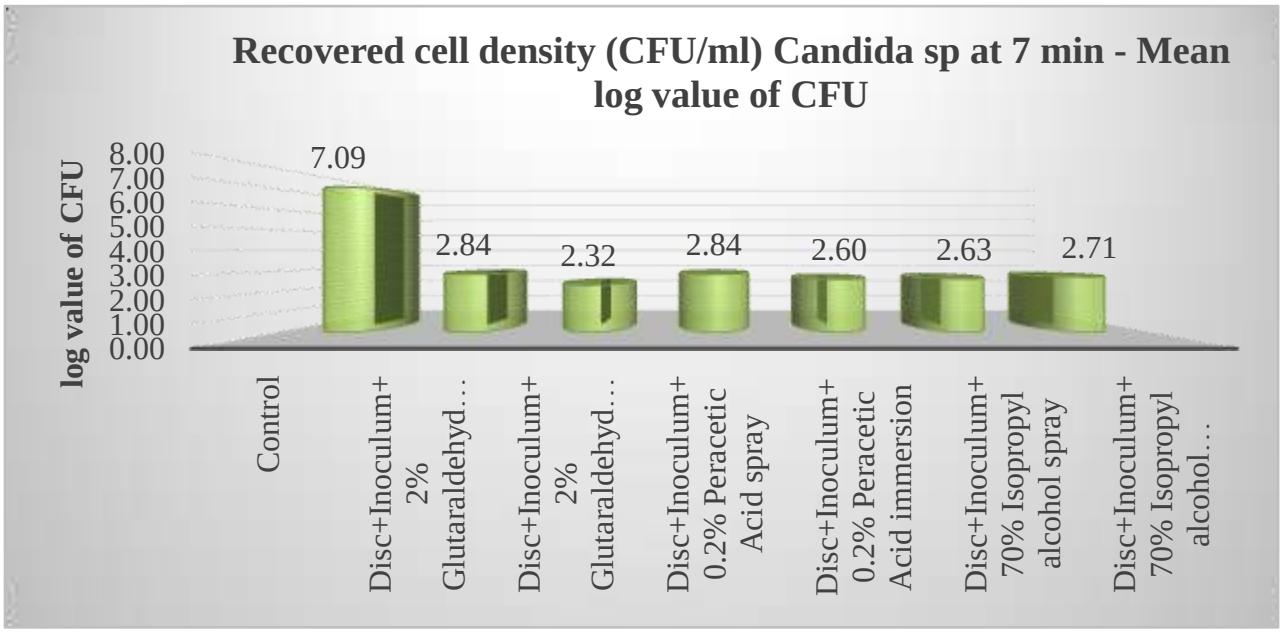

Fig 10:- Bar diagram showing mean log value of recovered cell density (CFU/ML) of Candida sp. at 3 min.

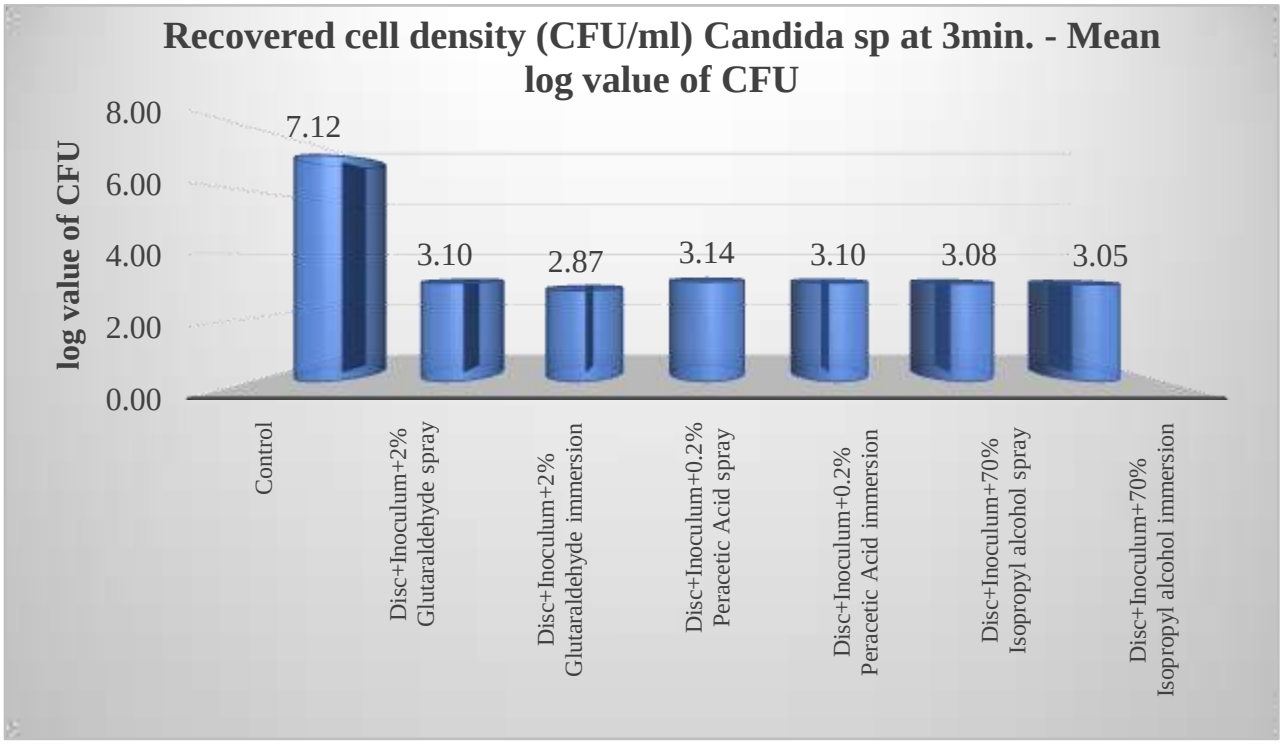

Fig 11:-Bar diagram showing mean log value of recovered cell density (CFU/ML) of Staphylococcus sp. at 7 min.

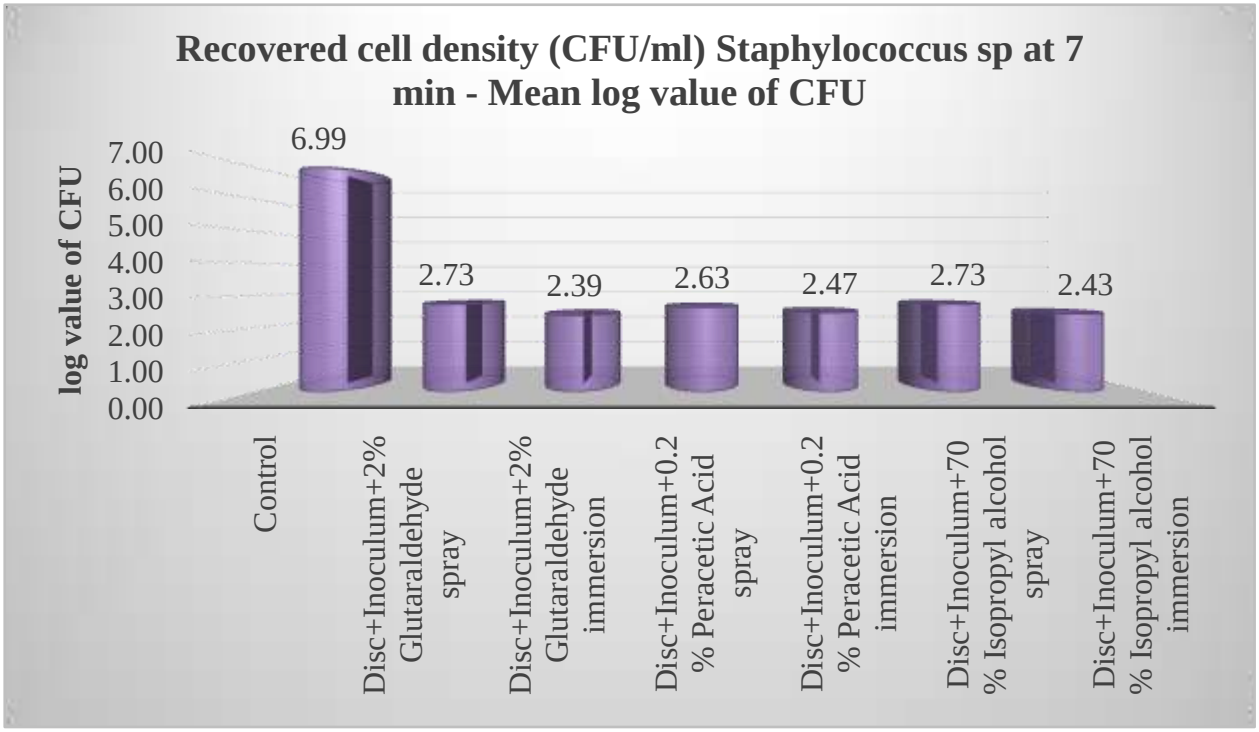

Fig 12:- Bar diagram showing mean log value of recovered cell density (CFU/ML) of Staphylococcus sp. at 3 min.

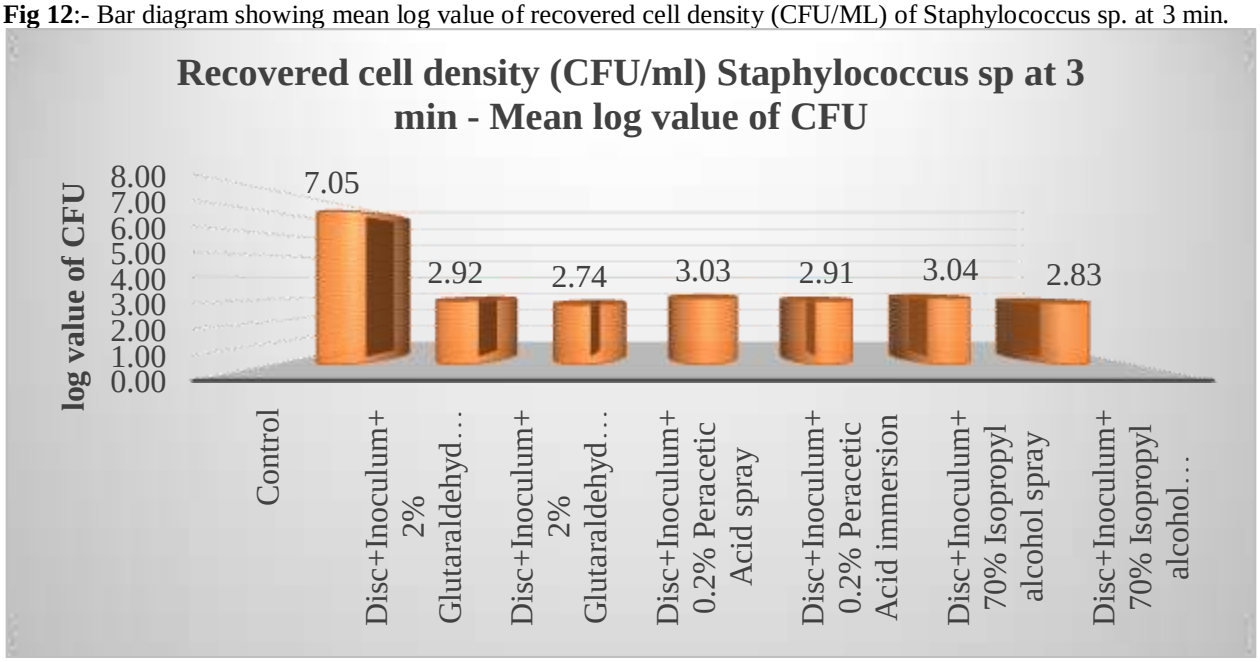

Fig 13

Groups at 7 mins	Mean CFU/ml	CFU/ml (S.D.)	Mean log value of CFU	Mean log value of CFU SD	Mean log reduction	% Mean reduction	P value
Control	12240000	93968 0.80	7.09	0.03	--	--	<0.0001

Disc+Inoculum+2% Glutaraldehyde spray=$Spray_{Candida}A_7$	690.00	54.77	2.84	0.04	4.25	99.994	Significant
Disc+Inoculum+2% Glutaraldehyde immersion=$Immersion_{Candida}A_7$	210.00	41.83	2.32	0.09	4.77	99.998	
Disc+Inoculum+0.2% Peracetic Acid spray=$Spray_{Candida}B_7$	710.00	207.36	2.84	0.14	4.25	99.994	
Disc+Inoculum+0.2% Peracetic Acid immersion=$Immersion_{Candida}B_7$	400.00	50.00	2.60	0.06	4.49	99.997	
Disc+Inoculum+70% Isopropyl alcohol spray= $Spray_{Candida}C_7$	430.00	75.83	2.63	0.07	4.46	99.996	
Disc+Inoculum+70% Isopropyl alcohol= $Immersion_{Candida}C_7$	520.00	97.47	2.71	0.08	4.38	99.996	

Groups at 7 mins	Mean CFU/ml	CFU/ml (S.D.)	Mean log value of CFU	Log value of CFU SD	Mean log reduction	% Mean reduction	P value
Control– $Staph_{Control\ 7min}$	9740000	991463.56	6.99	0.04	--	--	<0.0001 Significant
Disc+Inoculum+2% Glutaraldehyde spray= $Spray_{Staph}A_7$	540	89.44	2.73	0.07	4.26	99.994	
Disc+Inoculum+2% Glutaraldehyde immersion= $Immersion_{Staph}A_7$	260	89.44	2.39	0.16	4.60	99.997	
Disc+Inoculum+0.2% Peracetic Acid spray =$Spray_{Staph}B_7$	430	83.67	2.63	0.08	4.36	99.996	
Disc+Inoculum+0.2% Peracetic Acid immersion= $Immersion_{Staph}B_7$	300	50.00	2.47	0.07	4.52	99.997	
Disc+Inoculum+70% Isopropyl alcohol spray = $Spray_{Staph}C_7$	550	158.11	2.73	0.13	4.26	99.994	
Disc+Inoculum+70% Isopropyl alcohol	270	44.72	2.43	0.07	4.56	99.997	

immersion = $Immersion_{Staph}C_7$							

Fig 14

Comparison between	P value	Result
$Candida_{Control\ 3min}$ Vs$Spray_{Candida}A_3$	0.000	Significant
$Candida_{Control\ 3min}$ Vs$Immersion_{Candida}A_3$	0.000	Significant
$Candida_{Control\ 3min}$ Vs $Spray_{Candida}B_3$	0.000	Significant
$Candida_{Control\ 3min}$ Vs $Immersion_{Candida}B_3$	0.000	Significant
$Candida_{Control\ 3min}$ Vs $Spray_{Candida}C_3$	0.000	Significant
$Candida_{Control\ 3min}$ Vs $Immersion_{Candida}C_3$	0.000	Significant
$Spray_{Candida}A_3$Vs $Immersion_{Candida}A_3$	1.000	Not significant
$Spray_{Candida}A_3$ Vs $Spray_{Candida}B_3$	1.000	Not significant
$Spray_{Candida}A_3$ Vs $Immersion_{Candida}B_3$	1.000	Not significant
$Spray_{Candida}A_3$ Vs $Spray_{Candida}C_3$	1.000	Not significant
$Spray_{Candida}A_3$ Vs $Immersion_{Candida}C_3$	1.000	Not significant
$Immersion_{Candida}A_3$ Vs $Spray_{Candida}B_3$	1.000	Not significant
$Immersion_{Candida}A_3$Vs $Immersion_{Candida}B_3$	1.000	Not significant
$Immersion_{Candida}A_3$ Vs $Spray_{Candida}C_3$	1.000	Not significant
$Immersion_{Candida}A_3$ Vs $Immersion_{Candida}C_3$	1.000	Not significant
$Spray_{Candida}B_3$Vs $Immersion_{Candida}B_3$	1.000	Not significant
$Spray_{Candida}B_3$Vs $Spray_{Candida}C_3$	1.000	Not significant
$Spray_{Candida}B_3$Vs $Immersion_{Candida}C_3$	1.000	Not significant
$Immersion_{Candida}B_3$Vs $Spray_{Candida}C_3$	1.000	Not significant
$Immersion_{Candida}B_3$Vs $Immersion_{Candida}C_3$	1.000	Not significant
$Spray_{Candida}C_3$Vs $Immersion_{Candida}C_3$	1.000	Not significant

Conclusion:-

According to the results of the study the following conclusions can be made:

1. The mean overall reduction of microorganism(CFU/ml) was maximum for 2% Glutaraldehyde in which discs containing inoculum were immersed for 7 minutes for both Staphylococcus and Candida group.
2. Both immersion and spraying methods had similar reduction efficacy(99.99%) when compared with control.
3. The log reduction value increased as the time increased.(4.25 for 3 minutes and 4.77 for 7 minutes) for Candida and Staphylococcus species(4.31 for 3 minutes and 4.6 for 7 minutes).
4. 0.2% Peracetic acid ,which is non toxic and non irritant, can be used as an effective and cheaper alternative to 2% Glutaraldehyde and 70% Iso propyl alcohol for disinfection.

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