Original article

Are antiseptics and disinfectants commonly used against methicillin resistant staphylococcus aureus effective? A study in a tertiary care hospital

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Abstract:

Introduction:Strains sensitive to beta-lactam antibiotics are called methicillin sensitive Staphylococcus aureus (MSSA) and strains resistant to beta-lactams are defined as methicillin resistant Staphylococcus aureus(MRSA). The acquisition of extra gene has rendered them to be named asone of the serious emergent pathogens in the hospital environment.The aim of this study was to assess the efficacy of eight antiseptics and disinfectants used commonly against MRSA and MSSA in a tertiary care hospital.

Methods: Few of the commonly used disinfectants were tested against isolated strains of MRSA and MSSA from clinical samples at the stock strength and upto its 16th fold dilution according to the methods recommended in CLSI guidelines. The antiseptics and disinfectants used were 10%povidone iodine, 4% chlorhexidine gluconate solution, 4.8% chloroxylenol dettol,2% lysol, 2% glutaraldehyde, 1% sodium hypochlorite solution, 2% liquid phenol, chlorophenol , each in different dilutions for both MRSA ans MSSA strains.

Observations and Results: Chloroxylenol and chlorhexidine gluconate were most effective disinfectants on MRSA and MSSA strains, followed by povidone iodine, lysol, phenyl chlorophenol. While interestingly 1% sodium hypochlorite and 2% glutaraldehyde showed full resistance against both the strains.The most important finding being resistance of all the MRSA isolates against 2% liquid phenol but uniquely this is effective on MSSA isolates.

Conclusion:The wide spread use of these products in the hospital environment has prompted some speculation that these they can induce resistance gene in the microbes to be propagated to the hospital environment.

Keywords: Antiseptics, Disinfectant,MRSA, MSSA, Hypochlorite, Glutaraldehyde, Phenol

Introduction:

Sterilization is a process by which all forms of microbial lifes are destroyed.It is an absolute term whereas disin-fection refers only to the destruction of pathological flora.Chemicals used to destroy all forms of microbilogical life, can be called chemical sterilants. They can be used for shorter exposure periods as part of the disinfection process [1]. Antiseptics and disinfectants are part and parcel of hospital infection control management policy. Th-ese

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are extensively used in hospital and other health care setups for a variety of topical and surface application .[2]A wide variety of active chemical agent [biocides] are found in these products. Many of which have been use-d for hundreds of years including alcohol, phenol, iodine, chlorine. Though most of these active agents demons-trate broad spectrum antimicrobial activity, the mode of their action is still evolving in comparision to antibiotics. The wide spread use of these products has shortly induced the developement of microbial resistance towards them. 'Biocide' can act as 'cidal' and 'static' form for microbes.Antiseptics are biocides that destroy or inhibit growth of microorganisms on living tissue, mainly used for hand washes and surgical scrubs[3] .Alcohol is used for sterilizing skin whereas povidone iodine is applied on skin before any operating manuevers [3].The different disinfectants and antiseptics used in this study were10% w/v povidone iodine, 4% chlorhexidine gluconate solu-tion, 4.8% w/v chloroxylenol(dettol),2% lysol(cresol 50% v/v + soap 50% v/v) , 2% glutaraldehyde, 1% sodium hypochlorite solution, 2% liquid phenol(carbolic acid), chlorophenol 5% v/v (phenyl liquid/black disinfectant fl-uid/per-fenol). Phenolics(carbolic acid) which are intermediate level disinfectants act as bacteriocidal, fungicidal , virucidal, tuberculocidal and clean floor, walls, hard surfaces and equipements that doesnot touch mucous membrane. Phenol leaves residual film on environmental surfaces. In some cases, they have been used for handwa-shing, cleaning in low dose.Phenol acts specifically on the cell membrane and inactivates intracytoplasmic enzy-mes by forming unstable complexes[3].Among the phenolics 2% liquid phenolis the effective concentration for spillage and cleaning in general bacteriology in presence of organic and tuberculous materials [3]whereas Lysol (c-resol 50% v/v + soap 50% v/v) acts as a surfactant disrupting the cytoplasmic membrane.Povidone iodine acts as bacteriocidal, fungicidal, virucidal, sporicidal with the help of its activeated iodine.Other phenol derivatives like chlorhexidine gluconate and chloroxylenoldamages cell membrane irreversibly[3].Among the two high level disinfectants1% sodium hypochlorite acts as bacteriocidal and sporicidal interfering in the cytoplasmic membr-ane integrity with an irreversible enzymetic inhibitionand 2% glutaraldehyde alters RNA , DNA, and protein sy-nthesis. Chlorop-henol 5% V/V (blackfluid)which is a low level disi-nfectant inhibits the membrane bound part of the electron transport chain, and also induces leakage, causes protoplast lysis, and inhibits respiration.

Aims and objectives: To assess the efficacy of eight antiseptics and disinfectants used commonly against MRS-A and MSSAin a tertiary care hospital.

Materials and methods:

It is a laboratory based interventional study conducted during one year period. This study focuses on Staphylo-coccus aureus with its MRSA and MSSA variants. Pure cultures of MRSA and MSSA were isolated from vari-ous clinical samples received in bacteriology laboratory in the Department of Microbology in B.S.M.C.H. The test organisms were identified to the species level based on standard microbiological methods such as ; culture characteristics[4], cellular characteristics (microscopic examination)[4]and biochemical tests [4]and susceptibility patterns wereidentified as per CLSI guidelines 2015[3].Strains were differentiated into individual isolates accor-ding to their different resistogram patterns. The antiseptic and disinfectant soluteons were procured from differ-ent manufacturing companies and they were purchased as hospital supplied .Phenol / carbolic acid(80% w/v)was from Indian Drug House, Sonarpur,24Pgs, West Bengal whereas hypochlorite solution (4%) was from Stan

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Bio Reagent Pvt. Ltd,Kolkata.. Both lysol and Chlorophenol(5% V/V) were manufactured by P. H. Laboratory, Ma-dhyamgram. 2% glutaraldehyde and chloroxylenol (dettol) were manufactured from D.P.Enterprise,Kolkataand from Hootagali Ind area, Mysore respectively. The source of povidone iodine was Win-Medicare Pvt. Ltd., New Delhi. Lastly the manufacturer of the chlorhexidine gluconate was 3M India Limited, Ranjangaon, Pune. All th-ese disinfectants and antiseptics had been used according to the procedure as per recommended by the manufa-cturer. The antimicrobial activities of these tested agents were measured against all the isolates of MRSA and MSSA by disc diffusion method and minimum inhibitory concentration method.

Susceptibility testing by disc diffusion method: For each antiseptic and disinfectant 6mm discs were prepared following standard protocol, where Whatman no 1 filter paper had been used to absorb approximately the whole volume of one drop (0.01ml) from micropipette[5].After impregnation, discs were dried by leaving them in an in-cubator for 2hrs. For each of the eight different types of disinfectants and antiseptics discs were prepared upto 1-6th fold of dilution[6].Disc diffusion methods of susceptbility testing was performed following standard method of Kirby-Bauer disc diffusion method as per CLSI guideline 2015 [2][3]where standardisation of the bacterial sus-pension was done by adjusting the turbidity with 0.5Mc Farland standard (i.e., 1.5×10^8 CFU/mL)[4].

Susceptibility testing by minimum inhibitory concentration (MIC) method: This MIC test was determined by broth microdilution technique according to the method recommended in CLSI guidelines 2012 depending on the turbidity of the bacterial growth in overnight culture suspensions of individual bacterial isolate as measured by colorimeter in terms of optical value [4][7][8].The MIC was recorded as the lowest concentration of the tested disinfectant/antiseptic that will inhibit the visible growth of the organism or that prevents bacteria under test to grow after overnight incubation.

Observations and Results: In order to assess the efficiency of commonly used antiseptics and disinfectants in various fields of healthcare setup, eight of them were tested towards the two emerging strains of MRSA and M-SSA. Results are depicted in table (1) and table (2).

Results of discdiffusion method: If thezone of inhibitions were compared between MRSA and M-SSA for the eight disinfectants and antiseptics they were (8-14)mm and (9-19)mm; (9- 25)mm and (10-19)mm ; (11-26)mm and (14-26)mm ; (7-23)mm and (10-25)mm; (7-18)mm and (7-28)mm for povidone-iodine [Figure 1], black dis-infectant fluid [Figure 2], chlorhexidine gluconate [Figure 3] , lysol [Figure 4], chloroxylenol [Figure 5] respectively.Secondly, as found in the disc diffusion method the doses of resistance for the tested disinfectants and anti-septics for MRSA and MSSA were 12.5mg/ml and 6.5mg/ml ; 8mg/ml and 16mg/ml; 130mg/ml and 65mg/ml; 6mg/ml and 1.5mg/ml for povidone-iodine, blackfluid, lysol and chloxylenol respectively. Chlorhexidne gluco-nate showed full susceptibility against both MRSA and MSSA strains till its 16th fold dilutions with a variable zone of inhibition. 2% liquid phenol was established as totally resistant against MRSA whereas for MSSA only the stock strength ie. 20mg/ml showed susceptibility and rest all the dilutions came to be resistant [Figure 6]. 1 % hypochlorite and 2% glutaraldehyde expressed their full resistant for both MRSA and MSSA strains even at their stock strengths interestingly.

Results of minmum inhibitory concentration method: Comparision of the MIC values between MRSA and MSSA for the eight tested disinfectants showed values were 2.5mg/ml,12.5mg/ml,

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33mg/ml in case of chlorhex-idine gluconate, povidone-iodine and lysol respectively. Whereas MIC values of the phenyl chlorophenol liquid for MRSA and MSSA it was 16mg/ml and 8mg/ml respectively. Chloroxylenol (dettol) showed full susceptibi-lity even at its 16^{th} dilution. In MIC method 2% liquid phenol revealed the complete resistance against MRSA b-ut showed MIC value of 20mg/ml for MSSA strains. In case of 1% hypochlorite and 2% glutaraldehyde both M-RSA and MSSA showed complete resistance even at their original stock strengths as depicted in MIC procdure.

In this short study the tested isolates of MRSA and MSSA were selected depending on their significant recurre-nce in nosocomial infection (nosocomial infection with MRSA is of 16%) Comparision of the results of the disc diffusion method itself, showed hospital supplied commercial povidone iodine (10% w/v), lysol (cresol + soap solution), chlorhexidine gluconate,chloroxylenol (dettol), blackfluid (chlorophenyl) were variably susceptible against both MRSA and MSSA strains. It varied not only with the type of disinfectants but also with the differe-nt concentrations of each disinfectant. For an individual isolate and for a particular type of disinfectant's streng-th MSSA showed larger zone size in comparision to MRSA in case of povidone-iodine, lysol, dettol, chlorhexi-dine gluconate. Only in phenyl blackfluid zone size of MSSA remains unchanged in comparision to MRSA. Di-sc diffusion also showed that for all the disinfectants including povidone-iodne, lysol, chlorhexine gluconate, c-hloroxylenol(dettol) , 2% liquid phenol showed resistant to MRSA at a lower concentration (higher dilution) in comparision to MSSA for each of the strengths.Only the blackfluid/ phenyl chlorophenol showed the alteration. Among the eight disinfectants comparative results of the MIC values showed MSSA strains having lesser MIC values than that of MRSA in case of blackfluid and 2% liquid phenol. Rest all the disinfectants were having the same MIC values for both MRSA and MSSA strains.

Table no. 1 shows variability of sizes of zone (mm) of inhibition in 8 different disinfectants and antiseptics (ave-rage values of 10 MRSA and 5 MSSA strains were calculated) concluded complete resistance in both MRSA a-nd MSSA against 2% glutaraldehyde and 1% hypochlorite while both the strains were resistant against 2% liqu-id phenol except MSSA is susceptible to the stock strength of 2% liquid phenol only. Among rest of the 5 disin-fectants MSSA showed a higher range of zone of inhibition in comparison to MRSA.Considering 10 MRSA strains and 5 MSSA strains positive control for MRSA and MSSA were 1.82-1.84 and 1.87-1.9 respectively. Negative control for povidone iodine, chlorhexidine gluconate, dettol, blackfluid, 1% hy-pochlorite, 2% glutara-ldehyde, lysol and 2% phenol were 0.3, 0.12, 0.24, 1.11, 1.2, 1.0, 0.33, 1.0 resp-ectively. Table no 2 showing variability of the optical values for 8 different disinfectants and an-tiseptics against MRSA and MSSA strains (average of 10 different MRSA and 5 different MSSA taken) determined the MIC values of lysol, povide iodine , chlorhexidine gluconate as 33mg/ml,12.5mg/ml, 2.-5mg/ml. Dettol showing susceptibility in all streng-ths. Blackfluid having MIC at 16mg/ml for MRSA and 8mg/ml for MSSA.1% hypochlorite and 2% gl-utaraldehyde both were showing full resistance for MRSA and MSSA where 2% liquid phenol showed the on-ly sensitivity at the stock strength for MSSA.

Discussion:

Disinfectants are usually tested against Salmonella typhi in Rideal-Walker coefficient test but with an emergen-ce of resistant strains of different types of bacterial isolates it requires that a continuous

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evaluation should be do-ne for emerging and emergent organisms like ESBL, MRSA etc.The present study shows the emergence of resi-stance of MRSA to commonly used disinfectants specially in hospital environment being invaded by 16% of M-RSA. So this is required that antiseptics and disinfectants should be evaluated in this light.This study results sh-ow chloroxynelol (dettol) and chlorhexidine gluconate as the two maximally effective disinfectants against Stap-hylococcus aureus. Least susceptibility was found toward 1% hypochlorite and 2% glutaraldehyde solution. 2% hospital supplied liquid phenol(carbolic acid) is better suited for MSSA than MRSA.One of the previous studies where both disc diffusion and broth dilution method were used for study the effic-acy of disinfectants and antise-ptics results of MIC method showed chlorhexidine/hibitane as the most effective disinfectant on MRSA followed by chlox-ylenol,hydrogen peroxide, sodium hypochlor-ite , formaldehyde, sodium dichloro-isocyanurate and povidone-iodine, while chlorhexidine cetramide (savlon) showed no efficacy against MRSA[9]Some other study result established povidoneiodine anti-septicsolution as the most rapid bacteriocidal and having maximum pote-ncy till date where both chlorhexidine and hexachlorophene have persistent antibacterial effects even at low co-ncentration [10].Another study from WestBengal, that was done on testing the sensitivity of MRSA and MSSA to different antiseptics and disinfectants concluded that all the MRSA and MSSA were sensitive to dettol,lysol, ch-lorhexidine and 1% hypochlorite solution whereas 15% MSSA with 29% of MRSA were resistant to betadine a-nd 68% MSSA with (46-55)% MRSA had growth in presence of phen-ol[11].Limitation of this study was that the suscep-tibility pattern of the disinfectants were tested aga-inst one bacterial agent named Staphylococcus aur-eus only.

Conclusion:

Increasing prevalence (>16%) of MRSA infection in the hospital care environment has become an emerging pr-oblem worldwide. The antiseptics and disinfectants are part of cleaning maneuvers in different high risk areas li-ke emergency ward, operation theatre, ICCU, ITU etc.In the system of hospital infection control it is essential to make the hospital atmo-sphere absolutely free from dangerous orga-nism like MRSA. In this study certain com-monly used antiseptics and disinfectants were selected for hosp-ital infection control purposes to see their affect on MSSA and MRSA.Therefore inability to kill multi-drug resistant organisms like MRSA, MSSA even by two such high level disinf-ectants(1% hypochlo-rite and 2% glutaraldehyde) and also by another intermediate level disifectant 2% liquid p-henol carries an increased risk of occurance of uncontr-olled severe infections and its transmission in healt-h care set up. Regular follow up of the patients with their infection profile may uncover this fact. Disin-fectants are oft-en misused and rationalization of their use in hospitals is desir-able for control of inf-ections. Therefore, in health care settings with high prevalence of MR-SA4.8% chlorxylenol (dettol)& 4% chlorhexidine gluconate solution a-re the most effective disinfect-ants to avoid cross contaminat-ion in the hos-pital environment.Therefore in this modern era of antibiotics where invention of newer antibiotics having alter-native mode of act-ion agai-nst bacteria is seeking the major attention, findings of this short scientific study wou-ld pave the way of controlling the infections at its root level with the help of effective and suitable disinfectants. This study requires a further study to prove the efficacy of antiseptics and disinfectants toward another emerging pathogen like ES-BL.

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Table no.1: Disc diffusion method for selected disinfectants and antiseptics against MRSAand MSSA isolates

	Lysol			**Povidone iodine**			**Chlorhexidine gluconate**			**chloroxylenol (Dettol)**			**Blackfluid**			**1% hypochlorite**			**2% glutarylde hide**			**2% liquid phenol**		
Dilutions	Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition		Conc mg/ml	Zone of inhibition	
		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm		MRSA mm	MSSA mm
D	520	13	21	1000	12	16	40	18	22	48	19	23	63	R	7	10	R	R	20	R	R	20	R	10
D/2	260	6.0	10	50	10	13	20	17	17	24	18	18	32	R	5	5	R	R	10	R	R	10	R	R
D/4	130	R	R	25	8	9.6	10	16	16	12	13.2	10	16	R	R	2.5	R	R	5	R	R	5	R	R
D/8	65	R	R	12.5	R	9	5	14	15	6	R	7.6	8	R	R	1.25	R	R	2.5	R	R	3	R	R
D/16	33	R	R	6.25	R	R	2.5	14	15	3	R	7	4	R	R	0.62	R	R	1.25	R	R	2	R	R
D/32	17	R	R	3.12	R	R	1.25	13	14	1.5	R	R	2	R	R	0.31	R	R	0.63	R	R	1	R	R

Table no. 2:Minimum inhibitory concentration for selected disinfectants and antiseptics against MRSA and MSSAisolates

	Lysol			**Povidone iodine**			**Chlorhexidine gluconate**			**Chloroxylenol (dettol)**			**blackfluid**			**1% hypochlorite**			**2% glutarylde hide**			**2% liquid phenol**		
Dilution	Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value		Conc mg/ml	Optical density value	
		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA		MRSA	MSSA

D	520	0.333	0.333	100	0.330	0.330	40	0.12	0.12	48	0.24	0.24	63	1.11	1.11	10	1.51	1.3	20	1.3	1.4	20	1.2	1.O
D/2	260	0.333	0.333	50	0.330	0.330	20	0.12	0.12	24	0.24	0.24	32	1.11	1.11	5	1.57	1.5	10	1.57	1.5	10	1.3	1.32
D/4	130	0.333	0.333	25	0.330	0.330	10	0.12	0.12	12	0.24	0.24	16	1.11	1.11	2.5	1.68	1.6	5	1.68	1.54	5	1.5	1.57
D/8	65	0.333	0.333	12.5	0.330	0.330	5	0.12	0.12	6	0.24	0.24	8	1.40	1.11	1.25	1.71	1.72	2.5	1.71	1.6	3	1.52	1.76
D/16	33	0.333	0.333	6.25	1.111	0.667	2.5	0.12	0.12	3	0.24	0.24	4	1.52	1.60	0.63	1.8	1.79	1.25	1.78	1.7	2	1.72	1.78
D/32	17	1.78	0.54	3.12	1.770	1.63	1.25	1.70	0.79	1.5	0.24	0.24	2	1.77	1.79	0.31	1.8	1.79	0.63	1.8	1.74	1	1.80	1.79

Figures: Bar graphs showing the zone of inhibition(in mm)of the selected antiseptics and disinfectants towardMRSA and MSSA with their stock strengths (D) and 16th fold serial dilutions (D/2-D/32) .

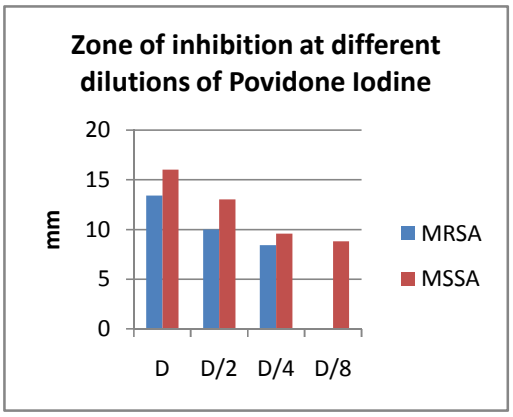

Fig.1

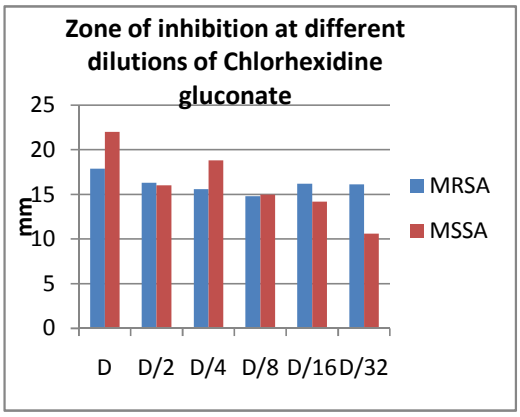

Fig.2

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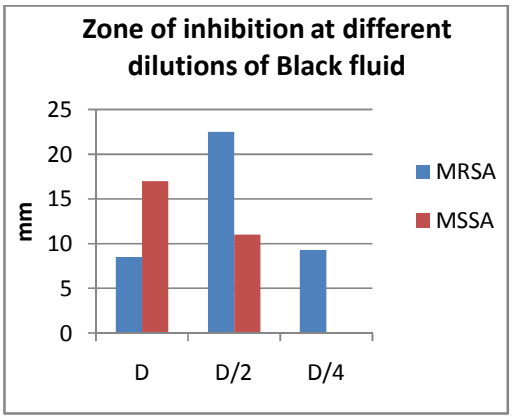

Fig.3

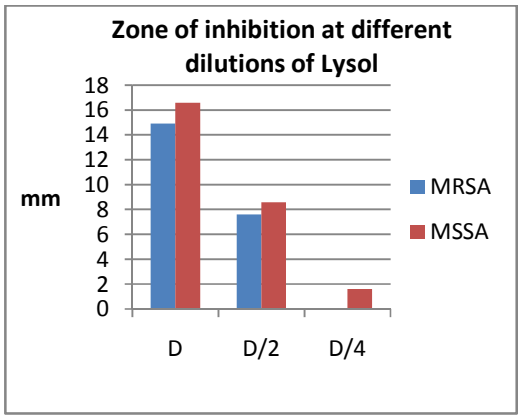

Fig.4

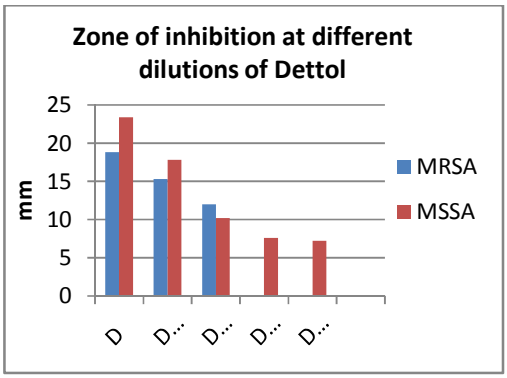

Fig.5

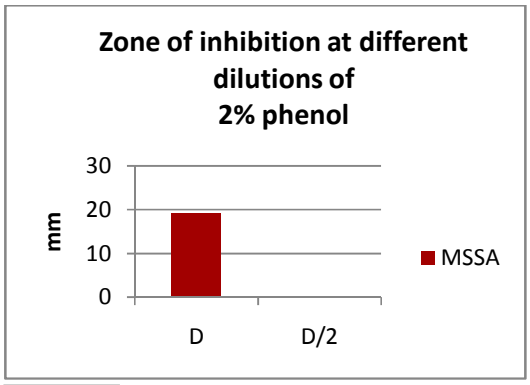

Fig.6

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