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ACTION OF NEWER DISINFECTANTS ON MULTIDRUG RESISTANT BACTERIA

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ABSTRACT: BACKGROUND: Current procedures for infection control in hospital environments have not been successful in curbing the rise in infections by multi-drug-resistant (MDR) pathogens. Emergence of resistance to chemical disinfectants is increasing steadily and has been reported worldwide. So prevention of multidrug-resistant health care associated infections (HAI) has become a priority issue and great challenge to clinicians. This requires appropriate sterilization and disinfection procedures and strict adherence to protocol in infection control policy. There is a need to evaluate the efficacy of newer disinfectants which have come into the market for better control of HAI. **AIMS AND OBJECTIVES:** The aim of this study was to evaluate and compare disinfection efficacy of three newer disinfectants– Novacide (didecyldimethylammonium chloride and polyhexamethylene biguanide), Silvicide a strong oxidizing agent (hydrogen peroxide and silver nitrate) and Virkon, a powerful oxidizing agent (a stabilized blend of peroxygen compounds and potassium salts), pitting them against two time-honored conventional disinfectants phenol and lysol and testing them against common MDR clinical isolates, reference strains and spores. **MATERIALS AND METHODS:** All the disinfectants at different dilutions were tested for bactericidal efficacy by liquid suspension time-kill tests. A heavy initial microbial load was simulated by preparing bacterial inoculum. Numbers of viable cells were counted and reduction in microbial colony counts before and after disinfectant exposure was expressed as log reduction. **RESULTS:** Among the disinfectants, Novacide was most effective. All clinical MDR bacterial isolates and reference strains were killed within 30 seconds of exposure at 0.156% solution, whereas spores got killed after 30 minutes of exposure at 2.5% solution which is the recommended concentration. For Silvicide all vegetative bacteria were killed at 5% solution after 20 minutes contact time and at 20% solution after 10 minutes contact time where recommended concentration is 20%. Spores also were killed at 20% solution after 1 hour contact time. Virkon was very effective for vegetative bacteria at 1% solution (which is the recommended concentration), killing within 30 seconds, but for exerting sporicidal action took 2 hours contact time. The conventional disinfectant phenol has currently restricted use because of its corrosive nature and high toxicity but still is considered as standard disinfectant for microbicidal efficacy testing methods. Lysol, the most widely used hospital disinfectant was found to be least effective among the five disinfectants. Both phenol and Lysol exerted poor sporicidal effect. **CONCLUSION:** Novacide is the most effective disinfectant in our study. Also Silvicide and Virkon being sporicidal agent can be considered as high level disinfectant, but Virkon required much greater time exposure inappropriate for routine hospital uses including instrument disinfection, floor cleaning and waste disposal.
KEYWORDS: Newer disinfectants, MDR bacteria, Liquid suspension time kill tests, HAI.
MESHTERMS: ("Disinfectants"[Mesh]) AND "Bacteria"[Mesh].

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INTRODUCTION: Nosocomial infections or Hospital-acquired/ Health care associated infections (HAI) occur worldwide and affect both developed and developing countries. In general, 5–10% of patients develop nosocomial infections.[1] During the last decade, there has been an alarming rise in HAI by multi-drug-resistant micro-organisms and are responsible for significant morbidity and mortality in today's healthcare settings. [2] Emergence of resistance to chemical disinfectants is increasing and has been reported worldwide[3-7] So, in spite of regular use of conventional disinfectants, surfaces and medical equipment are usually not effectively decontaminated and disinfected.

Disinfection in hospital practice is either by surface disinfection, sterilization of operation theatres or high level disinfection of surgical instruments and equipment, or disinfection of biomedical wastes. So, different disinfectant solutions have different applications. Also there is limited awareness among health care providers about choosing an appropriate disinfectant and is usually chosen based on the literature provided by manufacturers. In the backdrop of the above scenario, the following study was planned with the objective to evaluate bactericidal efficacy and to do comparative analysis of disinfection efficacy by performing simpler and practical tests in a very cost-effective way, with three newer hospital disinfectants - (a)Virkon -Triple salt of potassium monopersulphate, potassium sulphate and potassium hydrogen sulphate (strong oxidizing agent); (b) Novacide - 3%w/v polyhexamethylene biguanide and 10%w/v did ecyldimethylammonium chloride (fourth generation quaternary ammonium disinfectant with surfactant properties); (c) Silvicide - 0.01% silver nitrate and 10% hydrogen peroxide (H_2O_2) (strong oxidizing agent with stabilizer incorporated) and two conventional disinfectants – phenol (protoplasmic poison and causes enzyme inactivation) and lysol (cresol with added soap). The efficacies were tested against nosocomial clinical bacterial isolates those which commonly contribute to HAI and showed a multidrug resistant pattern on antibiogram testing.

MATERIALS AND METHODS: Efficacies of three newer generation disinfectants were studied in the backdrop of two conventional ones. It is a laboratory based interventional study. The conventional disinfectants were Phenol (80%v/v) manufactured by Indian Drug House, West Bengal, locally purchased and Lysol (50% cresol with 50% soap) manufactured by P.R.S. Chemical Works, Kolkata, purchased as hospital supply. Three newer ones were procured from the manufacturer: Virkon (Triple salt of potassium monopersulphate, potassium sulphate and potassium hydrogen sulphate and a strong oxidizing agent), a registered trademark of Antec International Limited, a subsidiary of DuPont; Novacide (3%w/v polyhexamethylene biguanide and 10%w/v didecyldimethyl ammonium chloride, a fourth generation quaternary ammonium disinfectant with surfactant properties) and Silvicide (combination of 0.01% silver nitrate and 10% hydrogen peroxide, also a strong oxidizing agent) both manufactured by BioShields, Tulip group, India.

The test organisms included in this study were nosocomial multidrug resistant Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Acinetobacter baumanii, Pseudomonas aeruginosa, (all ESBL, MBL or AmpC producers) Staphylococcus aureus (MRSA), MDR Enterococcus spp. and as an environmental contaminant Bacillus subtilis along with reference strains of Escherichia coli ATCC-25922, Staphylococcus aureus ATCC-25923 and Pseudomonas aeruginosa ATCC-27853. Disinfection efficacy was tested by in-house standardized procedures, which is based on liquid suspension time-kill tests.

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PREPARATION OF BACTERIAL SUSPENSION: All the nosocomial bacterial isolates were subcultured on nutrient agar to obtain isolated pure growth. Pure growth of the test organisms and reference strains were inoculated into peptone broth and incubated at 37^0 C aerobically for 24 hours.[8] Following incubation, turbidity of the suspension was adjusted to 2 McFarland (6 X 10^8 CFU/ml) standards, to start with heavy inoculum load.

PREPARATION OF BACTERIAL SPORES: From reference strain of Bacillus subtilis ATCC 6633, pure growth was obtained in nutrient agar plate, incubating for 24 hours at 37°C and after confirmation it is further sub cultured on nutrient agar slants in bigger test tubes (25 × 150 mm) with extra agar added and incubated horizontally for 10-14 days at 37ºC.Percent sporulation was determined microscopically daily by visual acuity count after staining with 5% malachite green 10 days onwards till the microscopy showed profuse sporulation i.e. spores >95% compared to vegetative cells. Following incubation, spores were harvested by adding 25 mL cold (2-5°C) sterile distilled water to nutrient agar slants. Using a bent glass rod, growth was removed and 4-6 sterile glass beads were added and gently shaken for 2 minutes to dislodge further bacterial growth. Finally spore suspensions were pipetted into sterile centrifuge tubes aseptically and the samples were heat-treated in 70 °C in water bath for 30 minutes with occasional shaking. Next tubes were centrifuged at 5, 000 rpm for approximately 10 min at room temperature. After discarding the supernatant, it was repeated two more times. Finally pellets were re-suspended in 5 ml distilled water. It is enumerated by standard plating method and CFU/ml calculated after incubating for 24 hours at 37^0C. It is finally stored at 2^0C and spore suspension was made ready for the disinfectant challenge test.[8, 9] Before the test, it was further diluted with distilled water to form an inoculum of 10^8 spores/ml.

METHOD OF EVALUATION OF BACTERICIDAL EFFICACY BY LIQUID SUSPENSION TIME KILL TEST: From the prepared bacterial suspensions (2 McFarlands), 0.25ml of it was added with 4.75ml of different dilutions of disinfectants and deionized water. Deionized water with test strain bacteria only without disinfectant is taken as positive control. So, final initial bacterial load was $3X10^7$ ($7log10$) CFU/ml. All test tubes were incubated at 37°C for 0.5, 1, 5, 10, 15, and 20 min. After each time exposure one loop full suspension with disinfectants and control was taken, sub-cultured in nutrient agar and after 24 hours of incubation at 37°C, colony count was done. All the organisms were inoculated in triplicate and the mean colony count was calculated. So, number of viable bacterial colony and the end point was determined by semi-quantitative culture technique.[8] End point is the point which is the lowest time of exposure for different dilutions of inoculated disinfectant solutions showing no surviving bacteria on nutrient agar plate after inoculation and incubation. Bactericidal efficacy was thus determined by broth dilution method and liquid suspension time kill test and determination of end points.

METHOD OF EVALUATION OF SPORICIDAL EFFICACY BY LIQUID SUSPENSION TIME KILL TEST: From the prepared spore suspension (10^8/ml), 0.5ml was added with 4.5ml of different dilutions of disinfectants and deionized water in centrifuge tubes, so final initial load was 10^7spores/ml. These were incubated at 37°C for 5, 10, 15, 30, 60, 120 mins. After incubation, the tubes were centrifuged at 5, 000 rpm for 5.0 min. The supernatant was discarded and to the sediment, 5 ml of glucose broth was added and were incubated for 24 hours at 37ºC. After incubation, the number of viable spores

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that had turned into vegetative cells was determined by the semi-quantitative culture technique. One loop full of suspension was taken and sub-cultured in nutrient agar plate. Colonies were counted on the plates after incubation at 37ºC for 24 hours.[8]

RESULTS: Mean viable colony count for each test organisms was determined against each time interval for different dilutions of disinfectants and statistically analyzed. The statistical analysis and the graphs were done by Graph Pad Prism software and appropriate graphs have been chosen. Data were expressed as the mean ± standard deviation (SD). Statistical procedures were performed using non-linear regression analysis. From the time kill curves, among the bacterial isolates tested for efficacy testing for phenol, Klebsiella pneumoniae was found to be most resistant (Figure-1), Lysol showed least efficacy among Acinetobacter baumanii (Figure-2), Novacide was least effective for Acinetobacter baumanii as well (Figure-3), Silvicide was found to be more resistant for gram positive bacteria than the gram negative one, showing maximum resistance for Enterococcus spp (Figure-4) and Virkon least susceptible for Pseudomonas spp (Figure-5). Time kill curves showing sporicidal efficacy of five disinfectants were shown from Figure-6-10.

Comparative analysis was done by column statistics. Statistical significance was defined as P < 0.05. From the comparative efficacy graph, Novacide (0.04%) is most efficient in killing vegetative form of bacteria, followed by Virkon (0.5%), then Phenol (1.25%), Silvicide (5%) and Lysol (5%). (Figure-11) All are less or equal to recommended concentration. Whereas from the comparative graph showing sporicidal effect, it is seen that Virkon (1%) at 2 hours contact time, is most efficient in killing spores, followed by Novacide (1.25%), then Silvicide (5%), all within recommended concentration, and Phenol and Lysol are least effective. (Figure-12) Phenol and Lysol were sporicidal at much higher concentration than the recommended concentration. Overall bactericidal efficacy of multidrug resistant clinical isolates and sporicidal efficacy against each disinfectant were shown in Table: 1-5.

DISCUSSION: This study was done with the primary aim of evaluating and comparing the practically achievable disinfectant efficacy of five different disinfectants, three newer and two conventional disinfectants. Bactericidal and sporicidal efficacy testing of disinfectants can be done by various methods. In this study, a very simple method of liquid suspension time kill tests at different dilution of disinfectants was applied and also end point of no surviving bacteria was determined. This method was based on similar method followed by Aggarawal et al. [8] Because of increase in resistance to antimicrobials and even disinfectants in recent times as evident in current international scientific literature,[3-7] it is essential to perform efficacy testing of disinfectants regularly in health care settings. As we are more concerned about prevention and control of HAI, so proper bio-medical waste disposal and sterilization of reusable items and equipment in CSSD are our target. So, heavy microbial load of 10^7 CFU/ml was taken in this study with all MDR strains with special importance to ESBL, MBL, AmpC and MRSA strains.

Among the many factors affecting efficacy of chemical disinfectants, most important are the time exposure and concentration used. As users tend to follow the available manufacturer's instructions, taking the recommended concentration and along with it other higher and lower concentrations, this study has been designed. Secondly the contact time specified on the label of the product is often too long to be practically followed for environmental surface disinfection in a health-

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care setting, because for floors or surfaces, commonest practice is to apply a disinfectant and allowing it to dry within one minute. Also there is always a chance of drying of the disinfectant on the surface even before exerting its disinfectant action at greater contact time. As environmental surfaces (e.g., bedside table) also could potentially contribute to cross-infection by health-care personnel, so disinfectants demonstrating significantly lesser contact time efficacy would be preferred in the health-care environment. However for high-level disinfection, contact time should be more and reusable instruments are immersed within disinfectant solution for sterilization. According to present guideline of CDC for disinfection(2007), the exposure time required to achieve high-level disinfection has been changed from 10-30 minutes to 12 minutes or more depending on the FDA-cleared label claim and the scientific literature.[10] Taking these into consideration we had designed the efficacy study for contact time starting from 30 seconds and upto 20 minutes for vegetative bacteria; and spores being more resistant intrinsically, efficacy tests were performed for upto 2 hours contact time.

The present study led us to the following conclusions: Among the disinfectants tested for bactericidal efficacy, Novacide was undoubtedly the most effective one, much better than the conventional ones which killed all vegetative bacterial isolates within 30 seconds of exposure at only 0.156% solution, hence most ideal for surface disinfection, with very rapid action within 30 seconds of contact time and with much lower concentration, hence also very cost-effective. This is also much less than the recommended concentration of 2.5% and recommended time exposure of 10 minutes. At greater contact time of 20 minutes, MBC was even lower i.e. 0.039%. Novacide can, however, also be used as a high level instrument disinfectant and sterilant for semi-critical items having broad spectrum of activity killing all vegetative bacteria and spores at 2.5% solution within contact time of 30minutes which is at par with the manufacturer's recommendation. Novacide is an aldehyde free high level disinfectant with much added advantages over glutaraldehyde. Hence is also an ideal replacement for glutaraldehyde.

Silvicide is an ideal aerial disinfectant and OT fumigant acting as a sterilant at recommended concentration of 20% at one hour contact time. Also as there are no toxic residual by-products produced by it, it is safe and can be done regularly. So it is an ideal replacement for formaldehyde fumigation. Silvicide can also be used as high level instrument disinfectant and sterilant but requires 20% solution for 1 hour contact time or 5% solution for 2 hours contact time for its sporicidal action. At shorter contact time of 1 minute, more resistance was seen for gram positive isolates than for gram-negative one. Among the gram-positive bacteria, Enterococcus was found to be more resistant as also seen in another study by Saurina et al.[11] Enterococcus was even more resistant than MRSA.

This may be due to decreased penetrability of the Enterococcal thick peptidoglycan cell wall as occurs in vancomycin resistance in Enterococci. For Staphylococcus, catalase production may function to inactivate toxic hydrogen peroxide explaining reduced susceptibility compared to gram negative bacteria at shorter contact time. But with increase in contact time, bactericidal effect increased for both gram positive and gram-negative isolates. At 20 minutes contact time, all isolates were killed at 5% solution. Silvicide can also be used as surface disinfectant at 5% solution but requires 20 minutes for its bactericidal action. Hence it is not an ideal surface disinfectant because of greater contact time. Similar results were also found in a study by Davoudi et al 2012 [12] where they used similar combination and killing effect seen after 15 minutes of exposure. Among gram negative

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bacteria both Pseudomonas and Acinetobacter were most resistant ones. According to CDC guidelines for disinfection and sterilization in health care facilities (2008), they have recommended not to perform fumigation or disinfectant fogging with chemicals like formaldehyde due to its several toxic side effects and carcinogenic potential. [10] Instead newer technologies involving fogging with hydrogen peroxide and silver nitrate have been encouraged. It is reported that a combination of Silver and Hydrogen Peroxide (1:1000) shows a higher inhibiting potency on E.coli growth than each individual agent. [13] From this study we can see that Silvicide not only effective for vegetative bacteria but also has shown sporicidal effect at 20% solution after 1 hour contact time. So, overall bactericidal and sporicidal efficacy was more time dependent for Silvicide, efficacy increasing with increase in time exposure.

Virkon has shown excellent bactericidal and sporicidal efficacy. At 1% solution, all vegetative bacterial strains were effectively killed within 30 seconds exposure while spores were killed after contact time of 2 hours. So Virkon as surface disinfectant is fast acting and ideal one for practical uses. Phenol and Lysol both have poor sporicidal action, requiring 20% solution which is 4 times the recommended concentration and 1 hour and 2 hours contact time respectively. Hence efficacies of these conventional disinfectants are poor. For surface disinfection purpose, phenol is better than lysol, but because of its toxic effects, use of phenol is limited.

In general gram negative bacteria were more resistant than gram-positive bacteria for the disinfectants tested except for Silvicide in which at shorter contact time gram positive bacteria were more resistant to the killing action than gram-negative bacteria.

Among the gram positive bacteria, MRSA was more resistant than the MDR strains of Enterococcus. Among the gram negative bacteria, non-fermentors, Pseudomonas aeruginosa and Acinetobacter baumanii were most resistant.

This study generated considerable data with results of bactericidal efficacy for the disinfectants which can be utilized for making hospital infection control policy. Further studies can be done using in-use test methods for efficacy testing of these hospital disinfectants. Tests against MDR Mycobacteria can also be included. These data will also enrich our knowledge regarding appropriate use of disinfectants. So, it can be concluded from these present studies that, action of newer disinfectants are definitely better than the conventional ones.

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↑Concentration

↑ Dilution

Time (mins)	1:512	1:256	1:128	1:64	1:32	1:16	1:8	1:4	1:2
0.5				E. coli Pseudomonas aeruginosa Enterococcus spp	Staphylococcus aureus Proteus mirabilis Acinetobacter baumanii	Klebsiella pneumoniae			
1				Proteus mirabilis Acinetobacter baumanii	Klebsiella pneumonia				
5									
10			Enterococcus spp						
15			Proteus mirabilis						
20			S. aureus K. pneumoniae						
30									
60								Spore	
120							Spore		

Table 1: Efficacy of phenol (80% v/v) as high level disinfectant (end point determined by no viable bacterial colony for particular dilution and contact time)

↑Susceptibility

↑Resistance
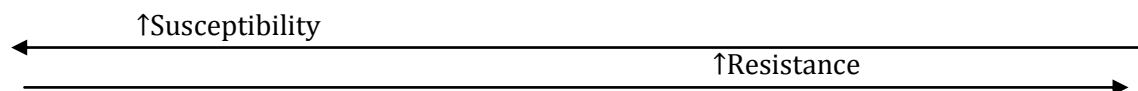
Table-1: The position of the bacteria denotes no viable colony count i.e. end-point upto that particular dilution and contact time. Dilutions to the right, also denotes no growth but to the left denotes positive growth for each bacteria and spores. The organisms mentioned in the individual cells mean that, this contact time and this concentration is required to kill that organism. If we increase the concentration or increase the contact period beyond this, the organism will definitely be killed. From this table, 10% (1:8) phenol X 2 hours or 20% phenol (1:4) X 1 hour is required to kill all bacteria and spores.

↑Concentration

↑ Dilution

Time (minutes)	1:160	1:80	1:40	1:20	1:10	1:5
0.5			Enterococcus spp	E. coli Proteus mirabilis	Staphylococcus aureus Klebsiella pneumoniae Pseudomonas aeruginosa Acinetobacter baumanii	
1				Klebsiella pneumoniae		
5						
10			Staphylococcus aureus	Pseudomonas aeruginosa		
15			E. coli	Acinetobacter		

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			baumanii		
20					
30					
60					
120					Spore

Table 2: Efficacy of Lysol (50%cresol +50%soap) as high level disinfectant (end point determined by no viable bacterial colony for particular dilution and contact time)
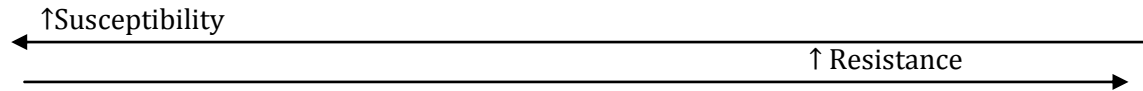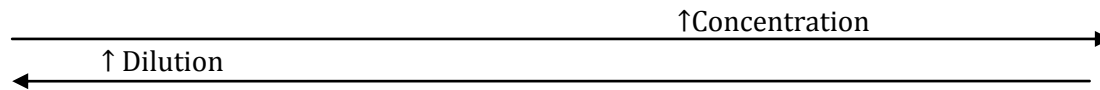
↑Susceptibility

← ↑ Resistance →

Table 2: The position of the bacteria denotes no viable colony count i.e. end-point upto that particular dilution and contact time. Dilutions to the right, also denotes no growth but to the left denotes positive growth for each bacteria and spores. From this table, 20% (1:5) lysol X 2 hours is required to kill all bacteria and spores.

↑Concentration →

← ↑ Dilution

Time mins	1:5120	1:2560	1:1280	1:640	1:320	1: 160	1:80	1:40	1:20	1:10	1:5
0.5			Enterococcus spp Klebsiella pneumoniae	Staphylococcus aureus E. coli Pseudomonas aeruginosa Proteus mirabilis Acinetobacter baumanii							
1	Staphylococcus aureus Enterococcus spp		E. coli Pseudomonas aeruginosa	Acinetobacter baumanii							
5			Acinetobacter baumanii Proteus mirabilis								
10		E. coli Acinetobacter baumanii									
15	E. coli	Proteus mirabilis Klebsiella pneumoniae Pseudomonas aeruginosa									Spore

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20									
30							Spore		
60									
120							Spore		

Table-3: Efficacy of Novacide as high level disinfectant (end point determined by no viable bacterial colony for particular dilution and contact time)
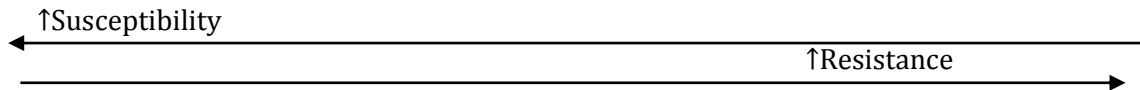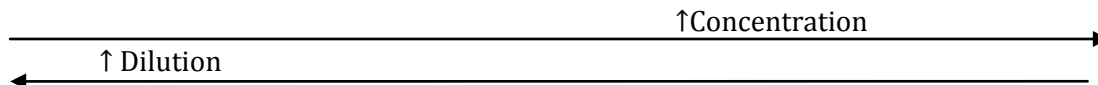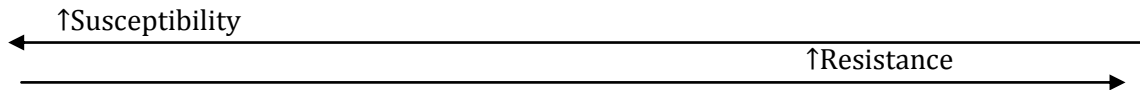
↑Susceptibility

←———————————————————————————————

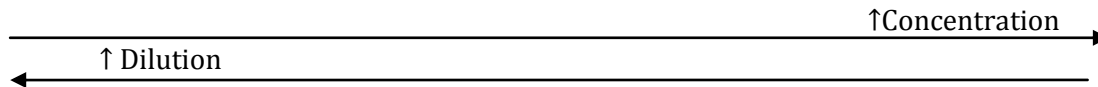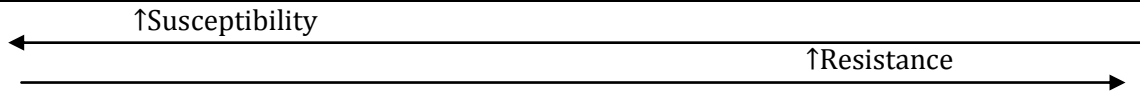———————————————————————————————→ ↑Resistance

Table 3: The position of the bacteria denotes no viable colony count i.e. end-point upto that particular dilution and contact time. Dilutions to the right, also denotes no growth but to the left denotes positive growth for each bacteria and spores. From this table, 2.5% (1:40) Novacide X 30 mins is required to kill all bacteria and spores.

↑Concentration ———————————————→

← ↑ Dilution ——————————

Time (mins)	1:160	1:80	1:40	1:20	1:10	1:5	1:2.5
0.5							
1						E. coli	S. aureus Enterococcus spp
5				S. aureus	S. aureus E. coli	S. aureus Klebsiella pneumoniae Proteus mirabilis Pseudomonas aeruginosa Acinetobacter baumanii	
10			S. aureus		Klebsiella pneumonia Proteus mirabilis Pseudomonas aeruginosa Acinetobacter baumanii	Enterococcus spp	
15		S. aureus		Enterococcus spp Proteus mirabilis Klebsiella pneumoniae	Enterococcus spp		

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20			E. coli	Pseudomonas aeruginosa Acinetobacter baumanii			
30							
60						Spores	
120				Spores			

Table 4: Efficacy of Silvicide as high level disinfectant (end point determined by no viable bacterial colony for particular dilution and contact time)

↑Susceptibility

←——————————————————————————

↑Resistance

——————————————————————————→

Table 4: The position of the bacteria denotes no viable colony count i.e. end-point upto that particular dilution and contact time. Dilutions to the right, also denotes no growth but to the left denotes positive growth for each bacteria and spores. For Silvicide, 20% (1:5) X 1 hour is required to kill all bacteria and spores.

↑Concentration

——————————————————————→

↑ Dilution

←——————————————————————

Time (minutes)	0.25%	0.5%	1%	2%	3%	4%
0.5			S. aureus Enterococcus spp E. coli Klebsiella pneumonia Proteus mirabilis Pseudomonas aeruginosa Acinetobacter baumanii			
1						
5						
10		S. aureus				
15		Enterococcus spp E. coli Proteus mirabilis Klebsiella pneumonia Pseudomonas aeruginosa Acinetobacter baumanii				
20	S. aureus					

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30					Spores
60				Spores	
120			Spores		
Table 5: Efficacy of Virkon as high level disinfectant (end point determined by no viable bacterial colony for particular dilution and contact time)					

↑Susceptibility

↑Resistance

Table 5: The position of the bacteria denotes no viable colony count i.e. end-point upto that particular dilution and contact time. Dilutions to the right, also denotes no growth but to the left denotes positive growth for each bacteria and spores. For Virkon, 4% X 2 hours is required to kill all microorganisms.

Graphs: Time kill curves:
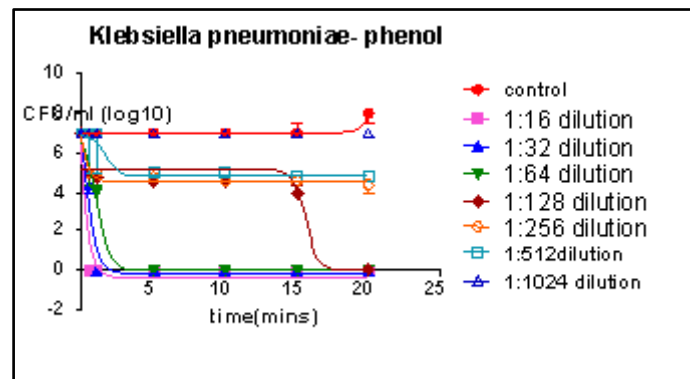
Figure 1

Figure 1: Time-kill curve: The plot shows the number of colony forming units per millilitre (CFU/ml) (mean, n =10) of Klebsiella pneumoniae for the control and after exposure to different dilutions of Phenol under study.
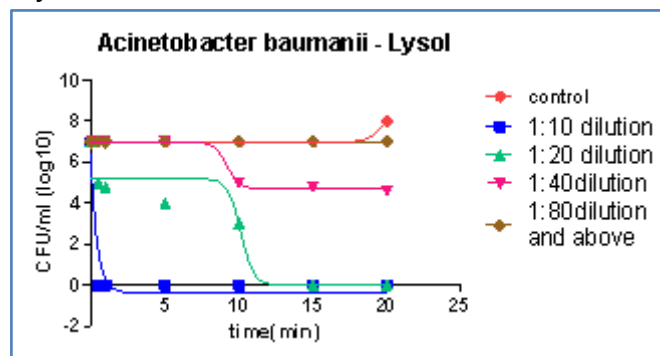
Figure 2

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Figure 2: Time-kill curve: The plot shows the number of colony forming units per millilitre (CFU/ml) (mean, n =10) of Acinetobacter baumanii for the control and after exposure to different dilutions of Lysol under study.
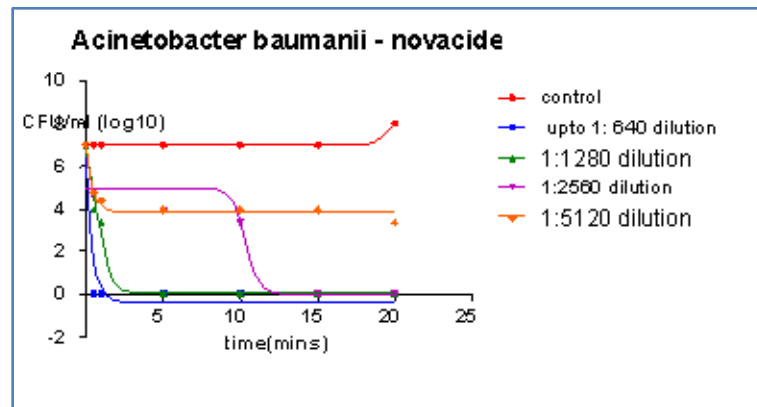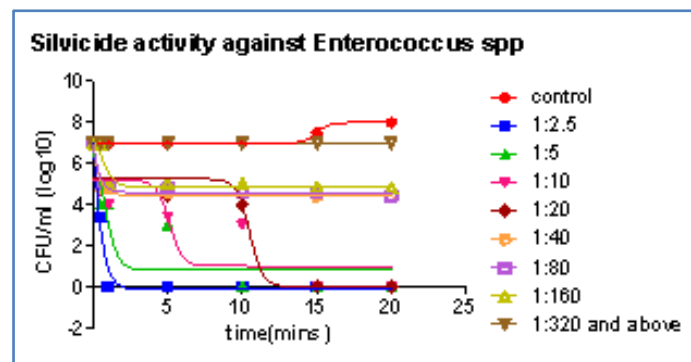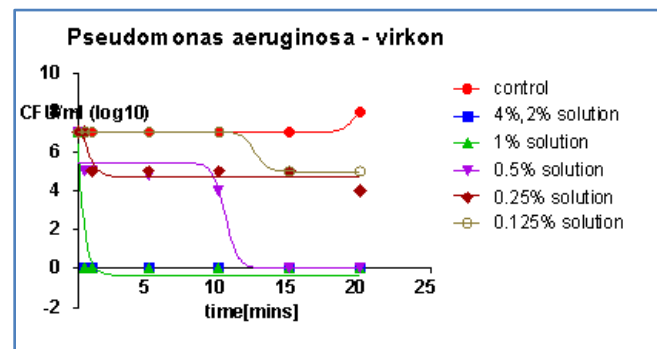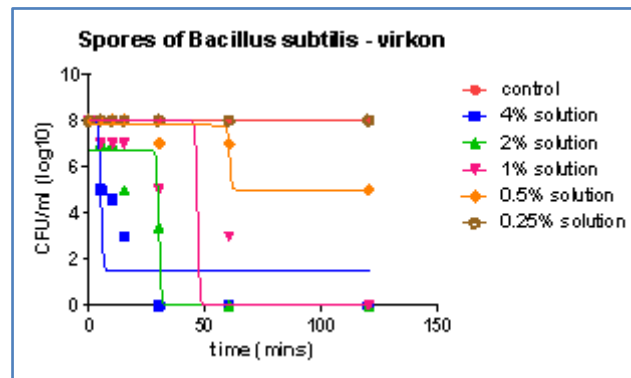

Figure 3

Figure 3: Time-kill curve: The plot shows the number of colony forming units per millilitre (CFU/ml) (mean, n =10) of Acinetobacter baumanii for the control and after exposure to different dilutions of Novacide under study.

Figure 4

Figure 4: Time-kill curve: The plot shows the number of colony forming units per millilitre (CFU/ml) (mean, n =10) of Enterococcus spp for the control and after exposure to different dilutions of Silvicide under study.

Figure 5

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Figure 5: Time-kill curve: The plot shows the number of colony forming units per millilitre (CFU/ml) (mean, n =10) of Pseudomonas aeruginosa for the control and after exposure to different dilutions of virkon under study.

Figure 6

Figure 6: Time-kill curve: The curve shows mean viable colony counts for each viable spore plotted against time interval for different dilutions of virkon taken in this study.
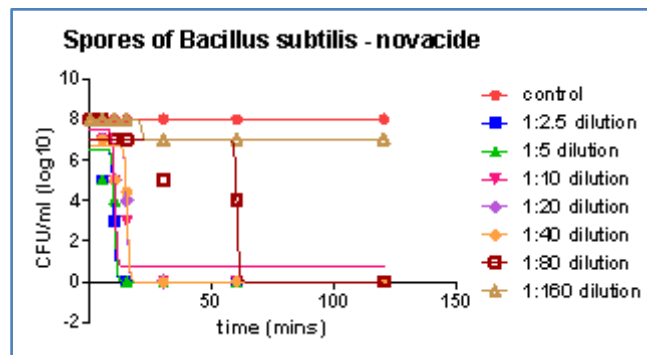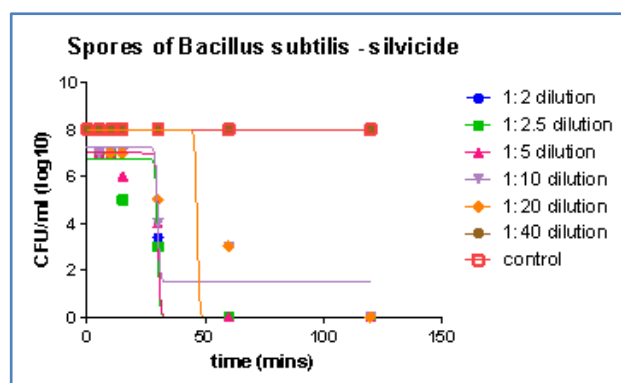

Figure 7

Figure 7: Time-kill curve: The curve shows mean viable colony counts for each viable spore plotted against time interval for different dilutions of Novacide taken in this study.

Figure 8

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Figure 8: Time-kill curve: The curve shows mean viable colony counts for each viable spore plotted against time interval for different dilutions of silvicide taken in this study.
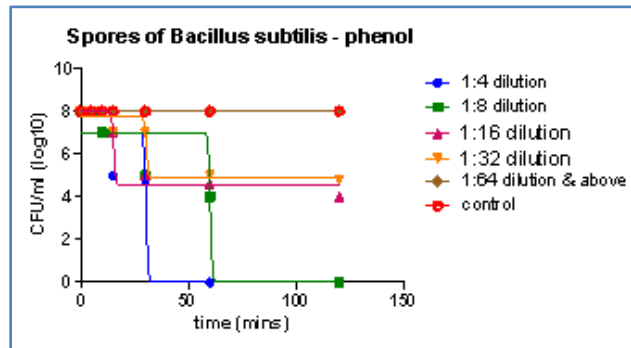

Figure 9

Figure 9: Time-kill curve: The curve shows mean viable colony counts i.e. number of viable Bacillus subtilis spores that have germinated (n=5), plotted against time interval for different dilutions of phenol taken in this study.
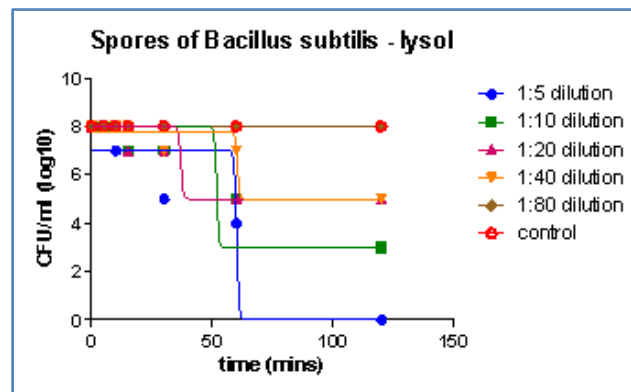

Figure 10

Figure10: Time-kill curve: The curve shows mean viable colony counts i.e. number of viable Bacillus subtilis spores that has germinated (n=5), plotted against time interval for different dilutions of lysol taken in this study.
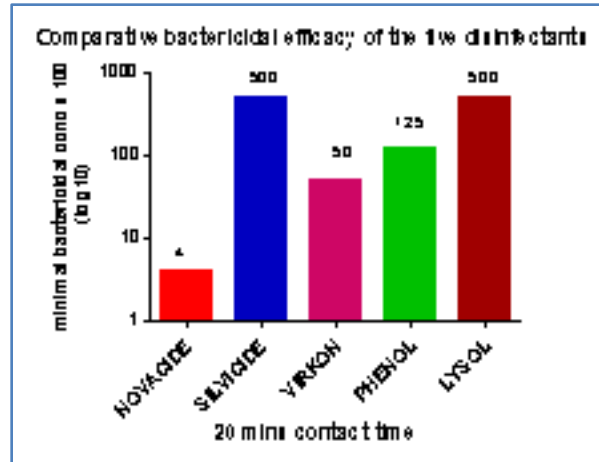
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Figure 11

Figure 11: Overall comparative efficacy of five disinfectants at 20 minutes contact time considering their highest dilution at which all isolates placed in this study were killed and there was no growth of viable colony on further sub-culture. In this graph, the minimum concentration or the highest dilution that killed all bacteria has been considered (the mean value has not been taken), as our objective is, to compare complete bactericidal efficacy of these disinfectants.
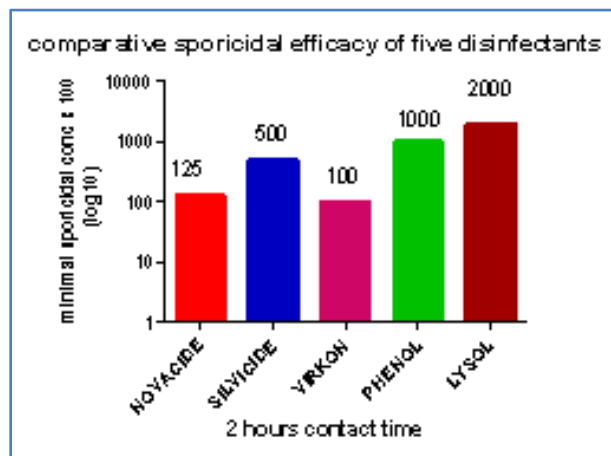

Figure 12

Figure12: Overall comparative efficacy of five disinfectants at 2 hours contact time considering their highest dilution at which all spores placed in this study were killed and there was no growth of viable colony on further sub-culture.

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