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EFFICIENCY DETERMINATION OF COMMONLY USED DISINFECTANTS OVER SEVEN DAYS OF STORAGE.

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ABSTRACT
Among commonly used broad spectrum disinfectants ethanol, isopropyl alcohol (IPA) and savlon are well known. Although their effectiveness against different group of microorganisms has already been established but till now effectiveness of old preparation of any of these is not established. This study was aimed to find out whether the efficiencies of prepared disinfectants solution remain same or reduced during storage. Effectiveness was tested by applying 70% ethanol, 70% IPA and 3.3% savlon solutions individually on surface of stainless steel sheets previously contaminated with *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Candida albicans* as well as one non-sterile sheet. Following the application of disinfectant after 30 seconds, 5, 10 and 15 minutes number of surviving organisms were counted. Same test was done on first, fourth and seventh day of solution preparation and their effectiveness were compared. In this study it was found that Ethanol and IPA effectively killed 99.99% populations of test organisms and most of the non-specific contaminants from non-sterile surfaces on both 1st and 4th day's test, but failed to kill 99.99% of test organisms at 7th day's test. Savlon was found effective against all the test organisms and non-specific contaminants on 1st, 4th and 7th day. So, the obtained results suggested that efficiency of prepared solutions remained constant up to 4th day of preparation for 70% ethanol and 70% IPA and at least up to 7th day for 3.3% savlon.

KEYWORDS: Disinfectant, effectiveness, storage, decontamination.

INTRODUCTION
As per the Environmental Protection Agency (EPA) disinfecting agents are antimicrobial pesticides and other substances used to control, prevent, or destroy harmful microorganisms (i.e., bacteria, viruses, or fungi) on inanimate objects and surfaces.[1] Antiseptics and disinfectants are used extensively in the hospitals and other health care settings on a variety of topical and hard-surface applications.[2] Alcohol is a powerful broad-spectrum germicide and ethanol is considered more superior than iso-propyl alcohol (IPA).[3] Alcohols have been used effectively to disinfect oral[4] and rectal[5] thermometers, hospital pagers,[6] scissors,[7] and stethoscopes.[8] In addition, alcohol is also used to disinfect external surfaces of equipments (e.g., ventilator, manual ventilation bags,[9] ultrasound instruments,[10] and medication preparation areas.[11] Alcohol is widely accepted as an effective chemical disinfectant if used appropriately.[3] Alcohol is also effective against a number of human viruses such as influenza virus,[12] rota virus,[13] hepatitis B virus[14] etc but not effective against hepatitis A virus[15] and poliovirus.[16] In general, isopropyl alcohol is considered slightly more efficacious against bacteria[17] and ethyl alcohol is more potent against viruses.[18] Savlon (2.8% v/v n-propyl alcohol, 0.3% w/v chlorhexidine gluconate and 3.0 w/v cetrimide)[19] is another chemical disinfectant commonly used in hospitals[20] found that savlon works as bactericide against various species such as *Salmonella typhi*, *Escherichia coli* although[19] found it less effective against *Candida albicans*. MICs of savlon were found from 1:160 (for *Staphylococcus aureus*) to 1:80 (for *Klebsiella sp*).[19] Effectiveness of these disinfectants depends on many factors such as type of chemical, preparation of disinfectant, storage condition etc. Prince & Ayliffe (1972) noted that disinfectant (phenolic) itself may be contaminated by non-fermentative bacilli, *Pseudomonas aeruginosa* etc., [21] however, there was no explanation whether contamination was due to an inadequate concentration of disinfectant or whether

organisms are surviving or growing at or above the recommended concentration. Onaolapo (1990) noted that in-use topping of old dilution and use of disinfectant lower than effective concentration have been identified as dangerous practice.[22] Although a number of studies have been carried out to test efficacy of chemical disinfectants but there is no data about the efficacy of prepared disinfectant upon storage. It is beyond any doubt that it is necessary to determine the efficacy of disinfectant initially after preparation and during storage, thus a validated shelf life of these disinfectants can be established. So objective of this study was to observe the efficacy of freshly prepared solutions of three disinfectants as well as to observe their efficacy after storage. The optimum bactericidal concentration of ethanol and IPA is 60%–90% solutions in water (volume/volume).[12, 23] Ethanol demonstrates the strongest disinfecting effect in a concentration of 70% in water. That is why 70% ethanol and 70% IPA have been used in this study. In hospital setting, 3.3% savlon solution is used for disinfection purpose.[24] So, in this study 3.3% aqueous solution of Savlon was used.

MATERIALS AND METHODS

Disinfectant solutions

Three individual disinfectants were used for this study i.e. 70% Ethanol, 70% Isopropyl alcohol and 3.3% Savlon. Ethanol manufactured by "Fisher Scientific, UK" Isopropyl alcohol manufactured by "Sigma Aldrich, Germany" and Savlon manufactured by "ACI Limited, Bangladesh" were used to prepare these disinfectant solutions. Disinfectants were prepared at their target concentration by diluting with purified water. In order to avoid the effect of light and temperature all these solutions were stored at dark and cool place in closed glass containers.

Organisms

Efficacy of these disinfectants were tested against three gram negative bacteria; *Escherichia coli;* ATCC: 8739, *Pseudomonas aeruginosa* ATCC: 9027, *Salmonella typhimurium* ATCC: 14028, one gram positive bacteria; *Staphylococcus aureus* ATCC: 6538 and one yeast; *Candida albicans* ATCC: 10231.

Method

Soybean casein digest broth was inoculated with fresh culture of respective organisms and incubated at 32.5^0C for 2-4 hours to obtain suspension having 10^4 to 10^5 cells/mL and all these suspensions were kept at 2-8^0C temperature until use (not more than 24 hours). As, type of surface may also affect the effectiveness of disinfection plan as porous, uneven, cracked, or pitted surfaces, especially wooden surfaces and earthen floors can hide microorganisms and are difficult to disinfect[1] and smooth surface is ideal for disinfection,[25] plain stainless steel surface was used in the study. For testing each of disinfectant solution seven stainless steel sheets (7cm×30cm) were employed on each of testing day. Every clean sheet of stainless steel was marked in five regions of approx. 25 cm^2 (5cm ×5cm) and labelled as (+)ve control, 30 sec, 5 min, 10 min, 15 min with a permanent marker and five sheets were wrapped with aluminium foil followed by sterilization by autoclave. Non-sterile sheet was intended to be used as source of non-specific wild microorganisms. Then at room temperature, each sterilized sheet was contaminated with each organism (five test organisms on five different sheets) by individually applying 0.1 mL suspension on previously marked 25 cm^2 areas. After drying, test disinfectant was sprayed all of these sheets including the non-sterilized one but 25 cm^2 area (labelled as (+)ve control) on every sheet was carefully left for positive control. At the same time a sterile stainless sheet was kept as negative control where neither organisms nor the disinfectant was applied. Following application of disinfectant swab sample were collected from previously marked 25 cm^2 area after 30 seconds, 5, 10 and 15 minutes along with the positive control for enumeration of surviving organisms. Then number of organisms challenged to disinfectant for varying time was compared with the number of organisms of positive control. In parallel, one stainless steel sheet was labelled, autoclaved and sampled but that received neither the any disinfectant solution nor contaminated deliberately, served as negative controlled. These procedures were repeated on first day, 4^{th} day and 7^{th} day of preparation of disinfectant in order to compare their effectiveness during storage.

RESULTS

Prepared 70% ethanol was found to be effective against vegetative cells of all the test organisms up to 4^{th} day as it reduce cell number by ≥99.99%. There was some growth in case of non-sterile control and those reassembled fungal colony. But on the 7^{th} day, efficacy dropped significantly (Table-1).

Almost similar pattern was found in case of prepared 70% IPA. Up to 4^{th} day, it was as effective as it was at first day. Great reduction of effectiveness was observed on 7^{th} day. It failed to eliminate contamination on non-sterile control which gave fungal like colony although colony count on treated non-sterile surface was much lower than that was on untreated surface (Table-2).

Unlike prepared ethanol and IPA solution there was no decline in effectiveness of prepared aqueous solution of savlon during storage. On each of test day it eliminated not only by ≥99.99% of test organisms but also killed all the contamination on non-sterile control within minute.(Table-3).

Table 1: Effectiveness of 70% ethanol against target microorganisms on first, fourth and seventh day of preparation

Test item	Colony count at different time interval (CFU/plate)														
	Day 1					Day4					Day 7				
	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min
Negative control	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Wild/environment control	80	3	1	2	Nil	54	Nil	4	2	Nil	>100	10	Mat	7	Mat
C. albicans	4.7×10^4	Nil	Nil	Nil	Nil	9.8×10^4	Nil	Nil	Nil	Nil	1.24×10^4	10	Nil	Nil	Nil
E. coli	1.7×10^4	Nil	Nil	Nil	Nil	5.4×104	Nil	Nil	Nil	Nil	6.4×10^4	71	Nil	Nil	Nil
S. typhimurium	1.3×10^4	Nil	Nil	Nil	Nil	3.4×10^4	Nil	Nil	Nil	Nil	9.1×10^4	<100	19	Nil	Nil
S. aureus	5.6×10^4	Nil	Nil	Nil	Nil	3.5×10^4	Nil	Nil	Nil	Nil	2.4×10^4	<100	24	Nil	Nil
P. aeruginosa	2.9×10^4	Nil	Nil	Nil	Nil	1.3×10^4	Nil	Nil	Nil	Nil	1.9×10^4	3	Nil	Nil	Nil

Table 2: Effectiveness of 70% IPA against target microorganisms on first, fourth and seventh day of preparation

Test item	Colony count at different time interval (CFU/plate)														
	Day 1					Day4					Day 7				
	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min
Negative control	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Wild/environment control	53	3	3	5	Nil	46	Nil	1	4	Nil	50	7	Mat	6	4
C. albicans	4.7×10^4	Nil	Nil	Nil	Nil	9.8×10^4	Nil	Nil	Nil	Nil	1.24×10^4	Nil	Nil	Nil	Nil
E. coli	1.7×10^4	Nil	Nil	Nil	Nil	5.4×10^4	Nil	Nil	Nil	Nil	6.4×10^4	15	Nil	Nil	Nil
S. typhimurium	1.3×10^4	Nil	Nil	Nil	Nil	3.4×10^4	Nil	Nil	Nil	Nil	9.1×10^4	25	6	Nil	Nil
S. aureus	5.6×10^4	Nil	Nil	Nil	Nil	3.5×10^4	Nil	Nil	Nil	Nil	2.4×10^4	Nil	Nil	Nil	Nil
P. aeruginosa	2.9×10^4	Nil	Nil	Nil	Nil	1.3×10^4	Nil	Nil	Nil	Nil	1.9×10^4	3	Nil	Nil	Nil

Table 3: Effectiveness of 3.3% savlon against target microorganisms on first, fourth and seventh day of preparation

Test item	Colony count at different time interval (CFU/plate)														
	Day 1					Day4					Day 7				
	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min	(+)ve control	30 sec	5 min	10 min	15 min
Negative control	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Wild/environment control	17	Nil	Nil	Nil	Nil	70	Nil	Nil	Nil	Nil	49	Nil	Nil	Nil	Nil
C. albicans	4.1×10^4	Nil	Nil	Nil	Nil	4.3×104	Nil	Nil	Nil	Nil	3.1×104	Nil	Nil	Nil	Nil
E. coli	1.2×10^4	Nil	Nil	Nil	Nil	7.0×104	Nil	Nil	Nil	Nil	1.3 ×104	Nil	Nil	Nil	Nil
S. typhimurium	4.7×10^4	Nil	Nil	Nil	Nil	3.7×104	Nil	Nil	Nil	Nil	4.7×104	Nil	Nil	Nil	Nil
S. aureus	2.7×10^4	Nil	Nil	Nil	Nil	4.0×104	Nil	Nil	Nil	Nil	2.7×104	Nil	Nil	Nil	Nil
P. aeruginosa	2.0×10^4	Nil	Nil	Nil	Nil	1.2×104	Nil	Nil	Nil	Nil	2.0×104	Nil	Nil	Nil	Nil

DISCUSSION

From the results obtained it has been confirmed that effectiveness of prepared solution of disinfectants were affected by storage. Both of the alcoholic solutions have been proved to be satisfactory on first to 4th day of preparation, but on the 7th day efficacy of them considerably reduced. It might happen because of evaporation of ethanol and IPA which in turn altered the concentration nevertheless both solutions were kept in closed container. Both of them have been found equally effective against both Gram-positive and Gram negative bacteria. As ethanol and IPA are not sporocide, growth on non-sterile surface could be from the fungal spore. In a previous study by Boyce effectiveness of chlorohexidin (component of savlon) against both Gram positive and Gram negative bacteria was proved[26] and in another study by Awodele savlon was proved ineffective against *C. albicans*.[19] Nonetheless, in this study savlon solution eliminated not only the test organisms (which were freshly cultured hence vegetative cell instead of spore) but also killed all the contaminant from the non-sterile surface which harbor non-specific environmental organisms (not surprisingly including spores) up to 7th day of preparation. So, effectiveness of prepared solution of savlon has been found to satisfactory throughout its storage of 7 days.

In this study, storage period was targeted as 7 days. It should be noted that disinfectants were tested on clean, smooth surface so there was no interference of dirt. In parallel to each test a negative test was done which at least partly assured the validity of study. Moreover, it is noteworthy that these disinfectants are also used as antiseptic, but this study was concerned only with their role as disinfectant. In parallel to each test a negative test was done which at least partly assured the validity of study. In addition, growth on positive controls which were not treated with any of disinfectant ensured that organisms received no external pressure. As mentioned earlier, use of old dilution of disinfectant has been proved dangerous so, study on effectiveness of prepared dilution of disinfectant during storage was necessary to avoid devastating outcome. But there was no available information regarding their efficacy during storage. In this sense this study was unique. However, the results obtained suggest that storage and use of prepared aqueous solution of ethanol and IPA must not more than four days and prepared solution of savlon can safely be used up to seven day for disinfection purpose.

CONCLUSION

The study was designed to determine whether effectiveness of prepared solutions of disinfectants will decrease during storage or not. From the findings of this study it can be proposed that 70% ethanol and 70% IPA can be used up to 4days of storage and 3.3% savlon can be prepared on weekly basis for disinfection purpose. It can be suggested to repeat this work targeting viruses.

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