Journal of Pharmaceutical and Health Sciences 2014;2(2), 93-98.

Original Article

JPHS

Study of microbial contamination of hospital antiseptics and disinfectants

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Abstract

Nowadays, the prevalence of nosocomial infections are considered as a serious problem in health systems. Use of appropriate antiseptics and disinfectants play major role in the control of nosocomial infections. In this study, we examined microbial contamination of hospital antiseptics and disinfectants and the effect of dilution on their rate of contamination to gain insight into their rational use in controlling nosocomial infections.

Samples from three types of antiseptics and disinfectants, each from 2 different brands, were prepared (A & B). Hand samples were prepared in the form of solution and were sampled for microbial control after removing the cap. Instrument and surface samples were in concentrated form and dilutable, that were tested in four different times. The mentioned samples were assessed by plate count method and membrane filtration. We also assessed the efficacy of the mentioned samples by adding 10^6 microorganisms per ml to six different genera, including *S. aureus, P.aeruginosa, E. coli, B. subtilis, C. albicans, A. niger.* After passing the golden time the samples were cultured. The bacterial and fungal count tests, the sterility tests and the efficacy tests of mentioned samples showed no contamination.

Keywords: Disinfectant, Efficacy, Microbial Contamination, Dilution, Antiseptic, Microorganisms

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Introduction

Prevalence and risk of infectious diseases during treatment and diagnosis procedures is a usual problem in medical centers. In particular, hospitals are places with potential risk of spread of infectious diseases, which leads to extra cost of illness treatment, treatment delay and longer stay. Therefore, to create safe environment for patients and staff in medical centers careful planning for prevention and control of nosocomial infections is essential. Taking some health precautions such as hand washing techniques (the most important factor) besides using appropriate, standard antiseptics and disinfectants, following correct consumption model in view of choosing the right type and density, will help us to control nosocomial infections.

The objectives of this study include:

-Examining presence of contamination in concentrated and ready-to-use samples of antiseptics.

-Examining diluted antiseptics regarding microbial contamination at the beginning of dilution.

-Examining diluted antiseptics regarding microbial contamination during timely use of diluted samples.

- Examining diluted antiseptics regarding microbial contamination before the end of the useful time of using diluted samples.

Materials and Methods

Samples

Three types of disinfectants were tested with two different brands (A and B) Table 1.

Table 1: Specification of tested disinfectants

Name of material	Chemical Composition
A-1 :Hand disinfectant (Ready to use)	2 -Propanol 1 - propanol, benzyl - alkyl dimethyl ammonium chloride along with skin protective materials, oils and color
A-2 :Surface disinfectants (in concentrated form)	Didecyl dimethyl ammonium chloride, N,N-Bis-(3-aminopropyl)-dodecylamine, coco propylenediamine-1.5-bis-guanidinium acetat compounds with surfactant,the complex factors ,essence and color
A-3 :Instrument disinfectants (in concentrated form)	Coco propylenediamine-1.5-bis-guanidinium acetat, N,N-Didecyl-N-methyl-poly (oxyethyl) ammonium propionate compounds with surfactant, anti-corrosive materials, essence and color
B-1 :Hand disinfectant Ready to use))	Ethanol and 2- phenoxyethanol
B-2 :Surface disinfectants (in concentrated form)	Ammonium quaternary and alkyl amin
B-3 :Instrument disinfectants (in concentrated form)	Quaternary ammonia propionate, polyhexanide

Hand disinfectant samples were prepared in the form of solution to use and sampling for microbial control test was carried out after removing the cap. Instrument and surface samples were in concentrated form and dilutable and were tested in four different times (immediately after opening the caps of samples, at first, in the middle and close to the finishing golden time of the diluted sample). Each test was repeated three times. These samples were checked by membrane filtration and plate count methods. (Black, 1999; USP31, 2008). Filter papers of 0.45 μ made of cellulose nitrate were used for filtration.

Stage1

Bacterial and fungal counts were performed in aseptic conditions by filtration for counting probable contamination of above samples (USP31, 2008). From each sample 10 ml was filtered for bacterial count and the same amount was filtered for fungal count. Washing was then performed in three stages using normal saline solution (0.9% NaCl). One filter was then transferred to TSA culture media (Tryptic Soy Agar) and the other to SDA culture medium (Sabouraud Dextrose Agar). Bacterial plates were read after three days of incubation at 32.5 ^{o}C and fungus plates were read after 7 days of incubation at 22.5 ^{o}C.

Stage 2

To make sure that anti-septic solutions are sterile, a sterility test was carried out on the samples (USP31, 2008). Ten ml of each sample was filtered under aseptic conditions. The filter was then washed in three stages using normal saline solution. One filter was transferred to FTG culture medium (Thioglycollate Medium Fluid) and the other to TSB culture mediaum (Tryptic Soy Broth). FTG culture medium was incubated and read after 14 days at 32.5 ^{o}C and TSB culture media at 22.5 ^{o}C.

Stage3

10^6 CFU/ml from 6 types of microbe strains was added to the samples to test the efficacy of the samples (Black, 1999).When the efficient time of consumption ended (according to the time indicated on the catalogue), samples were transferred to the culture media. Diluting was done according to manufacturer's instruction.

Table 2: Test microorganisms

	Microorganism	ATCC
1	*S. aureus*	6538
2	*E. coli*	8739
3	*B. subtilis*	6633
4	*P.aeruginosa*	9027
5	*C. albicans*	10231
6	*A. niger*	16404

At this stage, firstly microbial suspensions of above microorganisms (up to 10^8 CFU/ml) were prepared. These suspensions were prepared after 18 to 24 hours of culturing the bacteria and 48 hours of culturing *C. albicance* and seven days of culturing *A. niger*. Optical density of the suspensions was properly adjusted by a spectrophotometer. Then the pour-plate method was used and the suspensions were diluted to control the number of microorganisms. The plates were read after 24 hours for bacteria, 48 hours for *C. albicance* and 5 days for *A. niger*, and the average was used for positive control.

One ml of 10^8 CFU/ml suspension was added to 99 ml antiseptic solutions (Black, 1999). When the due time according to the catalogue was finished, the microbial surface culture was considered to be accomplished (Black, 1999). Results were recorded after incubation.

Result

The results of microbial count and sterility test of samples showed no microbial contamination. Table (3)

Table 3: Results of microbial control in test samples by filtration method

Name of sample	Bacterial count results (CFU)		of sterility test results	
	TSA Medium	SDA Medium	FTM Medium	TSB Medium
A-1	0	0	No growth	No growth
A-2	0	0	No growth	No growth
A-3	0	0	No growth	No growth
B-1	0	0	No growth	No growth
B-2	0	0	No growth	No growth
B-3	0	0	No growth	No growth

Study of A-1 efficacy

Efficacy of A-1 (hand disinfectant) was studied after adding 106 CFU/ml of test microorganisms and their culture after 15 and 60 seconds. In another word, the contact time between the disinfectant and microorganisms was considered 15 to 60 seconds following the guideline in the catalogue. Relevant performance of disinfectant A-1 was confirmed by observing a zero count in the first 5 strains. However, the count of A. niger, was reduced about two Logs after 15 seconds and three Logs after 60.

Study of A-2 efficacy

A-2 efficacy (surface disinfectants in concentrated form) was examined after dilution with 0.1% and 0.25% concentration and by adding 10^6 CFU/ml from mentioned strains. The strains were cultured with a 0.1% concentration after 6 minutes and with a 0.25% concentration after 5 minutes. The results showed no contamination in the first 5 strains, confirming the good performance of A-2 disinfectant. With regard to A. niger, with a 0.1% concentration and 60 minutes contact time, almost 2 Logs and with the % 0.25% concentration and 5 minutes contact time, almost 1 Logs was reduced.

Study of A-3 efficacy

A-3 efficacy (Instrument disinfectants in concentrated form) was investigated after dilution with a 0.25% concentration and by adding 10^6 CFU/ml of the mentioned strains (due to the durability of a diluted form for 30 days). First, it was cultured during 10 days and then after 30 days.

About the first 5 strains, culture results after 30 and 60 minutes were specified by adding mentioned microbes in the first 10 days and 30 days after the dilution. No contamination was observed and indicating the good performance of A-3 disinfectant. However, *A. niger*, culture results were specified after two contact times of 30 and 60 minutes by adding mentioned fungus in the first 10 days and after 30 days of dilution procedure. Contamination had increased progressively in the first 10 days; however, after 30 days of re-contamination and re-culture, microbial count was zero after the contact times of 30 and 60 minutes (Table 4).

Table 4: Test results of checking the efficacy of A-3 with A. niger

Name of sample	Microorganism	Microorganism adding time	Initial inoculum (CFU/ml)	0.25% Concentration	
				30 minutes	60 minutes
A-3	*A. niger* ATCC 16404	Microorganisms were added in the first 10 days	10^6	0	0
				5	0
				30	0
				43	0
				40	0
				60	0
				6×10^2	10
				8×10^2	10
				10^4	10^2
				10^4	10^2
		Microorganisms were added after 30 days durability		0	0

Study of B-1 efficacy

B-1 efficacy (hand disinfectant) was examined after adding 10^6 CFU/ml test microorganisms and their culture after 30 seconds. In another word, contact time between the disinfectant and microorganisms was 30 seconds according to the time specified on the catalogue. Observation of no contamination indicated the good performance of the disinfectant.

Study of B-2 efficacy

B-2 efficacy (Surface disinfectants in concentrated form) was performed after dilution with a concentration of 0.25% and by adding 10^6 CFU/ml from mentioned strains. Aafter a contact time of 5 minutes showed that in the first 5 strains, disinfectant had a good action. However, the *A. niger* count reduced about 2 Logs after 5 minutes.

Study of B-3 efficacy

B-3 efficacy (Instrument disinfectants in concentrated form) was examined after dilution with a concentration of 0.25 % and by adding 10^6 CFU/ml of the mentioned strains. The result after 5 minutes contact time showed that in the first 5 strains, disinfectant had a good action. However, *A. niger* count reduced about 2 Logs after 5 minutes.

Discussion

To check the efficacy of each disinfectant, our study advises using isolated strains on the area. In addition, efficiency of each disinfectant should be rechecked in every hospital, according to the programs of WHO to control nosocomial infections.

It ishould be emphasized that hospital staff have to trained with regard to diluting methods of disinfectants periodically and regularly. Training should be done under the supervision of an expert to reduce nosocomial infections.

Acknowledgement

We wish to give sincere thanks to Young Researchers Club of the Pharmaceutics Sciences Branch and laboratory of microbiology of Jaber Ebne Hayyan Pharmaceutical Co., for their valuable support from this survey.

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