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Assessment of microbiological species on selected inanimate surfaces in a pharmaceutical parenteral (sterile injections) manufacturing company

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Abstract
In general, environment of the pharmaceutical company consists of water, air and surfaces, which are often contaminated with microorganisms introduced from humans and environment. These germs represent microbial reservoirs that may pose a risk of product contamination. In the current study, we determined the microbiological quality of the surfaces in a selected inanimate surfaces in a pharmaceutical parenteral (sterile injections) manufacturing company, Chennai, in order to prevent microbial contaminations. A total of 231 samples were collected in the study over a period of one year. The samples were collected from seven different surfaces. An overall microbial identification showed the presence of 11 species which were alienated into gram positive (6 species) or gram negative (2 species) bacteria, fungi (2 species) and yeast (1 species) respectively. The current study results confirmed the need to implement systematic measures in pharmaceutical company surveillance to be adapted as a comprehensive policy for the prevention of microbial contaminations including a systematic surface treatment protocol

Keywords: Disinfectant; surface; microbiological contamination; virosil pharma disinfectant.

Introduction
Disinfectants used in pharmaceutical companies and laboratories must be tested periodically to ascertain their potency and efficacy. Certain disinfectants lose potency on standing and addition of organic matter, their efficacy must be tested intermittently (Reybrouck, 1998) [23]. Further introduction of new disinfectants also needs selection based on efficacy. The term disinfection is generally used for a process in which microorganisms present on nonliving or inanimate objects and surfaces are killed using chemical substances (Mohonmanuselis, 2007; Carter, 2011) [18, 7]. The process does not necessary free the surfaces from the bacterial spores. The commonly used disinfectants belong to the categories namely phenol and its derivatives, compounds of heavy metals, mercury compounds, organic chemicals, soaps, synthetic detergents and alcohols (Cheah, 2009) [8]. The killing or growth inhibition to the microorganisms occurs by denaturation or coagulation of cell components.
Surfaces act as reservoirs for microorganisms and could contribute to the transmission of pathogens, increasing the risk of cross-contamination through indirect contact with the worker (Hota, 2004; Kramer *et al*., 2006; Otter *et al*., 2013) [12, 15, 22]. To reduce such risks, sanitation procedures are applied to every surface that directly or indirectly may come in contact with people. Despite experimental evidence suggesting that a reasonable use of disinfectants is recommended, their routine use is still controversial and not universal (Rutala and Weber, 2005; Dettenkofer and Spencer, 2007) [24, 10]. Nevertheless, a proper surface disinfection is recommended by all international guidelines as an important procedure for preventing contaminations (Rutala, 1996; Mangram *et al*., 1999; WHO, 2002; Rutala and Weber, 2008) [27,16,29], and considerable evidence exists concerning the benefits of surrounding cleanliness towards reducing Healthcare-Associated Infections (HAIs) (Dancer, 2009) [9]. Indeed, failure to ensure proper cleaning and sterilization or disinfection may lead to person-to-person transmission of contamination which in truth may cause injection and increase in microbial load (Weber and Rutala, 2013) [29]. On the other hand the widespread use of chemical disinfectants presents risks towards the environment and the safety of personnel. Thus a judicious use of the disinfectants or other chemicals is mandatory. Further the microorganisms can adapt to a variety of environmental physical and chemical conditions,

which includes not surprisingly the development of resistance to some of the extensively used antiseptics and disinfectants (McDonnell and Russell, 1999; Frabetti *et al*., 2009) [17, 11].

The waste generated in the pharma industry either during the production or maintenance can include infectious microbiological material. In many cases this would require additional use of disinfectants (Singh *et al*., 2012) [28]. Many hospitals and pharmaceutical companies, which are among the important generators of such wastes, are still using phenolic disinfectants in India. On the other hand advanced countries of the world are discouraging the use of phenolic disinfectants. Similarly toxicity issues have led to discontinued use of gluteraldehydes in some developed countries (BSG, 2008) but, in developing countries, its used very frequently. HAIs are one of the most frequent complications occurring in healthcare facilities and represent a concern for safety and quality of healthcare worldwide (Burke, 2003) [6], as also stated in a recent report by the World Health Organization estimating company-wide prevalence in high-income countries at 8% (Allegranzi *et al*., 2011) [1]. The main objective of this work was assessment of the efficacy of disinfectant Virosil pharma by in-use testing and to identify bacterial isolates contaminating even during the use of disinfectant in different surfaces.

Material and Methods

A prospective study was conducted at the Microbiology laboratory of Parenteral (Sterile injections) manufacturing pharmaceutical company located in Chennai, Tamilnadu, over a period of twelve months spanning from March 2016 to February 2017.Minimum inhibition concentration and efficacy of Virosil pharma derived from tube dilution study was challenged on inanimate surfaces of pharmaceutical industry to check the efficacy and reactivity of virosil pharma on practical scenario. The samples of seven surfaces viz., SS Coupon, Glass Coupon, Acrylic surface. Wall paper surface, Epoxy floor, Tyvek paper and Derlin surfaces were tested. The selection of testing surfaces was based on the material used in different places in the manufacturing area, simulating pharmaceutical manufacturing environment.

Testing techniques

MIC Efficacy challenge Test

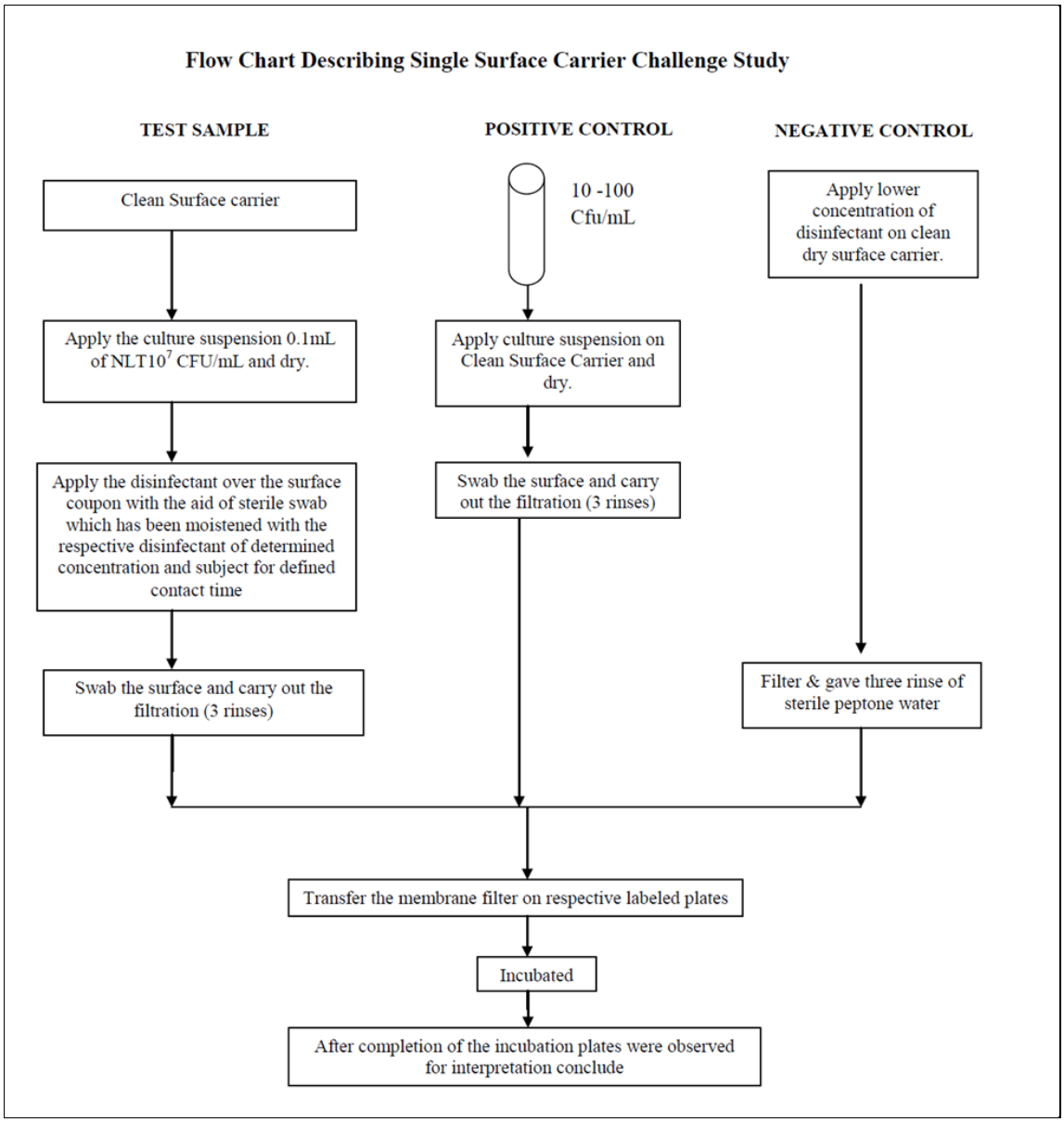
Sterilized clean and air dried surface carrier of about 24 to 30 cm^2 size representing each surface coupon was made available on Bio safety LAF cabinet. 0.1mL of 10^7CFU/mL of challenge microbial culture was applied on the sterile surface carrier of each coupon and allowed to be air dried under Bio safety cabinet. MI concentration of disinfectant solution was applied on each duplicate surface coupons with the help of same disinfectant moistened sterile swab. Surface coupons are kept without disturbing for pre-determined MI contact time derived in tube dilution method using stop watch. The surface coupons were swabbed to recover the microorganisms by dipping in 10ml of sterile saline test tube and vortexed vigorously. Then swab was removed by squeezing gently to the sides of test tube and content in test-tube was filtered through sterilized 0.45µm pore size, 47mm (diameter) membrane filter. Membrane filter was rinsed thrice by passing 100mL of sterile peptone water (0.1%). Aseptically membrane filter was transferred on to the surface of pre-incubated SCDA plate and incubated at 30-35 ˚C for 2-3 days followed by 20-25 ˚C for 3 – 5 days. The whole process is repeated for each disinfectant at MI concentration and contact time with each selected microbial challenge organism.

Positive Control

10-100 CFU/mL of challenge microorganism was spread on the sterilized surface coupon and allowed to air dry under Bio safety cabinet. Then surface coupons were swabbed to recover the microorganisms. Recovered swabs were dipped in 10 mL of sterile peptone water and vortexed vigorously and removed by squeezing to the sides of test tube. Then swab was removed by squeezing gently to the sides of test tube and content in test-tube was filtered through sterilized 0.45µm pours, 47mm (diameter) membrane filter. Membrane filter was rinsed thrice by passing 100mL of sterile peptone water (0.1%). Aseptically membrane filter was transferred on to the surface of pre-incubated SCDA plate and incubated at 30-35 ˚C for 2-3 days followed by 20-25 ˚C for 3 – 5 days.

Negative Control

Negative control test was performed in duplicates. Each of the MIC disinfectants are applied on to the surface coupons and allowed to air dry in bio safety cabinet. After drying, surface coupons were swabbed and transferred to the saline test tubes. Test tubes are vortexed vigorously and swabs are removed by squeezing to the sides of test tube. Vortexed solutions in test tubes are filtered through sterilized 0.45µm pores, 47mm (diameter) membrane filter. Membrane filter was rinsed thrice by passing 100mL of sterile peptone water (0.1%). Aseptically membrane filter was transferred on to the surface of pre-incubated SCDA media plate and incubatedat 30-35 ˚C for 2-3 days followed by 20-25 ˚C for 3-5 days.



Results

Annual trend of microorganism in pharmaceutical clean rooms was performed to identify the recurrent microbial contamination in clean room zones. Microorganisms identified in clean rooms are categorized into gram positive, gram negative, fungi and mold. Percentages of microbial species identified in clean rooms are explained in Figure 1.

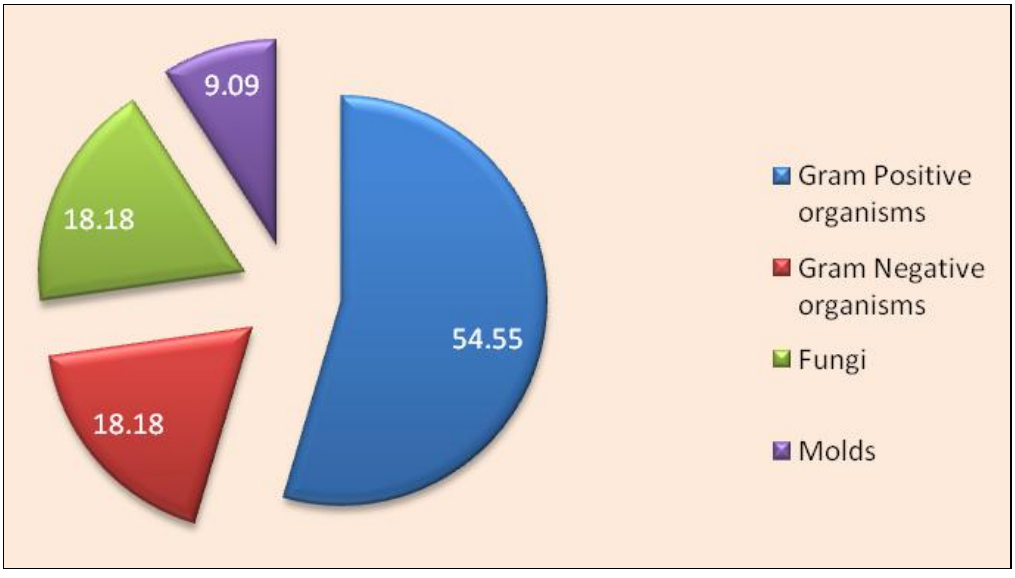
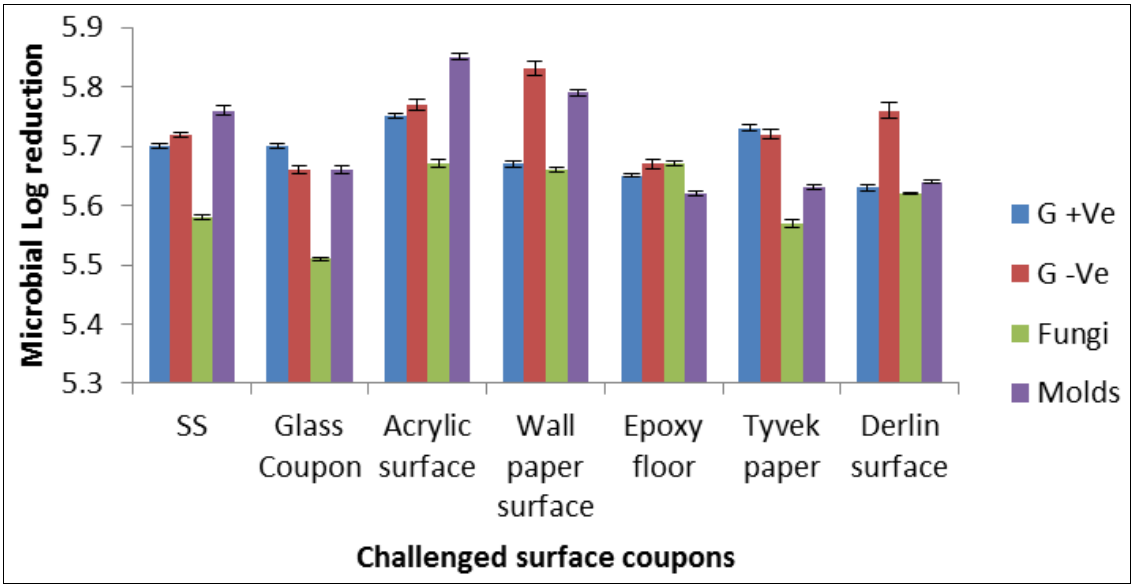


Fig 1: Categoriesed% microorganisms showed recurrent contamination with phenol based disinfectants.

Recurrent prevalence of polymorphic flora was identified in clean rooms, with the 11 different species identified including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus brasiliensis*, and *Candida albicans* (Table.1). The analysis showed a diversified micro flora. The predominance of Gram positive bacteria with 54.55%, whereas gram negative bacteria and fungi represented 18.18%, while molds 9.09%. These recurring organisms only were challenged in the study with the novel disinfectants (table 1 and figure 1).

Table 1: Microorganisms challenged in Surface challenge method

Test Cultures	Microorganisms
Gram Positive organisms	*Staphylococcus aureus* *Bacillus subtilis* *Micrococusluteus* *Staphylococcus hominis* *Kytococcussedentarius* *Kocuriakristinae*
Gram Negative organisms	*Escherichia coli* *Pseudomonas aeruginosa*
Fungi	*Aspergillus brasiliensis* *Aspergillus tamari*
Molds	*Candida albicans*



X-axis: Challenged surface coupons, Y-axis: Microbial Log reduction

Fig 2: Challenged culture results after Microbicidal activity

Table 2: Surface challenge results at Virosil Pharma 5.0% concentration and contact time of 6 minutes

Surface Coupon		Gram +ve	Gram -ve	Fungi	Molds
SS Coupon	A	5.70	5.72	5.58	5.76
	B	G	G	G	G
	C	NG	NG	NG	NG
Glass Coupon	A	5.70	5.66	5.51	5.66
	B	G	G	G	G
	C	NG	NG	NG	NG
Acrylic surface	A	5.75	5.77	5.67	5.85
	B	G	G	G	G
	C	NG	NG	NG	NG
Wall paper surface	A	5.67	5.83	5.66	5.79
	B	G	G	G	G
	C	NG	NG	NG	NG
Epoxy floor	A	5.65	5.67	5.67	5.62
	B	G	G	G	G
	C	NG	NG	NG	NG
Tyvek paper	A	5.73	5.72	5.57	5.63
	B	G	G	G	G
	C	NG	NG	NG	NG
Derlin surface	A	5.63	5.76	5.62	5.64
	B	G	G	G	G
	C	NG	NG	NG	NG
Note: Where, A: Challenged culture results after Microbicidal activity, B: Positive control, C: Negative control					

Table 3: Virosil Pharma disinfectant concentration efficacy study

Challenged cultures	Challenged population	Mean log reduction of challenged cultures											
		2.5%				5%				7.5%			
		Time in minutes											
		2	4	6	8	2	4	6	8	2	4	6	8
Gram Positive bacteria	7 log	TNTC				TNTC		6.25	7.0	No growth			
Gram Negative bacteria	7 log	TNTC				TNTC		5.24	6.86	No growth			
Fungi	7 log	TNTC				TNTC		5.48	6.80	No growth			
Yeasts and molds	7 log	TNTC				TNTC		5.31	6.97	No growth			

TNTC: Too numerous to count

The results show reduction of 5.51 to 5.85 log reduction in the number of microorganisms in the various categories tested. The disinfectant was most effective on Acrylic surface where a total reduction of 23.04 was obtained, in the total microbial load. Followed by total 22.95 log on wall paper surface and 22.76 log on stainless steel. Individually least reduction was obtained with 5.51 log on glass surface, although it was quiet satisfactory from the point of view of disinfection efficiency, as 4 log reduction is required when challenged with 7 log population in the pharmaceutical company. The most important surface in the clean room is glass surface which gave a result of total 22.53 log reduction in microbial load. The positive control in the experiments showed growth on all the challenged surface coupons. Negative control showed no growth on any of the surfaces.

Discussion

The products and procedures for disinfection or sterilization of clean room surfaces, as described in the literature may not be able to adequately disinfect or decontaminate items. As the disinfection depends on the prevailing conditions such as the contamination of surfaces with microorganisms, especially with highly resistant or unusual organisms, or if the bio-load of microorganisms is very heavy (Singh *et al*., 2012) [28]. When choosing a disinfectant for specific pharma company use, it may be necessary to know the expected number and the types of organisms likely to be present on the surface. It is critical that the disinfectant be selected based on its ability to be effective against the prevalent microorganisms that can be transmitted by direct or indirect contact with the environment (Kawamura-Sato *et al*., 2010) [13]. An ideal disinfectant should have a broad antimicrobial spectrum, should be non-irritating, less toxic, noncorrosive and inexpensive (Rutala and Weber, 2001) [21].

Some researchers in India (Singh *et al*., 2012) [28] observed that phenolics showed poor activity on rough surfaces that represent cracks and grooves on the floors and walls, very commonly seen in developing country health care settings. A few other researchers (Awodele *et al*., 2007; Okesola *et al*., 2011) [3, 21] showed that antimicrobial activities of disinfectants were concentration dependent. This observation will mean that if appropriate concentrations are not used even in the in-use testing they will be contaminations of disinfectants. Some other workers (Atoyebi *et al*., 1999; Niemogha, 2003) [2, 19] confirmed contamination of disinfectants in their different studies. Disinfectants in constant and prolonged use gradually become contaminated thus raising the microbial load. The need to prevent this has been emphasized by some researchers (Oie and Kamiya, 1996) [20].

There was no policy about the In-use disinfectants in the pharmaceutical company where we carried out this study. Both the important factors i.e. recurrent organisms and the various surfaces, were tested for achieving good disinfection. The bacteria in an area of pharmaceutical company protected from recontamination were greatly reduced in numbers after each of the methods of cleaning and disinfection tested. In contrast 40-50% reduction in floor bacteria obtained by using dry methods (Babb *et al*., 1963) [4], whereas the reduction rate has increased about 80%,with the use of present disinfectant.

The microbial inhibition and killing effect of this Virosil disinfectant were observed to be increasing as the concentration of the disinfectant were increased. This conforms to the study by (Kortenbout, 1982; Okesola *et al*., 2011) [14, 21] where they observed that the higher the concentration of the solution, the more potent and effective the solution will be. The result of the capacity test showed that non-sporulating, non-mycobacterial, gram positive and gram negative organisms were equally susceptible to disinfectants, but slightly gram negative bacteria recovery results higher than other category of microbes. This observation is in line with the of Mamman *et al*., 2005, which showed that Gram negative positive were more resistant to disinfectants than Gram negative bacteria generally because the outer membrane of gram positive bacteria acts as a barrier that limits the entry of many chemically unrelated types of antimicrobial agents (McDonnell and Russell, 1999) [17].

Conclusion

This study showed the disinfectant efficacy on different surfaces studied. It will be necessary to always evaluate new disinfectants on inanimate surfaces (Table 1) before their application in pharmaceutical manufacturing environment and also check same periodically in-use to ensure efficacy (Table 2). This study recommended that the pharmaceutical companies and health care industries must be aware about the reality of the concept of environmental bacterial load and the need for respect of bio-cleaning procedures and choice of effective bio-cleaning disinfectants.

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