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Assessment for Sporicidal Activity of Two Types of Peroxygen/Silver-Based Disinfectants: A Comparative Study

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Abstract: Numerous cases have been reported globally showing outbreaks, dissemination of infections and/or spoilage of medicinal products, food and other consumables that affects human life and may lead to death in some cases. One of the most critical measures of microbial contamination that should be taken into consideration is the use of proper disinfectant depending on the type of activities and the work load of the healthcare institution, subjects or media for microbial transfer and the affected population. The current study shows significant difference in sporicidal activity between two types of commercial peroxygen/Silver based disinfectants obtained from same manufacturer and delivered through same distributor at the same time. The peroxygen component in one of the disinfectant products is Hydrogen peroxide while in the other product is Peroxyacetic acid/Hydrogen Peroxide mixture. Preliminary rapid assessment of the disinfectants activity was required using the most resistance microbial form (bacterial spore) as a reference microorganism to challenge the biocidal products. Bacillus pumilus and B. subtilis spores were exposed to both disinfectants at three different concentrations levels covering the range recommended by the manufacturer: 1, 3 and 5% (v/v). The first formula did not exceed 0.5 logarithmic reduction (LR) even after 30 minutes. While the other product achieved more than two folds LR (more than 100 times reduction in microbial population) after ten minutes contact time. Appropriate initial screening of biocidal activity in commercial disinfectants market is critical step that should be performed by the healthcare facilities before practical application. Otherwise, inefficient control on bioburden may lead to devastatingconsequences on human health. An initial, non-laborious,

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time-saving and non-expensive screening test using the most resistance microorganisms is encouraged to be performed by healthcare facilities prior to practical application of disinfectant rather than reliance solely on random selection of biocidal agents using informational data without confirmatory experiments.

Keywords: Colloidal Silver, Peroxygen, Bacillus pumilus, Bacillus subtilis, logarithmic reduction.

1. INTRODUCTION

Microorganisms have been long known for their ability to survive and disseminate in different climates and even in the harsh conditions where other living organisms cannot live. At the same time, this survival capability of microbial life is a drawback to those professionals working in the healthcare industry (Clontz, 2008). Direct-contact human products and materials such as food, drugs, cosmetics, medical devices and equipments are prone to microbial contamination from vast sources including human and environment and eventually gain access to the final consumer and/or patient (Denyer and Baird, 2007).

Disinfectants are prone to microbial contamination but at lower frequency compared to antiseptics, most probably due to ineffective application. There have been many reports for outbreaks and/or pseudo-outbreaks that have been originated from microbial contamination of disinfectant products (Weber *et al.*, 2007). Examples include *Bacillus cereus* contamination of Ethanol, Glutaraldehyde loaded with *Mycobacterium chelonae*, *Methylobacterium mesophilicum* (Kressel and Kidd, 2001) and *Mycobacterium* species (Laskowski *et al.*, 1977; Tyras *et al.*, 1978). In addition, phenolic products were intruded with *Pseudomonas* species (Cragg and Andrews, 1969; Elliott and Masters.,1977; Newman *et al.*, 1984), *Pseudomonas aeruginosa* (Baird and Shooter, 1976; Newman *et al.*, 1984) and *Alcaligenes faecalis* (Simmon and Gardner, 1969) and quaternary ammonium compounds (QACs) were contaminated with *Burkholderia cepacia* (Dixon *et al.*, 1976; Ebner *et al.*, 2005), *Serratia marcescens* (Ehrenkranz *et al.*, 1980), *Achromobacterxylos oxydans* (Lehours *et al.*, 2002) and *Pseudomonas aeruginosa* (Shickman *et al.*, 1959; Olson *et al.*, 1999). Even *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* (Vanholder *et al.*, 1992) and *Klebsiella oxytoca* were found in Formaldehyde (Reiss *et al.*, 2000).

Accordingly, proper sanitization and disinfection program should be implemented using appropriately and scientifically designed validation protocol (Sandle, 2014). Bacterial spores are one of the strongest forms that support survival of microorganisms in dormant state against hostile and

extreme environmental conditions (Russell, 1982; Lambert, 2004). While fungal spores are part of the normal life cycle of the fungi (Clontz, 2008).

Due to the above described challenges, the present work aimed to study the suitability of two commercially available sanitizing agents which were purchased from disinfectants market in Egypt. Moreover, a simple, inexpensive and non-laborious yet effective methodology was investigated which can be used within healthcare facilities to assess the suitability of specific disinfectant for particular application. The test should aid in providing decision within suitable time frame based on scientific approach.

2. MATERIALS AND METHODS

2.1. Subject of the study

Rapid assessment was requested for new two types of oxidizing disinfectant formulae which were obtained from the same manufacturer through the same supplier at the same time. These disinfectants should be used - based on the preliminary evaluation - in the routine sanitization and disinfection program of healthcare facility. Disinfectant samples were stored and prepared according to the manufacturer instructions. The antimicrobial components of the first disinfectant is Hydrogen Peroxide/Colloidal Silver (HP/Agc) and the other composed of Peroxyacetic acid (Peracetic acid)/Hydrogen Peroxide/ Colloidal Silver (PAA/HP/ Agc).

2.2. Preliminary neutralization parameters

The labeling system for traceability was as follows (disinfectant code/concentration % (v/v)/Bacillus spp.). The codes were given as follows: *Bacillus pumilus(p), Bacillus subtilis(s),* PAA/HP/ Agc (P) and HP/Agc (M). The basic principle of the preliminary neutralization study was conducted as described by Eissa and Nouby (2014). The method of conducting kinetics of spore inactivation study was performed as per reported detailed work (Eissa *et al,* 2014). Spore-forming microorganisms were purchased from ATCC (American Type Culture Collection, PO Box 1549 Manassas, VA_20108 USA, www.atcc.org). Standard strains that were used in the challenge test are *B. subtilis subsp.spizizenii* (ATCC 6633) and *B. pumilus* (ATCC 14884) and handled according to a standard procedure. All the culture media were purchased from OXOID (Basingstoke, Hampshire) and chemicals from Sigma-Aldrich (St. Louis, MO 63103).

2.3. Microbial suspension preparation

Standardized stable suspensions of test strains were prepared and used as stated by their suppliers. Seed-lot culture maintenance techniques (seed-lot systems)

were used so that the viable microorganisms used for inoculation are not more than 5 passages removed from the original master seed-lot. All culture media used in the current study were subjected to growth promotion (GP) test as per compendial method (USP38-NF33, 2015). Suspensions were quantified by making serial dilutions and performing duplicate plate counts using conditions and media suitable for each microorganism. Microbial test suspensions should be used as soon as the results of serial dilutions could be enumerated using a digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai).

2.4. Instruments and tools

All organisms were stored at -80°C in a validated -86°C Ultra low temperature freezer (-86 Degree ULT Freezers, Qingdao Shandong, China) in validated cryogenic environment and reactivated only prior to the study conduction using standard method illustrated by the supplier. All media were sterilized by autoclaving in a steam sterilizer (FEDEGARI FOB3, Fedegari Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). All pH measurements and weighing procedures were done using Mettler-Toledo S20 SevenEasy™ pH Meter and XPE Analytical Balance respectively (Mettler-Toledo, LLC 1900 Polaris Parkway Columbus, OH 43240). Plastic 9 mm sterile plates were purchased from Sterilin Limited (solaar house, 19 mercers row, Cambridge, UK). Biological safety cabinet (BSC) (Jouan MSC 9 Class II A2 BioSafety Cabinet, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, California 95134) was used for all microbiological processing techniques.

2.5. Testing and technique

Environmental monitoring (EM) samples from surfaces and air within test area were taken according to Eissa, 2014 with every campaign test performed in BSC to monitor the quality of cleaning, disinfection and aseptic behavior under laminar air flow conditions (Eissa, 2014). Bacterial visualization was facilitated using colorless Triphenyltetrazolium Chloride (TTC) dye which is reduced from colorless to red insoluble1,3,5-triphenylformazan (TPF) in viable cells by various dehydrogenasesas indicated by some manufacturers of culture media (BD Difco™ , 2016). Cultures purity and identity were confirmed by isolation and identification (Estridge *et al.,* 2000; Ashour *et al,* 2011). Acceptance criteria of the test results were decided based on what is stated (Clontz, 2008). Acceptance criteria for significance of microbial reduction and spore count reduction were decided based on United States Pharmacopoeia (USP)31-National Formulary (NF)26 (USP31-NF26-Chapter <1072>, 2008; USP31-NF26-Chapter <51>, 2008).

2.6. Data interpretation

All statistical analysis tests and the kinetics of microbial death were performed using Graph Pad Prism version 6.01. Any interpretation or complex calculation was performed using Microsoft Excel 2007.

3. RESULTS AND DISCUSSION

Figure 1 showed that disinfectant P could achieve more than two logarithmic reduction (LR) within ten minutes and hence met the compendial requirement for sanitizing agents. While M was not able to meet this requirement even after 30 minutes, although it has been claimed to be effective against wide spectrum of microorganisms including spore-formers. On the other hand, Table 1 demonstrated that at all points and concentrations P had significant impact on bioburden reduction as LR exceeded 0.3 to 0.5 variability from the initial values. Aberrant results required further analysis, statistical comparison was performed using One-Way Analysis Of Variance (ANOVA) at $p<0.05$.This is in contrast of M as there was slight difference observed at 3% dilution (v/v), which may indicate that the slow action requires longer time. Strangely, an

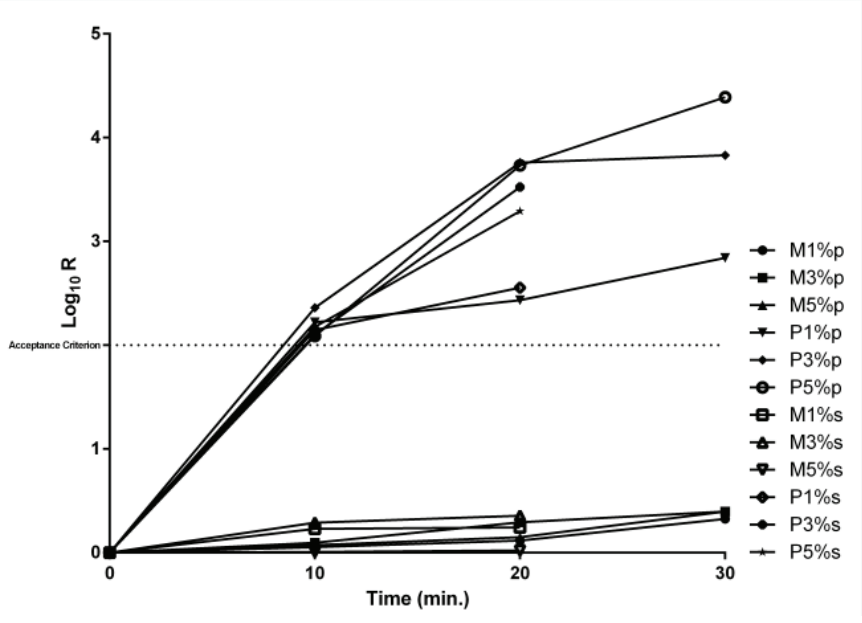

Figure 1: Inactivation kinetics of bacterial spores exposed to peroxygens and expressed as logarithmic reduction (LR to base ten) from the initial inoculum, with dashed line showing the acceptance criterion.

exception odd results of M 5% (v/v) concentration may be excluded although it was not found to differ significantly from the other values of M. It is not clear if M can affect spore population significantly after 30 minutes or not, as it is known that HP is slow acting sporicidal disinfectant in contrast to PAA (Cotruvo *et al.*, 1999; WHO, 2004; Tamime, 2009; LabSafety, 2009). However, it will not be practical to allow for longer contact time because it is not reasonable for activities and heavy and frequent work load. Accordingly, M was excluded from further applications or studies. Meanwhile, P was incorporated in further antimicrobial studies parallel to its application at 1% (v/v) concentration for ten minutes on utensils and surfaces in the healthcare buildings.

Sagripanti and Bonifacino, 1996 have demonstrated that the rate of spores inactivation by PAA are much higher than that of HP. So, it is not strange in view of other researchers results to find such contrast between both products. Based on this outcome proper selection and application of disinfectant should be tested and evaluated before its practical use, regardless of the labelled spectrum of activity and use that are claimed by the manufacturer of the disinfectant. However, the limitation of application of this challenge test is the large array of microorganisms displayed by guidelines of this test (Clontz, 2008), which may be hindering barrier for application especially for healthcare facilities in the developing countries. Accordingly, very limited microorganisms can be carefully selected (as shown in the current study) to represent the most challenging microbes for specific industry and/or activity. The microorganisms can be referred to as "marker" or "indicator" microbe for specific situation.

Table 1: Comparative study for the significance of the reduction of spore population at different concentrations intervals covering the recommended range of dilutions.

Microorganism	Time (min.)	M1%	M3%	M5%	P1%	P3%	P5%
	10	0.054	0.097	0.073	0.000	0.000	0.000
Bacillus pumilus	20	0.117	0.295	0.150*	2.222	2.362	2.089
	30	0.327	0.398	0.398	2.434	3.760	3.732
Bacillus subtilis	10	0.230	0.291	1.009*	2.146	2.102	2.178
	20	0.244	0.358	1.022*	2.555	3.523	3.292

*Odd results required further analysis using One-Way Analysis Of Variance (ANOVA) at $p<0.05$ to compare it with the other dilutions of the same disinfectant (M) and the difference was found to be not significant.

4. CONCLUSIONS

Disinfectant selection screening study showed that PAA/HP/Agc combination was very effective as sporicidal agent and was capable in meeting the acceptance criteria within ten minutes contact time with spores by achieving more than 100 reduction folds in the recovered population in CFU. Accordingly, it can be applied efficiently as biocidal agent to control bioburden at the dedicated concentration range 1-5% (v/v). On the other, HP/Agc formula from the same manufacturer was not able to yield effective results within 30 minutes of contact. Thus, it cannot be used safely to control microbial population when relatively fast action is required. The present case highlights the importance of conducting preliminary evaluation study of commercially available sanitizing agents before using it blindly, even if the instructions for storage, preparation and dilution were done literally as recommended by the manufacturer. The availability of varieties of antimicrobial products in the open world trading market requires careful and controlled approach from healthcare providers in selecting and using biocidal products that impact consumer health and the quality of life.

5. ACKNOWLEDGEMENT

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