Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 479-481
Received: 17-01-2018
Accepted: 19-02-2018

Meenal Khana
Shri Venkateshwara University, Gajraula Distt. JP Nagar, Uttar Pradesh, India

UVS Teotia
Shri Venkateshwara University, Gajraula Distt. JP Nagar, Uttar Pradesh, India

Yogendra Singh
Zydus Cadilla Ltd, Baddi, Himachal Pradesh, India

Correspondence
Meenal Khana
Shri Venkateshwara University, Gajraula Distt. JP Nagar, Uttar Pradesh, India

Effect of storage on microbial quality of non-sterile liquid dosage form

Meenal Khana, UVS Teotia and Yogendra Singh

Abstract
This study attempted to evaluate effect of packaging system and storage on the microbiological quality of non sterile oral liquid dosage form. A total 14 sample, representing seven category of drug packed in different packaging system were subjected to microbial evaluation using: total aerobic bacterial count and identification of microbial pathogens. The samples were kept at 25^0C ± 2/60 % ± 5 for one year and microorganism growth rate of samples were evaluated at 0, 6 and 12months. *Bio burden altered among the samples* 42.85 % were free from microbial contaminants whereas the remaining held bacteria in levels below 10^2 cfu/mL *and highest microbial load in liver tonic. The isolated organisms were* gram positive bacteria including *Staphylococcus* spp. and *Bacillus* spp., and gram negative bacteria including *Proteus* spp, *Klebesilla* spp and *Pseudomonas* spp, there was no growth of fungi. The bacterial count of the products and the number of isolated contaminants increased after storage for 6 and 12 months, none of sample exceeded the acceptance criteria. The microbial quality of plastic pack were not significantly different from glass pack ($p > 0.05$).

Keywords: microbial load, stability, non-sterile, packaging, total aerobic bacterial count

Introduction
The use of contaminated pharmaceutical preparations has turned out unsafe to the health of patient. There have been cases regarding drug-borne human infection worldwide [1, 2]. Contamination of drug product with microorganism can also give rise alteration in physicochemical properties of pharmaceutical products [3]. Reports of infections triggered by pharmaceutical contamination of microorganism results to the establishment of a special committee at the international pharmaceutical federation (FIP) which was intended with composing guidelines regulating manufacturing. The works resulted in the development of Good Manufacturing Practices (GMP) guidelines. The GMP objectives were introduced, to ensure quality drug products and assure patient safety. Moreover microbiological standard were established and the need of final microbiological control was introduced [4]. Currently manufacturers of pharmaceuticals have improved the quality of nonsterile pharmaceuticals such that today products contain only minimal bioburden. The finding of microbial contamination has been well documented and contaminants range from true pathogens such as *Clostridium titani* to opportunistic pathogens such as *Pseudomonas aeruginosa*. The major health concern arises when such microorganisms exceeded acceptable criteria (10^2 CFU/mL) [5, 6].

Oral liquid dosage forms represent large proportion of pharmaceutical product which are available in Indian market. Oral liquid dosage forms are prone to microbial spoilage and degradation. These may provide suitable environment and even may serve as nutrients for the growth and survival of microorganisms as they consist of high amount of sugar and moisture [7, 8]. Therefore there is a need to know the microbial content of all drugs all such dosage forms, whether they are sterile or non sterile [9].

Packaging system used for pharmaceutical products are various and changing continuously. Packaging is an important factor in preserving the quality of the product and maintaining its characteristics up to the end of their shelf-life. For instance, liquid dosage forms are more likely to interact with the packaging materials as compare to solid dosage forms. Considering the variety of the packaging available today, it is of great interest the study of the relationship between the drug itself and the packaging material used, with the aim to uncover the potential microorganism contamination [7]. The aim of this study was to evaluate microbiological status of oral liquid dosage forms on long term storage and investigate the effects of different packaging system on microbial status of pharmaceutical product.

Materials and Methods

Materials

A total number of 14 non sterile oral liquid dosage form samples were tested in this study. The samples comprised of seven categories of drugs, packaged in different packaging. Samples were randomly collected and stored under appropriate conditions. All procured samples were approved by Central Drug Standard Control Organisation (CDSCO). Sample was taken in triplicate.

The media used for the microbiological analysis include: Soya bean casein digest agar, nutrient broth agar, MacConkey agar, mannitol salt agar, blood agar, thioglycollate medium and Sabouraud dextrose agar. They were manufactured by Darmstadt, Germany. The media was prepared according to manufacturer's instructions.

Qualitative analysis of bacteria

Enrichment Culture

Ten ml of sample was diluted in the sterile buffered solution. Fifty ml nutrient broth in each conical flask (triplicate) was inoculated by 1 ml diluted sample (10^{-1}) and incubated at 30^0C for 24 hr to 48 hr in shaker incubator (for aerobic growth). After incubation, turbidity of the medium was observed as the incubation of microbe in the sample [10].

Sub Culture

One loop full of enrichment culture was transferred aseptically to an appositely labeled Nutrient agar and MacConky agar plates and inoculated by four way streak method on each medium. Inoculated plates were incubated for 24 hours at 30^0C and observed for the presence of discrete colony [10].

Isolation and Identification of bacteria

Isolated colonies from subculture were aseptically transferred and streaked on to nutrient agar slant tubes individually. Tubes were incubated for 24 hrs at 30^0C and then stored for identification test.Identification of bacterial colonies was carried out on the basis of microscopic morphology, staining characteristics and biochemical properties using standard microbiological manual [11].

Quantitative analysis of total viable bacteria

The quantitative estimation of bacteria was done by plate count method.Ten ml of sample was diluted in the sterile buffered solution. The diluted (10^{-1}) samples were immediately spread over the surface of Soyabean Casein Digest Agar (SCDA) for determination of total aerobic microbial count (TAMC) and incubated at 30^0C for 24 hr. Arithmetic average of the count was taken and the number of colony forming unit (CFU) per g was calculated [8].

Effect of storage on microbial load

The study sample was stored at 25^0C ± 2/60 % ± 5 for one year. Sample were estimated for microbial load at 0,1,6 and 12 months.

Result and Discussion

The total aerobic microbial count (TAMC) and isolation of specific microorganism were used as parameters for the evaluation of the protective function of two primary packaging system for non sterile liquid dosage form. The protective function in relation to microbial quality, of glass and plastic were examined in the study. The results shown in Table 1 exhibit that out of 14 samples examined, 6 (42.85 %) were free from microbial contaminants whereas the remaining 6 samples (42.85 %), held bacteria in levels below 10^2 cfu/mL. It is apparent from the table that only 14.28 % of the samples contained bacterial count between 10^2 and 10^3 cfu/mL. The highest contamination level seemed predominantly in the glass and plastic packs of liver tonic. This may be due to use of natural ingredients, which are likely to be more contaminated [1]. In different studies, oral liquid dosage samples were found to be highly contaminated with fungi and total viable bacteria exceeded the USP limit ($<10^2$ cfu/ml) in major samples [7, 10] but none of sample exceeded the acceptance criteria in our study. It unveils that the microbiological quality of the examined samples was, in general, decent and, in majority samples, excellent and which might due to better compliance to 'Good Manufacturing Practices' by pharmaceutical manufacturers in recent years.

Table 1: Total aerobic bacterial count (CFU/ml) of non sterile liquid dosage form during storage.

S. No	Category	Packaging	Total Aerobic Microbial Count (TAMC)			
			0 month	3 month	6 month	12 month
1	Antacid	Glass	1.6×10^1	1.8×10^1	2.3×10^1	3.1×10^1
2		Plastic	2.2×10^1	2.6×10^1	3.8×10^1	4.7×10^1
3	Liver Tonic	Glass	8.6×10^2	10.2×10^2	1.52×10^3	2.0×10^3
4		Plastic	10.2×10^2	1.4×10^3	1.78×10^3	1.98×10^3
5	Multi Vitamin	Glass	3.8×10^1	4.2×10^1	5.4×10^1	7.2×10^3
6		Plastic	4.3×10^1	5.1×10^1	6.6×10^1	7.5×10^1
7	Amoxycilline Suspension	Glass	ND	ND	ND	1.5×10^1
8		Plastic	ND	ND	ND	2.3×10^1
9	Lactulose	Glass	6.8×10^1	7.3×10^1	8.2×10^1	1.03×10^2
10		Plastic	7.2×10^1	7.6×10^1	8.5×10^1	1.15×10^2
11	Antitussive	Glass	ND	ND	ND	ND
12		Plastic	ND	ND	1.2×10^1	2.3×10^1
13	Expectorant	Glass	ND	ND	2.4×10^1	3.2×10^1
14		Plastic	ND	1.4×10^1	2.1×10^1	2.8×10^1

Table 2 represents the different microorganisms isolated from each sample. It is clear that *Staphylococcus* spp was the most commonly found Gram positive bacteria followed by *Bacillus* spp, while *Proteus* spp, *Klebesilla* spp and *Pseudomonas* spp were the Gram negative bacteria recovered. Statistical analysis of the bacterial counts indicated that no significant difference in the microbiological quality of the two packaging system. *Staphylococcus* spp and *Bacillus* spp were the most common gram positive microorganism recovered in various studies is consistent with our observations. *Staphylococcus* spp. might carry from environment and worker during the manufacturing of drugs, their presence does not always imply

that the administration of drugs are potentially be unsafe to patient as not all the strain of *Staphylococcus* spp. produces endotoxin and higher infectious dose (10^5-10^6cfu/ml) is required (1, 12). On the other hand, *Bacillus* species are ubiquitous, but undesirable because of their spoilage potential, and their presence in a product suggests poor environmental hygiene during processing or heavily contaminated or adulterated raw materials (6, 13). There was no significant difference ($p>0.05$) in microbial load of glass and plastic packs.

Table 2: Bacterial profile of non sterile liquid dosage form during storage.

S. No	Category	Packaging	Isolated Microorganism			
			0 month	3 month	6 month	12 month
1	Antacid	Glass	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp* *Bacillus Spp*
2		Plastic	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp*
3	Liver Tonic	Glass	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*
4		Plastic	*Staphy. Spp* *Pseudo. Spp*	*Staphy. Spp* *Pseudo. Spp*	*Staphy. Spp* *Pseudo. Spp*	*Staphy. Spp* *Pseudo. Spp*
5	Multi Vitamin	Glass	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*	*Staphy. Spp* *Bacillus Spp*
6		Plastic	*Staphy. Spp* *Klebesilla Spp*	*Staphy. Spp* *Klebesilla Spp* *Bacillus Spp*	*Staphy. Spp* *Klebesilla Spp* *Bacillus Spp*	*Staphy. Spp* *Klebesilla Spp* *Bacillus Spp*
7	Amoxycilline Suspension	Glass	ND	ND	ND	*Staphy. Spp*
8		Plastic	ND	ND	ND	*Staphy. Spp*
9	Lactulose	Glass	*Proteus Spp*	*Proteus Spp*	*Proteus Spp*	*Proteus Spp*
10		Plastic	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp*	*Staphy. Spp*
11	Antitussive	Glass	ND	ND	ND	ND
12		Plastic	ND	ND	*Bacillus Spp*	*Bacillus Spp*
13	Expectorant	Glass	ND	ND	*Bacillus Spp*	*Bacillus Spp*
14		Plastic	ND	*Bacillus Spp*	*Bacillus Spp*	*Bacillus Spp*

All the preparations showed growth after storage except antitussive in glass bottle**.** The bacterial count of the products increased after storage for 6 and 12 months, respectively. For most of the preparations, the number of isolated contaminants increased with duration of storage. In a study, Gad et.al studied effect of storage on microbial quality of non-sterile pharmaceuticals available in Egyptian market and result was similar to observation of our study.

Conclusion

This study has concluded the acceptable quality of non sterile liquid dosage form manufactured by Indian pharmaceutical companies in relation to microbial count and the isolation of specified microorganisms. The effectiveness of the protection provided by the two primary packaging was not significantly different. Microbial quality of non sterile liquid dosage form remained within acceptable limit and revealed that good adherence to GMP by Indian pharmaceutical manufactures.

References

1. Gamal Fadl M Gad, Reham A Ibrahem Aly, Ashour MSE-d. Microbial Evaluation of Some Non-sterile Pharmaceutical Preparations Commonly Used in the Egyptian Market Tropical Journal of Pharmaceutical Research. 2011; 10(4):437-45.
2. Coker M. An assessment of microbial contamination during drug manufacturing in Ibadan, Nigeria. Eur J Scientific Res. 2005; 7:19-23.
3. Shaikh D, Jamshed TA, Shaikh R. Microbial contamination of pharmaceutical preparations. Pak J Pharm Sci. 1988; 1(1):61-6.
4. Ratajczak M, Kubicka MM, Kamińska D, Sawicka P, Długaszewska J. Microbiological quality of non-sterile pharmaceutical products. Saudi Pharmaceutical Journal. 2015/07/01/. 2015; 23(3):303-7.
5. Obuekwe CO, Obuekwe IF, Rafiq M. Surface contamination in some common available dosage forms. Med Princ Pract. 2000; 9(4):290-9.
6. Mugoyela V, Mwambete KD. Microbial contamination of nonsterile pharmaceuticals in public hospital settings. Therapeutics and Clinical Risk Management. 2010; 6:443-8.
7. Khanom S, Das K, Banik S, Noor R. Microbiological analysis of liquid oral drugs available in Bangladesh, 2013.
8. Kaniz Fatema, Sowmitra Ranjan Chakraborty, Tohora Sultana, Md. Mokhlesur Rahman, Niaz Muhammad Kamali, Kamal Kanta Das, *et al*. Assessment of microbiological quality of the pediatric oral liquid drugs. Journal of Pharmacognosy and Phytochemistry. 2014; 3(1):165-71.
9. Parker MS. editor. Microbiological contamination and preservation of pharmaceutical preparations. Hong Kong, China: Churchill Livingstone, 2000.
10. Islam MS, Alam MN, Kabir MAA, Nasrin T, Mia Z. Qualitative and quantitative microbial load in oral liquid drugs in Bangladesh. International Journal of Natural and Social Sciences. 2015; 2:54-9.
11. Phillips I. Cowan and Steel's Manual for the Identification of Medical Bacteria. Journal of Clinical Pathology. 1993; 46(10):975-.
12. Beys L, Hoest B. Investigation for staphylococci in foods, dietetic products and oral drugs. Rev J Food Protec. 1971; 25:26-33.
13. Christenson JC, Byington C, Korgenski EK, Adderson EE, Bruggers C, Adams RH, *et al*. Bacillus cereus infections among oncology patients at a children's hospital. American Journal of Infection Control. 1999; 27(6):543-6.