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EXPERIMENTAL EVALUATION OF PRACTICAL VIABILITY OF GOOD MANUFACTURING PRACTICE (GMP) IN PREPARATION OF ORAL LIQUID FORMULATIONS IN SMALL SCALE PHARMACEUTICAL INDUSTRIES

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ABSTRACT

Oral liquid formulations have a much greater possibility of getting contaminated by airborne bacteria than other pharmaceuticals. Such bottles should be closed immediately after opening to avoid the chances of contamination. However, due to their alkalinity antacids are highly prone to contamination. According to international regulation, bacterial count in liquids should not exceed 100 bacteria per ml and also that packaging area should have air handling unit (AHU). To determine practical importance of AHU, standard antacids were prepared in AHU, inside laminar air flow (LAF) system and in the general microbiology laboratory (ML) fitted with air conditioners. Samples were kept in coloured polyethylene terepthalate bottles and bacterial count was evaluated following standard procedures. Bacterial counts were determined simultaneously from two common antacids available in the market. Although number of bacteria varied from 2.33 to 4.66 in 10^{-1} dilution, the number was 0.33 in 10^{-2} dilution of antacids prepared in AHU and LAF and the number was 1 in the ML, proving thereby the expertise of the technical persons responsible for preparing the antacids. Isolated bacteria were identified as *Staphylococcus* spp. in all preparations, while *Bacillus* spp. could be detected in the antacids prepared in LAF and ML. An almost identical finding was observed in antacids purchased from the market. Thus this study very clearly proves that the use of AHU may not be made an absolute mandatory for small industries. They may be allowed and advised to manufacture their oral liquid formulations in properly maintained LAF by expert pharmacists.

KEYWORDS: Antacid suspension, liquid formulation, Air handling unit, Laminar air flow, Bacterial count.

INTRODUCTION

Among all pharmaceutical formulations oral liquid preparations are highly liable to be contaminated by air borne bacteria unless they are closed tightly and carefully immediately after opening. Such contaminations may lead to formation of toxic substances leading to degradation of potency of the medicine. According to the guideline of World Health Organization (WHO) the permissible number of microorganisms in an oral liquid preparation is 100 colony forming units (CFU) per ml. Additionally according to schedule M of GMP guidelines the primary packaging should be carried out in an Air Handling Limit (AHU).

It is known that oral liquid preparations possess a greater chance of microbial contamination than other liquid formulations in an industry. There is every possibility that treatment failure in complicated cases could well be associated with such contaminated oral drugs (Kallings et al 1996). Therefore, the acceptable limit of $<10^2$ CFU/ml should be maintained in all liquid formulations. However, under certain conditions the limit may be exceeded, resulting in a much bigger threat to public health measures (United States Pharmacopoeia or USP, 2003).

In a total of 40 different types of oral liquid drugs manufactured by various pharmaceutical industries in Bangladesh. Khanom et al (2013) found that only except one syrup all the samples were contaminated with 10^3/ ml microorganisms. Furthermore, in 4 syrup samples the number exceeded the USP limit. However, the primary isolates were *Staphylococcus* spp. and *Bacillus* spp. while common Gram negative bacteria like *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Pseudomonas* spp. failed to grow from any bottle. Sudeshika et al (2014) evaluated microbiological quality of paediatric oral liquid drug formulations during consumption. The common drugs included paracetamol, salbutamol, cephalexin, amoxycillin and lactulose. They observed that the number of bacteria (CFU/ml) was either nil or very low on the day bottles of paracetamol and lactulose syrups were opened. In subsequent days the

number increased substantially. However, bottles of salbutamol, amoxycillin and cephalexin revealed remarkably low or no bacterium after opening for consecutive three days. Therefore the authors opined that extra care should be taken while opening paediatric oral liquid drugs. Contamination of non- sterile pharmaceutical liquid preparations by various microbes has been a major concern in public health and drug efficacy (Baird & Petris 1981; Mugoyela et al 2010). Routine study on the microbial load in syrups and suspensions is required for safety of consumers. Several such non-acid liquid drugs contain organic constituents including sugar leading to support of growth and proliferation of microorganisms (Hossain et al 2004). However, a liquid antacid formulation has probably the maximum chances of contamination since they are not sterilized during their manufacturing processes (Coker 2005, Bajaj et al 2012). Contamination by fungi, bacteria and viruses may be introduced from raw materials and water used during manufacturing. Implementation of Air Handling Unit (AHU) is now a regulatory mandate for all pharmaceutical industries, although its maintenance is both cost and labour intensive for small and even medium size industries.

Oral liquid formulations are highly prone to invasion by microorganisms. Among oral liquid formulations antacids suspensions are one of the extreme cases since their susceptibility to bacteria and fungi is greater due to their alkalinity and basic pH (Udeze et al 2012; Urmi et al 2014). This study was designed to find out the possibilities of an alternative safe method of formulating oral liquid antacid suspensions without the guidance of AHU, as every small pharmaceutical company may not be in a position to purchase and maintain an AHU system right from the beginning. Moreover, it is sometimes found that bottles containing some oral formulations tend to bloat and then burst on storage after five to six months. In this study ten antacid bottles were left as such in the Microbiology laboratory for full one year to determine if any of the bottles burst out.

MATERIALS AND METHODS

Chemicals

Dried aluminium hydroxide, magnesium hydroxide, simethicone and menthol were the primary ingredients; the base used was sorbitol while sodium benzoate, sodium nipagin and sodium nipasol were used as preservatives. The anticaking agent was sodium citrate and suspending agent was sodium CMC while anisee oil was added for flavor. All the chemicals were obtained from various bulk drug manufactures in India; all these were certified as per Indian Pharmacopoeia norms by the respective manufacturers. The chemicals were dissolved in sterile distilled water.

Media

Solid media used were nutrient agar (NA; Oxoid) and Mueller Hinton Agar (MHA; Oxoid).

Defined areas of preparation of antacids

Inside Air Handling Unit (AHU), inside horizontal Laminar Airflow (LAF) bench (Klenz Flo) , inside Microbiology Laboratory (ML) fitted with Air-conditioners.

Formulation of antacids

Antacids were prepared in triplicates with the above chemicals following standard protocols separately and independently in the defined areas of the institution as mentioned above in 100 ml amounts. Magnetic stirrer used for stirring the suspension was procure for Remi (Thane, Maharashtra). These were kept in amber coloured sterile polyethylene terepthalate (PET) bottles. The bottles were screw capped soon after completion taking all precautions to avoid contamination (Table 1). In this suspension the anticaking agent was sodium citrate and the suspending agent was sodium nipagin. Amisee oil gave a nice flavor to the suspension.

Table 1. Composition of antacid suspension.

Chemical Compounds (IP)	Amounts
Dried Aluminium hydroxide	6 gm
Magnesium hydroxide	4 gm
Simethicone	500 mg
Sorbitol	20 gm
Sodium benzoate	200 mg
Sodium nipagin	100 mg
Sodium nipasol	20 mg
Sodium citrate	200 mg
Menthol	25 mg
Sodium CMC	1.1 gm
Aniseed oil	30 mg

The above chemical compounds were suspended in 100 ml of sterile distilled water contained in a sterile bottle.

Determination of total number of viable bacteria in antacids

Every antacid preparation was left on the working table for 10 days and then tested for presence of contaminating bacteria along with standard antacid preparations (Polycrol and Gelusil) purchased from a standard pharmacy shop. These are claimed to have been prepared under standard AHU.

From each bottle 1.0 ml was withdrawn with the help of a sterile pipette and added to 9ml of molten NA/MHA, mixed and poured in a 45 mm diameter sterile Petri dish, resulting dilution being 10^{-1}. In the same manner 0.1 ml was withdrawn and mixed with 9.9 ml of molten media and poured into sterile Petri dishes resulting in 10^{-2} dilution of the prepared antacid. As the media solidified the Petri dishes were inverted and kept in an incubator at 37^0C upto 100 hr. The process was repeated three times in both NA and MHA. The plates were observed after 18 hr for appearance of bacterial colonies and then every day upto 100 hr. Number of colonies appearing on the agar media were counted and mean value was calculated.

RESULTS

Enumeration of number of bacteria in antacids

The NA media inoculated with antacid suspensions prepared in three different locations of the microbiology department revealed the colonies on the surface of media (Table 2). The number of colonies started appearing after 18h. The result was finally recorded and tabulated after 100h. Mean values from 10^{-1} dilution were 2.33. 3.0 and 4.66 recovered from AHU, LAF and ML respectively. However, with 10^{-2} dilution the number of colonies reduced drastically. The mean value was 0.33 in 10^{-2} dilution of the suspensions prepared in AHU and LAF. However, the same value was 1.0 from the antacid prepared in the ML (Table 2). Very similar results were seen when the tests were performed in MHA.

Table 2. Microbial load of antacids prepared in three specific areas.

Specific area	Growth on NA inoculated from antacids prepared in different areas in the department							
	No. of colonies after 100hr on NA in two dilutions							
	10^{-1}				10^{-2}			
	Replicate 1	Replicate 2	Replicate 3	Mean value	Replicate 1	Replicate 2	Replicate 3	Mean value
AHU	2	1	4	2.33	0	1	0	0.33
LAF	6	3	0	3	1	0	0	0.33
ML	3	7	4	4.66	1	2	0	1

Identification of bacteria present in 10^{-1} dilution of antacid suspensions

Staphylococcus aureus could be detected in 10^{-1} dilution of all the antacids prepared in AHU, LAF and ML while *Bacillus* spp was found to be present in the suspensions prepared in LAF and ML. However, *Escherichia coli, Salmonella* spp, *Shigella* spp, *Vibrio cholerae and Pseudomonas aeruginosa* were definitely absent in all the antacid suspensions (Table 3).

Table 3. Detection of Gram positive and Gram negative organisms in antacids prepared in three areas.

Specific location	Total viable bacteria in 10^{-1} dilution	Bacteria found in 10^{-1} dilution of antacids prepared in different locations						
		Bacillus spp.	*Staphylococcus* spp	*E.coli*	*Salmonella* spp	*Shigella* spp	*Vibrio cholerae*	*Pseudomonas* spp
AHU	2.33	-	+	-	-	-	-	-
LAF	3	+	+	-	-	-	-	-
ML	4.66	+	+	-	-	-	-	-

AHU, Air handling unit; LAF, Laminar air flow; ML, Microbiology laboratory.

Presence of bacteria in Polycrol and Gelusil and their identification

The mean value of member of bacteria 10^{-1} dilution of polycrol in NA was 3.0 while it was 2.66 in gelusil. The only organism found in both the preparations was *S. aureus*. No Gram negative bacterium was detected from these samples, so was absent *Bacillus* spp. (Table 4). Almost identical results were observed in MHA plates.

Table 4. Determination of bacterial counts and specific organisms in 10^{-1} dilution of marketed antacids.

Antacid	Total viable bacteria in antacids purchased from market										
	No. of colonies on NA in 10^{-1} dilution				Bacteria detected						
	Replicate 1	Replicate 2	Replicate 3	Mean value	*Bacillus* spp.	*Staphylococcus* spp	*E.coli*	*Salmonella* spp	*Shigella* spp	*Vibrio cholerae*	*Pseudomonas* spp
Polycrol	4	2	3	3	-	+	-	-	-	-	-
Gelusil	2	2	4	2.66	-	+	-	-	-	-	-

DISCUSSION

Non- sterile pharmaceutical liquid products possess a much greater chance of contamination by both bacteria and fungi. Assessment of the number of bacteria and fungi present within various oral drugs like suspensions and syrups is required to ensure consumer safety before they are sent to market from the industries. Since such preparations often contain organic compounds and sweetening substances these are prone to support growth and proliferation of unwanted microorganisms (Hugo and Russel, 2007).

Antacid suspension commonly contain aluminium hydroxide, magnesium hydroxide and simethicone or magaldrate preparation as basic ingredients which may lead to microbiological spoilage due to low-cost production procedures as well as improper storage and distribution systems (Hossain et al, 2004). In this study aluminium hydroxide, magnesium hydroxide and simethicone have all been used in the preparation of the antacid in different locations in the microbiology department. However, necessary precautions were definitely taken every time to make sure that the prepared suspension is devoid of any contamination. Many pharmaceutical industries, big or small, often come across with complaints of adverse effects in oral drug consumption. The in-process quality control and the microbiological regulation of raw materials and finished products require routine monitoring to control bacterial and fungal contamination in the finished products in the industries. Such precautions would definitely help to reduce public health risks.

It is known that liquid preparations are less transportable and less stable. Careful attention is required to assume that the pharmaceutical product would not allow a microbial burden to develop on standing or under normal conditions of use once opened. It should be mentioned on the label of the bottle that special care must be taken while opening the bottle for consumption. It may be pointed out here that the antacid suspensions prepared in this study contained three standard antimicrobial preservatives for prevention of microbial contamination, namely sodium benzoate, sodium nipagin and sodium nipasol.

The present study evaluates the microbiological quality of antacid suspensions prepared in three different areas of the department. It has been observed that apparently there was no difference in the microbiological findings of the antacids prepared in AHU or LAF. The number of viable bacteria in either of the locations was 0.33 x 10^2 = 33 per ml. Although the number of bacteria in the antacid prepared in ML was higher, it was within permissible limits of the Indian/ British/ United States pharmacopoeias and therefore totally safe for human consumption. In this connection it is necessary to mention that none of the bottles showed any sign of bloating during the entire period of storage for one year. This again proves that careful and proper handling of oral liquid formulations is essential for proper maintenance of such pharmaceutical products.

According to schedule M (GMP guideline), the primary liquid packing area should be equipped with AHU. For oral liquid formulations, U.S. Federal Standard 209F has specified that the number of 10,000 with a size of 0.5 microns or larger (in the area where the particle count must not exceed a total of 10,000 particles per cubic foot or 353,000 particles per cubic meter) or 70 particles per cubic foot (2,470 particles per cubic meter) with a size 5.0 microns and larger should be maintained [https://set3.com/papers/209e.pdf]. However, implementation of AHU is both cost and labor intensive for small and medium scale industries, which nonetheless, has become a regulatory mandate for all pharmaceutical industries. In this study it has been repeatedly determined that the number of viable bacteria never exceeded limits as mentioned in the guidelines of GMP. It may therefore be concluded that implementation of such guidelines may not be made an absolute necessity for very small scale pharmaceutical industries trying to survive by manufacturing oral liquids only. Such industries may be strictly advised to follow the minimum requirements of properly maintaining a suitable laminar airflow system and sterility measures as described in this study. That would possibly help them to continue with production of oral liquids in their industry till they are able to get their own AHU for their own establishment.

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