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ORIGINAL RESEARCH ARTICLE

Assessment of Microbial Qualities of Some Cough Syrups and Multivitamins Marketed in Pokhara, Nepal

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ABSTRACT

Recently, the manufacturers of pharmaceuticals have improved the quality of non-sterile pharmaceuticals in such a way that such products contain only minimal bioburden. However, the production of sub-standard cough syrups and multivitamin syrups may cause non-therapeutic effect in patients, particularly in children. For this reason, this study was conducted to evaluate the microbiological quality of cough syrup and multivitamin syrups marketed in Pokhara, Nepal. Different brands of 15 cough syrups and 15 multivitamin syrups were collected from different vendors of Pokhara and the spread plate technique was performed to enumerate the microbial contaminant from the collected samples. Among 15 cough syrups, 12 samples were found to be contaminated with bacteria and nine samples were found to be contaminated with fungi. Similarly, among 15 multivitamin syrups, 10 were found to be contaminated with bacteria whereas 12 were found to be contaminated with fungi. Escherichia coli was not isolated in any samples. Overall, 14(93.33%) of cough syrup and 13(86.67%) of multivitamin syrups were found to be contaminated by either bacteria, fungi, or by both which exceeded the acceptance limit of International Pharmacopeia. The prevalence of these microorganisms in pharmaceutical products such as syrups samples may indicate the unhygienic condition, defect in production, poor adoption of Good Manufacturing Practice, ineffective preservatives and inadequate quality control. Though these products fall under non-sterile pharmaceutical products, so they need not require sterility but these drugs must conform to the microbiological purity criteria set in the appropriate pharmacopeial standard. These contaminated syrups explain the poor treatment and complicacy of the uncompromised people and the sick children.

KEYWORDS: Cough syrups, microbiological qualities, multivitamin syrups, parmacopeial standard

INTRODUCTION

Pharmaceuticals are used for the purpose of prevention, treatment and diagnosis of diseases (Denyer et al., 2004). In microbiological terms, pharmaceutical products can be divided into two groups; sterile and non-sterile. Non-sterile pharmaceutical products are not required to be sterile, but are subject to certain restrictions on the number and types of acceptable microorganisms to make sure their efficaciousness and safety (Jimenez, 2004).

Syrups are the non-sterile liquid dosage form that contain active medicaments and constitute the most convenient dosage form for babies, children and the elderly. They are generally prepared for oral administration in children since tablets and capsules cannot be easily or suitably administered to them. The administration of contaminated syrups to these people pose a real danger, even at low levels of contamination because their immune system is poorly developed (Mendie et al., 1993; Muhammed & Umoh, 2009).

Vitamins are a group of organic nutrients required in small quantities for a variety of biochemical functions that, generally, cannot be synthesized by the body and must therefore be supplied in the diet. The member of vitamin B complex plays an important role in various metabolic activities and their deficiency results in various symptoms in human body. Vitamin B syrups are an aqueous preparation for the oral use and usually marketed in the non-sterile dosage form (Bender, 2009).

The contamination of oral liquid pharmaceuticals with microorganisms not only makes them hazardous from the infectious standpoint, but may also change the physical, chemical and organoleptic properties of the drugs, alter the contents of active ingredients, or convert them to toxic products that lead to reduction in their shelf life and efficacy (Shaikh et al., 1988; Moniruzzaman et al., 2012). The microbiological quality of a pharmaceutical product may represent the contamination from raw materials, industrial equipments, environment, workers and containers (Muhammed & Umoh, 2009).

In Pokhara, cough syrups and multivitamin syrups are among the most available syrups in the market, which suggests strong likelihood of variation at the level of microbiological purity among these brands. So it is necessary to carry out investigations to ascertain the microbial qualities of these preparations, which are currently in the market.

MATERIALS AND METHODS

Sample Collection

A total of 30 samples (15 cough syrups and 15 multivitamin syrups from different manufacturers) were collected from different vendors of Pokhara valley. Each sample was examined for expiry dates, labelled properly and transported to microbiology laboratory of Prithvi Narayan Campus for further analysis.

Physical Properties Examination

The appearance was assessed by visual examination to determine the color. The taste was assessed by using the appropriate relevant sense organs. The pH value was measured by pH meter.

Assessment of Microbiological Qualities

Total Aerobic Microbial Count (TAMC)

One mililitre from each sample was withdrawn aseptically and transferred into 9 ml normal saline for serial dilution to 10^5. Diluted samples were thoroughly mixed for proper dissolution of the drug. 0.1ml of each samples (from 10^{-2} and 10^{-4}) were spread

aseptically onto nutrient agar and MacConkey agar for the isolation of total viable bacteria and *Escherichia coli*. Inoculated plates were then incubated for 24 hours at 37°c. Bacterial colonies were counted by colony counter and average number of colony forming unit (cfu) was determined for each ml of syrup sample.

Total Mold and Yeast Count (TYMC)

The mold and yeast count was performed by plating 0.1ml of dilution on Potato Dextrose agar. Plates were then incubated at 25°c for 5-7 days. Fungal colonies were counted by colony counter and average number of cfu was determined for each ml of syrup sample.

Identification of E coli

The characteristic colonies grown on MacConkey agar plates were isolated and purified. They were then characterized on the basis of morphological, biochemical and cultural characteristics (Harley & Prescott 2002).

Data Analysis

Data generated from the study was tabulated as Microsoft Excel sheet and statistical analysis was performed.

RESULTS

The bacterial contamination of syrup and suspension can cause the spoilage of the products and lead to serious clinical hazards particularly in children and elderly people (Takon & Antai, 2006). A presence of microbial contaminants becomes the major health concern when their number exceeds the acceptable limit recommended by the standards. The following are the results obtained from the laboratory as compared with the microbiological assay:

Table 1

Distribution of Bacterial and Fungal Isolated from Cough Syrups and Multivitamins

Sample categories	**Number of contaminated dosages from with**					
	Bacteria				**Fungi**	
	Gram positive rods	Gram positive cocci	Gram negative rods	Gram negative cocci	Yeast	Mold
Cough syrup	0	14	1	0	0	9
Multivitamin syrup	2	12	1	0	0	12
Total	2	26	2	0	0	21

Table 2

Comparison of Microbial Load of Cough Syrup with Standard

Total aerobic microbial count (TAMC) No. of samples with		**Total mold and yeast count (TYMC) No. of samples with**		***E coli***	**Recommended acceptance criteria for microbiological quality of non-sterile dosage forms Acceptable limit cfu/ml (IP 2019)**		
TAMC value within the acceptable limit	TAMC value beyond acceptable limit	TYMC value within acceptable limit	TYMC value beyond acceptable limit				
3	12	6	9	Nil	Absence of *E coli* (1 g or 1ml)	$<10^2$	$<10^1$
Total=15		Total=15					

Table 3

Comparison of Microbial Load of Multivitamin Syrup with Standard

Total aerobic microbial count (TAMC)		**Total mold and yeast count (TYMC)**		***E coli***	**Recommended acceptance criteria for microbiological quality of non-sterile dosage forms**		
No of samples with		**No of samples with**			**Acceptable limit cfu/ml (IP 2019)**		
TAMC value within acceptable limit	TAMC value beyond acceptable limit	TYMC value within acceptable limit	TYMC value beyond acceptable limit		Specified microorganism	Bacteria	Fungi
5	10	3	12	Nil	Absence of *E coli* (1 g or 1ml)	$<10^2$	$<10^1$
Total=15		Total=15					

Table 4

Pattern of Contamination in Cough Syrup and Multivitamin Syrups

SN	**Category**	**No of samples**	**Number of presence of growth**			**Number of absence of growth**	**% Growth**
			Bacterial	Fungal	Both		
1	Multivitamin syrup	15	1	3	9	2	86.67
2	Cough syrup	15	5	2	7	1	93.33
	Total		6	5	10	3	

DISCUSSION

In this study, the results showed that the samples tested had unsatisfactory total microbial count levels compared to the International Pharmacopoeia IP (2019) specification. Overall 14(93.3%) of cough syrups and 13(86.67%) of multivitamin syrups were found to be contaminated by either bacteria, fungi or by both. This level of contamination rate is similar to the finding of Mugoyela and Mwambete (2010) who showed 100% contamination in some selected cough syrup samples. However, these values of contamination are higher than the findings of Kabir and Hossain (2013) and Jameel (2017). In the case of multivitamin syrups, 86.67% were found to be microbially contaminated. There has been observed variation in the contamination rates of multivitamin syrups (Shaikh, 1998; Daniyan, & Sangodere 201; Kabir & Hossain 2013; Tukur et al., 2012).

A higher contamination rate might be due to defect in production, poor adoption of Good Manufacturing Practice, ineffective preservatives and inadequate quality control (Al-Kaf et al., 2015). In addition, loosely cocked and not firmly closed lids can serve as a source of contamination. The microbial contamination also depends upon the storage temperature as shown by Nwachuku and Olugbose (2009) who showed that the total viable count of bacteria and fungi increases in ambient temperature as compared to refrigeration temperature.

Similarly, the multivitamin syrup was found to be less contaminated than the cough syrup samples. This difference can be attributed to the presence of antioxidants and minerals present in multivitamin syrups (Chugh & Lhamo, 2012). Though the higher percentage of contamination were observed in our findings, these products fall under non-sterile pharmaceutical products, so they need not require sterility, but these drugs must conform to the microbiological purity criteria set in an appropriate pharmacoopeial standard (Ratajczak et al., 2015).

In our study, overall 12(80%) and 9(60%) of cough syrup exceeded the bacterial and fungal acceptable limit of International Pharmacopeia. Similarly, overall 10(66.6%) and 12(80%) of multivitamin syrup exceeded the bacterial and fungal acceptable limit. These findings are in accordance to Mendie et al. (1993) and Khanom et al. (2013). Mugoyela and Mwamb (2010) have found heavy microbial contamination of 50% in non-sterile pharmaceutical products. Raw materials, ingredients, unhygienic environmental condition and lack of aseptic handling would be the main factors for the observed microbial growth in the sample studied (Parker, 2000; Gad et al., 2010).

Though sterility is not requirement in the official compendia for non-sterile pharmaceutical products, the microbial load of such products should be within the acceptable limit (Mugoyela & Mwamb, 2010). This is because of the consumption of those contaminated syrups by children or infants and critically ill who are highly prone to infection may present a potential hazard (Nwachuku & Olugbose, 2009).

The absence of indicator organism like *E coli* is an absolute requirement (IP, 2019). This clarifies that water used by pharmaceutical industries may not be contaminated by coliform bacteria. The organisms of these types are water–borne and frequently contaminate liquid pharmaceutical product (Denyer et al., 2004). Our findings of absence of *E coli* are in accordance to Jameel (2017) and Nwachuku and Olugbose (2009).

Although the specific gram negative enteric bacteria were not found in the tested samples, the presence of viable bacteria especially gram positive ones along with fungi claims a sort of health risk associated with the consumption of those drugs (Khanom et al., 2013). Although the organisms that are detected in the syrup samples are not pathogenic, they are "objectionable" since they can bring about the destruction of active ingredients. Thus, they may interfere the function of the therapeutic product (Shaikh et al., 1988).

CONCLUSION

To conclude, this study has revealed the heavy microbial contamination in 93.33% of cough syrups and 86.67% of multivitamin syrups. Since a higher number of cough syrup and multivitamins exceeded the bacterial and fungal acceptable limit of International Pharmacopeia, the manufacturers should be stringent in terms of the product manufacturing, packaging and distribution of such medications. It is also important that the consumers should be aware of proper handling and storage of oral suspensions.

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