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Determination of Microbial Load in Multivitamin and Cough Syrups Sold in Dhaka City

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ABSTRACT

The production of substandard drugs and use of inappropriate methods in manufacturing medical products in pharmaceutical industries may cause non-therapeutic effect in patients particularly in children. Hence this study was conducted to determine the microbiological quality of multivitamin and cough syrups of different brands sold in local pharmacy in Dhaka city. Microbiological analysis was carried out using spread plate technique on different culture media including nutrient agar, mannitol salt agar, MacConkey agar, mFC agar, TCBS agar and SS agar for the determination of total viable bacteria, *Staphylococcus aureus*, *Escherichia coli*, total coliforms, *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. respectively from eleven multivitamin and twelve cough syrups. The results revealed that 50% of the cough syrup and 91% of the multivitamin syrup showed compliance with the official requirement of microbiological quality as they did not show any growth or their microbiological count is within the USP permissible limit ($<10^2$ cfu/ml). While the major contaminants in cough syrup were *Staphylococcus aureus* (75%), *Escherichia coli* (17%) and total coliforms (42%), multivitamin syrup (9%) contained only *Staphylococcus aureus*. Other pathogens like *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. could not be detected in both cough and multivitamin syrups. Although most of the multivitamin syrup (91%) samples are free from potential microbial threat, some of the cough syrup (75%) samples are contaminated with pathogenic bacteria. Hence potential safety measurement should be taken during the production and use of cough syrups to maintain the microbiological quality.

Keywords: Syrup, microbiological quality, contamination, pathogenic bacteria, pharmaceuticals.

INTRODUCTION

Pharmaceutical products are commonly used in many different ways in the prevention, treatment and diagnosis of diseases. In recent years, quality of the non-sterile products has been improved by many manufacturers to minimize the bio-burden. [1] There are several reports concerning the microbiological contamination of the pharmaceuticals products like syrup, suspension, oral pediatric drugs etc. have been published. [2-4] A number of factors including availability of nutrients, oxygen and presence of microorganisms are responsible for spoilage of any pharmaceutical product. The outcome of the consumption of contaminated medicament will depend on the type and degree of microbial contamination, the extent of deterioration, route of entry and also on the patient's immune status. [5] The presence of microbial flora, whether pathogenic or non-pathogenic, in the pharmaceutical products forces the consumers to lose faith in the manufacturing company; that also may cause the considerable financial defeats of the manufacturers due to microbes mediated changes in the stability of the pharmaceuticals. [6]

Syrups, most convenient dosage form for babies, children and the elderly, are non-sterile liquid dosage forms that contain active medicaments. Since tablets and capsules cannot be taken conveniently by children, syrups are generally prepared as alternative dosage forms for oral administration in children. [7] Bacterial contamination of medicaments like syrup and suspension can cause the spoilage of the products and lead to serious clinical hazards particularly in children and elderly people. Metabolic versatility of bacteria helps them to utilize and transform variety of ingredients of the formulation of the pharmaceutical products. [8] Therefore it is essential to determine the microbial load of all pharmaceutical products whether sterile or non-sterile to ensure good quality of the product. [9] Good Manufacturing Practice (GMP) is necessary to ensure that products are consistently manufactured to a quality appropriate for its intended use particularly in the production of non-sterile preparations like syrups which are especially meant for children. To decrease the microbial load

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and to ensure the quality of these products it is necessary to follow the rules of GMP. Microbial load can be monitored starting from the raw materials to the finished products to determine the sources of contamination. Patients being treated with contaminated syrups can cause secondary infections from the pathogenic microorganisms and may complicate the treatment procedure. [10] This study therefore is aimed to determine the microbial load and presence of common pathogenic bacteria in cough and multivitamins syrups manufactured by different pharmaceuticals in Dhaka city.

MATERIALS AND METHODS

Sample Collection

Twelve different brands of cough syrups labelled C1-C12 and eleven different brands of multivitamins labelled M1-M11 were purchased from the local pharmacy stores around Dhaka, Bangladesh between September 2013 and March 2014.

Enumeration technique

Spread plate technique was used to enumerate the microbial contaminant from the collected syrup samples. One milliliter from each sample was withdrawn aseptically and transferred into 9ml normal saline for serial dilution to 10^{-3}. Diluted samples were thoroughly mixed for the proper dissolution of the drug. 0.1ml of each sample was then inoculated into different culture media plates by spread plate technique

Quantitation of total heterotrophic bacteria, *Staphylococcus aureus, Escherichia coli* and total coliform

0.1 ml of the each diluted sample was spread aseptically onto nutrient agar, mannitol salt agar (MSA) MacConkey agar and mFC agar media plates (Himedia Laboratories Ltd., India) for the enumeration of total viable bacteria, *Staphylococcus aureus*, *Escherichia coli*, and total coliforms, respectively. Inoculated plates were then incubated for 24 hours at 37^0C. Bacterial colonies were counted manually and average number of colony forming unit (cfu) was determined for each ml of the syrup sample.

Determination of *Vibrio cholerae, Salmonella* spp. and *Shigella* spp.

1 ml sample was added to 9ml of Alkaline peptone water and incubated for 4-6 hrs at 37°C. 1-3 loop full sample was streaked on Thiosulfate-citrate-bile salts-sucrose agar (TCBS agar) plates and incubated at 37°C for 18-24 hrs. Sucrose fermenting colonies were further identified using standard biochemical tests. 1 ml sample was added to 9 ml of Selenite Cysteine Broth and incubated for 4-6 hrs at 37°C. Enriched sample was streaked on *Salmonella Shigella* Agar (SS agar) plates and incubated at 37°C for 18-24 hrs.

Identification of the isolated microorganisms

Cultural and biochemical tests based identification of the isolated microorganism was done following standard protocol. [11]

RESULTS

Table 2 and 3 shows the results of the total viable count of bacteria in the tested syrup samples. The bacterial count of the tested cough syrups ranged from 0 - 4.8×10^5 cfu/ml while the count for vitamin syrups ranged from 0 - 2.1×10^3 cfu/ml. The presence of total viable count in seven (58.33%) out of twelve cough syrup samples exceeded the USP permissible limit ($<10^2$ cfu/ml) for non-sterile pharmaceutical preparations. On the other hand, one out of eleven multivitamin syrups (9%) exceeded this limit. Total viable count in one of the cough syrups (C10) was within the acceptable limit as mentioned in the USP and rest of the cough syrups (42%) and multivitamin syrups (91%) did not show any growth of bacteria.

Distribution of different pathogenic bacteria in the syrup samples has been shown in Table 2 and 3. Nine (75.00%) out of twelve cough syrup samples were found contaminated with *Staphylococcus aureus* while two (16.66%) cough syrup samples were contaminated with *Escherichia coli* bacteria. Total coliforms were found in five (41.66%) cough syrup samples. *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. were absent in all cough syrups as well as in all multivitamin syrups. None of the multivitamin syrups was found to contain any member of the total coliforms and *E. coli* as contaminants. One (9.09%) of eleven multivitamin syrup samples was found positive for the growth of *Staphylococcus aureus.*

DISCUSSION

Contamination of pharmaceutical products can occur at any stage of production, processing, marketing and administration. Administration of contaminated pharmaceutical products can be harmful to the recipients such as, young and elderly patients. Survival and growth of microorganisms can deteriorate the product quality and production of metabolites/toxins may be harmful to the patient even they are present in minute quantities**.** [12] Presence of such microbial contaminants become major health concern when their number exceeds the acceptable limit (10^2 cfu/ml) recommended by the USP. [13]

Table 1: Summary of descriptive characteristics of the drugs collected

Sample Code	Active Compound	Manufacture Date	Expired Date
	Cough Syrup		
C1	Dextromethorphan	Jun 13	Feb15
C2	Dextromethorphan	Apr 13	Apr 15
C3	Ambroxol HCL	Jan 13	Jan 15
C4	Ambroxol HCL	Jan 13	Jan 15
C5	Ambroxol HCL	Jan 13	Jan 15
C6	Ambroxol HCL	Apr 13	Apr 15
C7	Dextromethorphan	Apr 13	Apr 15
C8	Dextromethorphan	Jan 13	Jan 15
C9	Paracetamol BP	Apr 13	Apr 15
C10	Paracetamol BP	Apr 13	Apr 15
C11	Ambroxol HCL	Jan 13	Jan 15
C12	Ambroxol HCL	Jan 13	Jan 15
	Multivitamin Syrup		
M1	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Oct 15
M2	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Oct 15
M3	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M4	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M5	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M6	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M7	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M8	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Dec 15
M9	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M10	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15
M11	Vitamin A, D, C, B_1, B_2, B_6, E & Cod liver oil	Nov 13	Nov 15

Table 2: Distribution of the bacterial contaminants in cough syrup samples

Sample Code	Total bacterial count (cfu/ml)	*Staphylococcus aureus* (cfu/ml)	*Escherichia coli* (cfu/ml)	Total coliforms (cfu/ml)	Present/Absent		
					Vibrio cholerae	*Salmonella* spp.	*Shigella* spp.
C1	3.6×10^5	2.6×10^5	Nil	4.7×10^3	-	-	-
C2	Nil	1×10^2	Nil	2×10^2	-	-	-
C3	Nil	1.51×10^5	Nil	Nil	-	-	-
C4	Nil	Nil	Nil	Nil	-	-	-
C5	6.2×10^3	1.5×10^4	2×10^2	4.4×10^4	-	-	-
C6	7.4×10^4	2.6×10^3	1×10^2	3.8×10^4	-	-	-
C7	Nil	Nil	Nil	Nil	-	-	-
C8	6.7×10^3	2.4×10^3	Nil	1.7×10^3	-	-	-
C9	4.5×10^5	1×10^2	Nil	Nil	-	-	-
C10	1×10^2	1×10^2	Nil	Nil	-	-	-
C11	Nil	Nil	Nil	Nil	-	-	-
C12	4.8×10^5	2.9×10^4	Nil	Nil	-	-	-

Legend: +=Present; -=Absent; Acceptable limit of total bacterial count $\leq10^2$ cfu/ml (USP)

Table 3: Distribution of the bacterial contaminants in multivitamin syrup samples

Sample Code	Total bacterial count (cfu/ml)	*Staphylococcus aureus* (cfu/ml)	*Escherichia coli* (cfu/ml)	Total coliforms (cfu/ml)	Present/Absent		
					Vibrio cholera	*Salmonella* spp.	*Shigella* spp.
M1	$2.1\text{x}10^3$	4×10^2	Nil	Nil	-	-	-
M2	Nil	Nil	Nil	Nil	-	-	-
M3	Nil	Nil	Nil	Nil	-	-	-
M4	Nil	Nil	Nil	Nil	-	-	-
M5	Nil	Nil	Nil	Nil	-	-	-
M6	Nil	Nil	Nil	Nil	-	-	-
M7	Nil	Nil	Nil	Nil	-	-	-
M8	Nil	Nil	Nil	Nil	-	-	-
M9	Nil	Nil	Nil	Nil	-	-	-
M10	Nil	Nil	Nil	Nil	-	-	-
M11	Nil	Nil	Nil	Nil	-	-	-

Legend: +=Present; -=Absent; Acceptable limit of total bacterial count $\leq10^2$ cfu/ml (USP)

However**, v**ery few studies were carried out on the microbiological quality of the pharmaceutical products like oral suspensions in Bangladesh. [14-15] This has encouraged us to carry out this research to assess the microbiological quality of non-sterile pharmaceutical products like cough and multivitamin syrups locally available in Dhaka city.

All the syrup samples used in this study were still within their shelf lives when the analysis was carried out. The study findings revealed that only a few syrup samples were contaminated microbiologically. The isolated aerobic bacteria in cough syrup were *Staphylococcus aureus* and total coliforms. All cough syrup samples were found free from *Vibrio cholerae*, *Salmonella* spp. and *Shigella* spp. Presence of opportunistic pathogens like *Staphylococcus aureus* is significant as they may cause potential deterioration in the health status of patient particularly those are immunologically compromised and infants with immature immune system. [16] Such contamination of the cough syrup may be attributed to poor manufacturing practice, the air in the manufacturing environment, from the raw materials, water used or personnel, packaging process or containers and equipment. [5]

Distribution of bacterial pathogens in multivitamin syrup was very narrow compared to the cough syrup; only 9.09% of the tested multivitamins were contaminated with *Staphylococcus aureus* and none of the other pathogens were found in any multivitamin syrup. Though, high sugar concentration prevents the growth of microorganisms, *Staphylococcus aureus* thrive well in fairly high concentration of sugar. [17] The heat resistance of *Staphylococcus* may contribute to their survival in processed products. [18]

The presence of *E. coli* and total coliforms in some of the cough syrup samples indicated fecal contamination that may be from production personnel through water as vehicle. *Escherichia coli* is an ideal indicator organism to test samples for fecal contamination as they are not always confined to the intestine and able to survive for brief period outside of the body. [19]

The microbiological analysis showed compliance with official requirement of microbiological quality for multivitamins syrup as 90.90% of the samples were found to be free from any potential microbial threat and in case of cough syrups microbial load of the 50.00% samples was within the USP permissible limits for non-sterile pharmaceutical products. Although microbiological quality of non-sterile products particularly multivitamin syrup is satisfactory, manufacturers should be stringent in terms of the product manufacturing, packaging and distribution of cough syrup. It is also important that the consumers are aware of proper handling and storage of oral suspensions.

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