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ORIGINAL ARTICLE

Traceability of Sources of Contaminants of some Preserved Oral Non Sterile Syrups

[1]Rania Abdelmonem Khattab[*], [1]Alaa El-Din Shawky Hosny and [2]Lobna Aref Deif

[1*]Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Kasr Al-Aini, Cairo, Egypt.
[2] Mamphis company for pharmaceutical and chemical industries, El-Sawah, Cairo, Egypt.

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Aspergillus flavus, Oral syrup, GMP, Preservatives, Sources of contamination and pharmaceutical products

ABSTRACT

Background: *Microbial contamination sources in the manufacturing process of some oral pharmaceutical syrups may be a leading cause of diseases in children, elderly and immunosuppressed individuals.* ***Objectives****: Traceability of some sources of contamination in manufacturing process of some oral pharmaceutical syrup.* ***Methodology****: Analysis of 400 oral syrup samples, raw materials, water and air samples using classical pharmacopeia techniques.* ***Results****: In this study, fungal contaminants were more common in pharmaceutical syrup than bacteria and air was an important source of contamination in finished product.*

INTRODUCTION

The control of microbial contamination of pharmaceutical products may be controlled through upgrading of GMP rules as well as preservation [1]. However there have been reports about drug borne human infection worldwide [2]. Moreover contamination of pharmaceuticals can cause changes in their physical characteristics, this include emulsion, thinning of creams, fermentation of syrups, appearance of turbidity or deposit and changes in odor and color [3]. Therefore a preservation may be included to minimize the risk of spoilage or to kill low levels of contaminants in multi dose preparations [4]. Identification as well as quantitation of pathogenic and opportunistic microorganisms in oral pharmaceutical products is recommended especially when the products intended for use by children, elderly and immune suppressed patients [5].

The objective of this study was to evaluate the number and type of microbial contaminants of some oral pharmaceutical products in the egyptian market and to trace the sources of contamination.

METHEDOLOGY

1- Preparation of the raw material sample:

The method for sample preparation depends on the physical characteristics of the material to be tested.

[*]***Corresponding Author:***
Rania Abdelmonem Khattab
Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Kasr Al-Aini 11562, Cairo, Egypt.
E-mail: rania.khatab@pharma.cu.edu.eg; khattab500@yahoo.com;
Tel.: +2 01005183478

a. ***Water-Soluble raw material:*** The raw material to be examined was diluted 1/10 in a soya bean casein digest broth. If necessary, pH was adjusted to 6-8. Further dilutions, when necessary, were prepared with the same diluent.
b. ***Non fatty raw material insoluble in Water:*** The raw material was suspended 1 in 10 in a Soya bean-Casein Digest Broth. A surface-active agent such as 1 g per L of polysorbate 80 may be added to assist the suspension of poorly water soluble substances. If necessary, pH was adjusted to 6-8.Further dilutions, when necessary, were prepared with the same diluent.
c. ***Fatty raw material:*** The raw material was mixed with the minimum necessary quantity of sterile polysorbate 80 or another non inhibitory sterile surface-active reagent, heated, if necessary, to not more than 40°C or, in exceptional cases, to not more than 45°C. Mixed carefully and if necessary the temperature in a water bath was maintained. A sufficient quantity of the pre warmed chosen diluent was added to make a 1 in 10 dilution of the original raw and mixed carefully, while maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial 10-fold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non inhibitory sterile surface-active reagent.

2- Cultivation by pour plate method:

One mL of the prepared sample was added to 15 to 20 mL of Soybean-Casein Digest Agar or Sabouraud Dextrose Agar.

The plates were incubated for the determination of total aerobic microbial count (TAMC) at 32.5°C for 3 days. For the determination of total combined yeast and molds count (TYMC), the plates were incubated at 22.5°C for 5 days. Pour plate Method at least in

duplicate for each medium was used, and the mean count of the colonies was obtained.

The mean count of the organisms was calculated and the number of CFU in the original inoculum was obtained.

3- Syrup sample preparation:

Membrane Filtration: Membrane filters having a nominal pore size not greater than 0.45µ m were used.

A suitable quantity of the prepared sample was transferred immediately, and the membrane filter was rinsed with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), the membrane filter was transferred to the surface of the Soybean-Casein Digest agar and incubated at 32.5°C for 3 days. For the determination of total combined yeast and molds count (TYMC), the membrane was transferred to the surface of the Sabouraud dextrose agar and incubated at 22.5 °C for 5 days.

4- Microbiological air monitoring using agar exposure plates (Passive Monitoring):

Petri dishes (90mm in diameter) containing Trypticase Soya Agar and Sabouraud Dextrose Agar were used. The entire agar surface, was completely exposed for at least 4 hours.

5- Water analysis:

Using membrane filtration, 100 ml of purified water were filtered and the membrane filter was transferred to plate count agar and incubated for 72 hour at 32°C [6].

6- Isolation and identification of microbial isolates:

The cultured plates from air, raw material, water and finished formula were selected and their representative colonies were picked up, and streaked using a sterile loop on Trypticase Soya Agar for bacteria and Sabouraud Dextrose Agar for fungi to obtain pure separate colonies. The plates were incubated aerobically at 32°C for 24 hours for bacteria and at 22°C for 14 days for fungi. The isolated colonies from each plate were spread on a clean slide and examined by the routine bacteriology methods.

RESULTS

A total of 39 fungal isolates were recovered from two pharmaceutical finished preserved products (200 samples for each), while the number of bacterial isolates were 3 organisms.

Air, water and raw material samples were examined for the presence of bacterial and fungal contaminants. The majority of isolates were obtained only from air samples. Recovered isolates from air were 64 fungal isolates and 188 bacterial isolates. The results are shown in tables 1, 2 and 3.

Table 1: Types of contaminants obtained from finished product and different possible contaminating sources.

Source Of isolate	*Types of isolates*	*number*
Air	*Aspergillus flavus*	31
	Aspergillus fumigatus	3
	Aspergillus niger	17
	Penicillium	13
	Gram positive rods	117
	Gram positive cocci	66
	Gram negative rods	5
Stored samples (A) (12 months)	*Aspergillus flavus*	10
	Aspergillus niger	1
	Aspergillus fumigatus	2
	Gram positive cocci	1
Stored samples (B) (12 months)	*Aspergillus flavus*	12
	Aspergillus niger	2
	Penicillium	3
	Gram positive cocci	1
	Gram positive rods	1
Fresh samples (A)	*Aspergillus niger*	1
	Aspergillus flavus	3
Fresh samples (B)	*Penicillium*	3
	Aspergillus niger	2
Water (A)	Gram negative rods	3
Sucrose (A)	Gram positive rods	4

Table 2: Result of raw materials and water samples analysis in product preserved with benzoic acid.

Sample	*Number of samples*	*TBC CFU/gm*	*TYC CFU/gm*
Calcium glubionate	3	0	0
		0	0
		0	0
Calcium lactobionate	3	0	0
		0	0
		0	0
Benzoic acid	3	0	0
		0	0
		0	0
Citric acid	3	0	0
		0	0
		0	0
Sucrose	3	3	0
		1	0
		0	0
Saccharine	3	0	0
		0	0
		0	0
Sorbitol	3	0	0
		0	0
		0	0
Water	3	2CFU/100 ml	0
		1CFU/100 ml	0
		0	0

Table 3: Result of raw materials and water samples analysis in product preserved with sodium benzoate.

Sample	*Number of samples*	*TBC CFU/gm*	*TYC CFU/gm*
Chlorpheniramine maleate	3	0	0
		0	0
		0	0
Sodium benzoate	3	0	0
		0	0
		0	0
Lactic acid	3	0	0
		0	0
		0	0
Sucrose	3	0	0
		0	0
		0	0
Neurily oil	3	0	0
		0	0
		0	0
Flavoring agent	3	0	0
		0	0
		0	0
Water	3	0	0
		0	0
		0	0

DISSCUSION

In developing countries, the possibility of the disease incidence is very high due to the unstable environmental condition, poor hygienic practices, and consumption of contaminated food and water [7]. Smaller numbers of opportunistic pathogens become infectious when resistance mechanisms are impaired, either by severe underlying disease, or by use of immunosuppressive drugs [8,9]. Microbial contamination in non-sterile oral drugs is more important because the patients, who are taking the drug, are already diseased. Therefore, it is very necessary to examine the efficacy and/or potency of some drugs which are commonly used.

In the current study, from 400 tested sample of two types of oral pharmaceutical syrups, 39 fungal isolates and 3 bacterial isolates were recovered and identified microscopically. Isolates from air, raw material and water were obtained.

Most of isolates in finished products were fungi (*Aspergillus flavus*) which may be due to high osmotic pressure of syrup. Microbial count in the pharmaceutical syrup samples, air, water and raw material did not exceed the United State Pharmacopeia limits. For the environmental and syrup samples, air was an important contamination source.

Some of the dosage forms of oral drugs, if stored in unfavorable environment, can serve as substrates for microorganisms [10,11,12,13]. Moisture and high amount of sugar in the oral liquid drugs in particular can support the microbial growth. Oral liquid drug formulations such as aqueous solutions, suspensions, emulsions and syrups are at a greater risk of microbial contamination due to sweetening agents, improper storage and handling defects. Microbial contaminations may ultimately contribute to secondary bacterial infections in patients [14,15,16].

In conclusion, in order to combat the microbial contamination in the oral drugs, proper implementation of good manufacturing practice (GMP) together with the total quality management (TQM) during product manufacturing in a microbiologically controlled environment must be done. Regular quality assessment during storage of the finished products, and appropriate aseptic handling of the drugs would be effectual[17,18,19,20,21].

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