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ORIGINAL ARTICLE

Microbiological Analysis of Marketed Available Cough Syrups in Karachi

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ABSTRACT

During this decade, various pharmaceutical companies have improved and gives the quality assurance of cough syrups in Karachi. The production of substandard cough syrups in pharmaceutical industries may cause non-therapeutic effect in patients particularly in children. For this purpose, this study was conducted to determine the microbiological quality of the cough syrup marketed available in Karachi .Different branded cough syrups were purchased from the local pharmacy stores. Spread plate technique was performed to enumerate the microbial contaminant from the collected syrup samples. Membrane Filtration technique was also performed in which sample was passed through a filter membrane paper and transferred into sabouraud dextrose agar and nutrient agar plates under aseptic conditions. In the result we found that all the samples that we have performed except two syrups were found to be the contaminated with a highly permissible number of CFU/ml. Fortunately, gram negative bacteria were completely absent in all tested samples except in Sancos, while the gram positive bacteria such as *S.aureus* and *Bacillus cereus* were found in both of samples (Sancos and Tixylix). The prevalence of these microorganisms in the pharmaceuticals products such as syrup samples may explain the un-hygiene condition followed by Karachi pharmacies. These contaminated syrups explain the poor treatment & complicacy of the immunocompromised people & sick children. So we can say the people who are consuming these contaminated syrups, they are highly at risk.

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cough syrup, membrane filtration, microbiological quality, pharmaceutical product.	*neelamstar77@gmail.com*	*Received: April 3, 2017* *Accepted: May 29, 2017*

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INTRODUCTION

Different pharmaceutical are available for selling the products of pharmaceuticals that are commonly used in many different purposes for the prevention, treatment and diagnosis of diseases (1). They follow a complex, multi-step processing system in which various risks from physical, chemical and microbial contamination occur from different sources, including raw materials, equipment, personnel, the environment, the facility etc. These contaminants can enter into the products from different several sources such as, poor facility design, personnel, poor hygiene conditions, incoming ventilation air, handling of the handlers, machinery and other equipment or tools for manufacturing and the production of the products (2).

The microbiological contamination in the pharmaceutical products is the common problem which has been reported for several times. It might be a little surprising that the problem of microbial contamination in cough syrups received an important attention in current situations. Bacterial contamination of medications like syrup and

suspension can cause the spoilage of the products and lead to serious clinical hazards particularly in children and elderly people. The presence of such microbial contaminants becomes a major health concern when their number exceeds the acceptable limit (10^2 cfu/ml) recommended by the USP (3). Majority of contaminants of pharmaceutical products are bacteria, yeast and filamentous fungi (mould). Some of these contaminants may be opportunistic and pathogenic while others grow as commensals even in the presence of preservatives (4).

The contamination of pharmaceuticals with microbes may bring changes in physiochemical characteristics of the syrup products , including the fermentation of syrups, breaking of emulsions, trepanning of the bottles, and appearance of turbidity or deposit, besides producing possible off odors and color changes. These changes will not only make the product aesthetically unacceptable, but can also affect the therapeutic effects on the human. 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. (3). Sometimes the presence of a small amount of the microbial doses in pharmaceutical products was completely proved to be potential hazard of the consumer's health (5). Metabolic versatility of bacteria helps them to utilize and transform a variety of ingredients of the formulation of the pharmaceutical products. 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 (5,6).

In recent times or year, the quality of the non-sterile products has been improved by many manufacturers to minimize the bio-burden (1). 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. This study, therefore is aimed to determine the microbial load and presence of common pathogenic bacteria in cough syrups manufactured by different pharmaceuticals in Karachi city.

MATERIALS AND METHODS

Sample collection: A cross sectional study was conducted in which six different brands of cough syrups were purchased from the local pharmacy and medical stores. Each sample labeled as C1-C6. Samples used were Sancos, Tixylix, Britnyl, Hydrillin, Acified and Pulmonol. They all were branded syrups and frequently used in all medical stores. The registration status, shelf life, and manufacturing and expiry date of these syrups were recorded.

Isolation and quantification of microbial contaminants: Standard plate technique and membrane filtration technique were performed for the screening of cough syrups. Each sample has been tested and performed at twice and all the samples were carried out under sterile conditions. Selective and nonselective culture media were used for quantification and isolation of the microbial contaminants.

Determination of Total bacteria, Escherichia coli, Staphylococcus aureus, and total coliform: By performing spread plate technique, 0.1ml of diluted sample was spread aseptically onto nutrient agar, mannitol salt agar (MSA) MacConkey agar and for the isolation of total viable bacteria, *Escherichia coli, Staphylococcus aureus,* and total Coliforms, respectively. Inoculated plates were then incubated for their respective time and temperatures. Bacterial colonies were counted manually and average number of CFU was determined for each ml of the syrup sample.

Identification of isolated microorganisms: The isolated microorganisms were identified and characterized on the basis of morphological, biochemical and cultural characteristics.

RESULTS

The results of our tested samples demonstrated that two samples out of the six cough syrups were contaminated with gram positive bacilli, while all samples were found to be free from *Escherichia coli, Pseudomonas aeruginosa and Salmonell*a fortunately. Summary of descriptive characteristics of the syrup collected is given in Table I. In

our study, we found 33% of the Syrups were contaminated. The load of bacteria in the syrups was 3.5 $<10^5$ CFU/ml which was the unacceptable amount of bacteria in cough syrup samples. *Bacillus* reported in our study to be the most predominant contaminants in pharmaceuticals products. Gram negative pathogens were completely absent from all the tested samples except in Sancos (Table II). Total fungal count and total aerobic count of the syrups was found within the range from $10<10^5$cfu/ml to $3.5<10^5$ cfu/ml. The total fungal load of $4<10^5$ cfu/ml was calculated in Sancos cough syrups that cannot be negligible. Our results showed that 33% of the cough syrups were undesirable because of contamination, and the predominant contaminants comprises of *Bacillus cereus, Klebsiella sp., S.aureus and Aspergillus specie*. Beside this, the results showed that the isolated *S.aureus* was resistant to Penicillin and intermediate to the vancomycin that indicated as VISA.

Table I: Summary of descriptive characteristics of the syrup collected

Sample code	Active compounds	Manufactured date	Expiry dates
C1 SANCOS	PSEUDOPHEDRINE HCL PHOLCHODINE	08-2016	07-2019
C2 PULMONOL	PROMETHAZINE HCL COUGH LICTUS	06-2016	06-2018
C3 TIXYLIX	PROMETHAZINE HCL COUGH LICTUS	MAY-2016	APRIL-2018
C4 ACTIFED –DM	PSEUDOPHEDRINE HCL DEXTROMETHORPHAN HYDROBROMIDE BP	05-2016	05-2018
C5 BRITNYL	TETRABUTALINE-SULPHATE PSEUDOPHEDRINE HCL	03-2016	03-2019
C6 HYDRILLIN	PSEUDOPHEDRINE HCL DEXTROMETHORPHAN HYDROBROMIDE BP	08-2016	05-2019

Table II: Microbial load in the branded cough syrup samples

Sample names	Company name	Total aerobic plate count cfu /ml	Total aerobic fungal count cfu /ml	*Fungal spp*	*E.coli*	*Bacillus spp*	*Salmonella spp*	*Staphylococcus spp*	*Klebsiella spp*
Sancos	Novartis	$3.5<10^5$ cfu/ml	$2.9<10^5$ cfu/ml	+	-	+	-	-	+
Pulmon-ol	Merk	-	-	-	-	-	-	-	-
Tixylix	Sanofi	$1.0<10^5$ cfu/ml	$4<10^4$ cfu/ml	-	-	-	-	+	-
Actififed-DM	Gsk	-	-	-	-	-	-	-	-
Britnly	Barrett Hodgson	-	-	-	-	-	-	-	-
Hydrylin	Sanofi	-	-	-	-	-	-	-	-

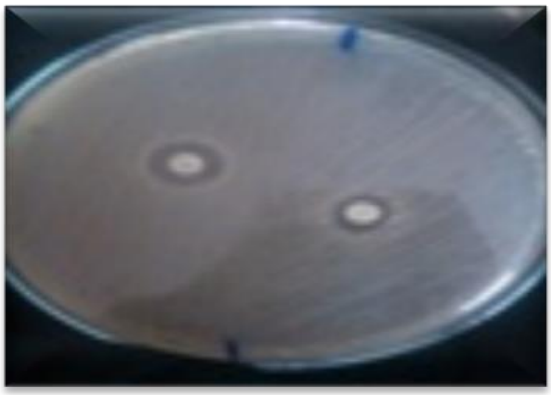

Figure 1: VISA on MHA in Tixylix.

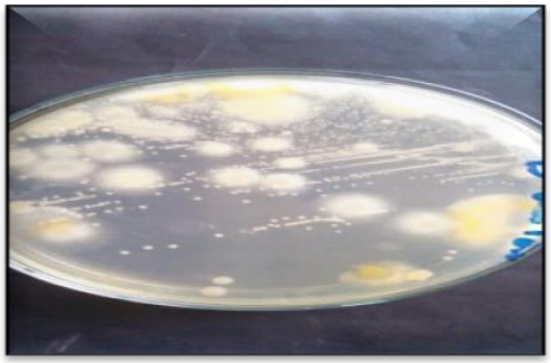

Figure 2: Fungal growth on SDA From Tixylix

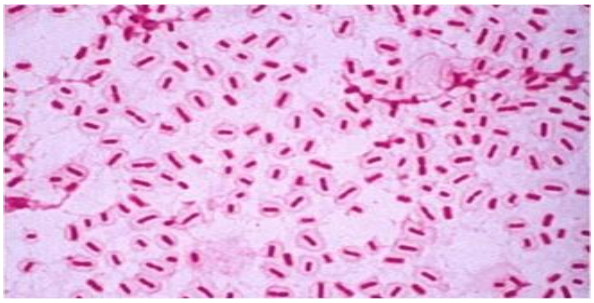

Figure 3: Gram negative rods in Cough syrups Sancos. The mucoid colony morphology and biochemical test later on indicated *Klebsiella specie*.

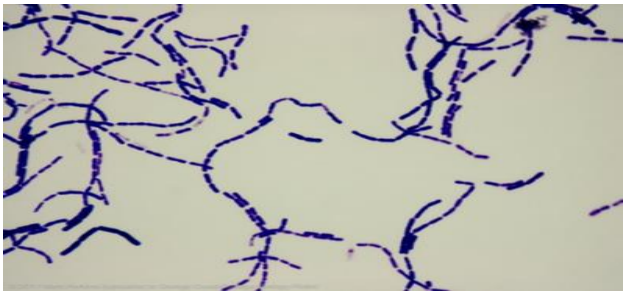

Figure 4: Gram positive chains of rods present in cough syrup Sancos. These rods after performing biochemical tests were later identified as *B. cerues*.

DISCUSSION

This study was attempted to find out the microbial contamination in the branded cough syrups that frequently used by the people. However, very few studies were carried out on the microbiological quality of the pharmaceutical products like oral suspensions in Pakistan. This has encouraged us to carry out this research to assess the microbiological quality of non-sterile pharmaceutical products like cough syrups locally available in Karachi city. The study findings revealed that only two syrup samples were contaminated microbiologically (7).

The microorganisms, including *Staphylococcus spp. and Bacillus spp*. might transmit from the environment (soil & water) and hands of the handler during the preparation of syrups, their incidence does not always mean that the consumption of syrups are potentially be hazardous to people as not all the strains of *Staphylococcus spp.* can necessarily produce enterotoxin and higher infectious doses (10^5-10^6cfu/ml) of *Bacillus sppis* required to cause diseases. Absence of coliform and pathogenic bacteria indicated that fecal contamination of water might not occur. Unhygienic environmental condition and improper handling of raw materials, ingredients and products might be the cause of contamination. Our results are matched with the existing studies, which showed that the majority of microbial contaminants in non-sterile pharmaceuticals are *Aspergillus* spp, *Bacillus* spps , and *Klebsiella* spp. This study showed a bioburden up to 10-fold of the unacceptable limits (<10^5 CFU/ml) in these final products (8). The pharmaceuticals companies do not maintain the sterile condition so the chances of the contamination occur in the products. This shows that these two syrups were microbiologically contaminated due to the improper procedures during repackaging into smaller packs, handling of the handlers, poor hygienic and dispensing of syrups. Underdeveloped countries, the chances of the incidence of the diseases and oral infections are very frequently reported just because of the poor hygienic practices, consumption of contaminated water & food and unstable environmental condition. Sometimes a minimum dose of contaminated pathogens could be infectious when resistance mechanisms and immune system are impaired and irregular, It may be happens by severe underlying disease, or by using of immunosuppressive drug. The contamination of the microorganisms in non-sterile syrups was claimed more significance for the patients, who are taking the type of contaminated syrup, is already sick. Therefore, it is very necessary to examine the efficacy and potency of these syrups that are very commonly used for the coughing.It is a very big problems in in developed countries that the people are not follow their rules for working like for examples Karachi's pharmacies should be follow a hygiene environment during the manufacturing & packaging of the pharmaceuticals products (9).

Consequently, A best quality control and compliance of techniques under aseptically during the preparation and packaging of the products of pharmaceuticals, and education on personal hygiene for the personnel, might be lower down the microbial and physical or any type of the contamination. Best hygiene conditions must follow during manufacturing of the products (10).

CONCLUSION

The cough syrups's efficacy and effectivity is questionable and doubtful, particularly in childern. The presence of any microorganism should not be negligible and considered undesirable for all samples. Although Gram negative enteric bacteria were not found in the tested samples, fortunately but the presence of viable bacteria, especially Gram positive along with fungi claimed a sort of public health risk associated with the consumption of these syrups. The quality control and quality assurance companies among the Karachi Pharmaceuticals should strictly deal and follow microbial stringency within the manufacturing, packaging, distribution and storage of pharmaceutical products. The people who are consuming such type of contaminated syrup they are highly at risk. So the microbiological study of such syrups is must suggested to check on the daily basis or routinely under hygiene conditions.

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