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The Microbiological Quality Control of Some Expectorant Syrups Found in the Market

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The Microbiological Quality Control of Some Expectorant Syrups Found in the Market

Summary: *Recently, successful results were obtained about the pharmaceutical preparations that they haven't been contaminated with pathogenic microorganisms. In spite of this, these products seem to contain microorganisms, since the formation of infections could not be prevented. From this point of view, we have studied 65 expectorant syrups from different manufacturers with different batch numbers for microbiological quality control. These expectorant syrups were made up of 13 different formulations. We tested five different batches from each formulation.*

The microbiological quality control of the syrups were carried out according to USP XXI and BP 1980 methods, that were somewhat modified by us. In the 65 expectorant syrups, the results were as follows : In 37 %, aerobic bacteria and in 4.6 %, fungi were found, which exceeds FIP standards. We isolated E.coli (4.6 %) in our three samples; P. vurgaris (3.1 %) in two samples; B.subtilis (1.5 %) in one sample; S. epidermidis (1.5 %) in one sample and C. albicans (4.6 %) in three samples.

We did not isolate Salmonella spp., Shigella spp., Klebsiella spp., Pseudomonas spp. and Streptococcus spp. in any of the samples.

Keywords *: Syrups, Expectorant, Microbiological quailty.*

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Piyasadaki Bazı Ekspektoran Şurupların Mikrobiyolojik Kalite Açısından İncelenmesi

Özet: *Son yıllarda farmasötik preparatların, patojen mikroorganizma içermemesinin temini konusunda, oldukça başarılı sonuçlar alınmıştır. Ancak mikroorganizma içeren preparatların kullanımı sonucu, çeşitli enfeksiyonların oluşumu da engellenememektedir. Bu noktadan hareketle araştırmamızda, değişik firma ve seri numaralarına sahip 65 ekspektoran şurup, mikrobiyolojik kalite kontrolu açısından ele alınmıştır. Bu şuruplar muhtelif semt eczanelerinden temin edilmiştir. Araştırılan şuruplar 13 farklı formülasyonda olup, her bir formülasyondan beşer numune denemeye alınmıştır.*

Şurupların mikrobiyolojik kalite kontrollerinde, USP XXI ve BP 1980'deki yöntemler tarafımızdan modifiye edilerek kullanılmıştır. Incelenen 65 şurubun % 37'sinin total aerob bakteri ve % 4.6'sının maya ve küf mantarı açısından FIP'in önerilerine uymadığı gözlenmiş, üç numunede E.coli (% 4.6); iki numunede P.vulgaris (% 3.1); bir numunede B.subtilis (%1.5); bir numunede S.epidermidis (% 1.5) ve üç numunede C.albicans (%4.6) izole edilmiştir.

Numunelerin hiç birinde Salmonella, Shigella, Klebsiella, Pseudomonas ve Streptococcus türlerine rastlanmamıştır.

Anahtar kelimeler *: Şurup, Ekspektoran, Mikrobiyolojik kalite kontrolu.*

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Introduction

There is no report in the literature until 1960s, of the possibility of drug products to contain microorganisms and therefore be an infection source[1,2]. First notice came to attention in the year 1963, when a Salmonella infection took place in Sweden, by tablets using a thyroid powder imported from Hungary. A further P.aeruginosa infection was caused in Sweden by ocular ointments[3,4]. Phillips reported four lung infections caused by using contaminated Lignokain ointment in 1966[5]. A further S.cubana infection was seen in United States and in England caused by gelatine capsules colored by contaminated carmin[6]. These and similar data showing that drug products can contain microorganisms, have focused attention ot the sources of such contamination and caused the development of various ways to prevent it.

Syrups, which contain large amounts of saccharose,

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are pharmaceutical products, although with difficulty, that can be contaminated by microorganisms during manufacture and use. Another source for microbiological contamination in syrups, is the use of water and/or fresh exracts prepared from crude drugs as solvents. The employment of crude drugs from natural sources, without any decontamination and the removal of surface microflora, has always caused serious problems,[3, 7-9].

Starting from this point, the aim of our study was to carry out the microbiological quality control and determine the microflora of expectorant syrups put on the market by various manufacturers for consumer use.

Materials and Methods

65 expectorant syrups of differing batch numbers and put on the market by a number of manufacturers, were obtained for this study from various local pharmacies. The syrups investigated belong to 13 different formalitons and five samples of each were employed. Each of these were considered as an individual sample and as shown in Table 1, were coded as: a: 1-13; b: 14-26; c: 27-39, d: 40-52; e: 53-65. In other words, samples 1, 14, 27, 40 and 53 are differend batches of the same formulation.

After the microbiological analysis of the samples, the isolation and identification of the growing bacteria was carried out.

The processess used during the microbiological analysis, should be performed in a suitable environment, in order to prevent outside contamination. However, it should be noted that, the measures taken for such purposes, can deleteriously effect the microorganisms in the sample[5]. For this reason, a laminar air flow cabin was used in the study. In addition to the laminar air flow cabin, ultaviolet lamps were also used in the sterilisation of the room, in order to prevent environmental contamination. Since lime absorbs 20 % of the radiation, oil paint was used for the painting of the walls of the room.

For the microbiological analysis, the methods in USP XXI were employed with modification. We took 1 mL, vs. 0.1 mL of the samples into sterile petri dishes and added 13-15 mL of the melted and cooled media at 40-45°C and mixed with a rotating motion. This modification allowed us to take more samples into the study.

1 mL of samples were taken from each syrup under aseptic conditions at the laminar air flow cabin. 1:10 dilutions were prepared in pure and sterile pH 7.0 phosphate buffer. 1:100, 1:1.000 and 1:10.000 serial dilutions were done with sterile pH 7.0 phosphate buffers.

Serial dilutions from samples were inoculated as given above, to Tryptone Glucose Extract Agar (Oxoid), Sabouraud Dextrose Agar (Oxoid), Vogel Johnson Agar (Oxoid), Cetrimide Agar (Oxoid), Salmonella - Shigella Agar (Oxoid), Eosin Methylene Blue Agar (Oxoid) and Staph. / Strep. Selective Supplement (Oxoid) media[9-17].

The petri dishes were incubated at 22°C for 5-7 days for yeast and mould counting; and at 35°C for 2-5 days for the rest. The total aerobic bacteria and yeast and mould counts were multiplied with dilution factors to obtain the microorganism counts per mL of samples[8, 9, 18, 19].

Other than total bacteria, yeast and mould counts, the identification of the isolated microorganisms were carried out with morphological, biochemical and cultural test[8, 9, 14, 17, 20, 21].

Results

The microbiological analysis data obtained in our study are given in Tables 1, 2 and 3. As can be seen from the analysis of the tables, 37 % of the 65 samples contain aerobic bacteria and 4.6 %, yeast and mould, which does not conform to the FIP recommendations. Additionally, 15 % of the growth is observed to be as pathogenic or potentially pathogenic microorganisms. E. coli was isolated from samples 2, 29(3c), 30(4c) (4.6%); P.vulgaris from samples 11 and 18(5b) (3.1%); S.epidermidis from sample 56(4e) (1.5%); B.subtilis from sample 19(6b) (1.5%); and C. albicans from samples 7, 17(4b) and 20(7b) (4.6%).

Although total aerobic bacteria count has been found to be considerably high, no pathogenic microorganisms could be detected in samples 5, 6, 8, 14

(1b), 15 (2b), 21 (8b), 22 (9b), 27 (1c), 33 (7c), 34 (8c), 37 (11c), 42 (3d), 45 (6d), 47 (8d), 53 (1e), 58 (6e), 60 (8e) and 63 (11e). Looking at the results from total yeast and mould counts, it was determined that, samples 2, 17 (4b) and 56 (4e) do not conform to FIP recommendations.

Additionally, E.coli, S.epidermidis and C.albicans were isolated from three differend batches (17, 30, 56) of formulation 4 and C.albicans from two differend batches (7,20) of formulation 7.

Table 1. Total aerobic bacteria count of the samples analysed (germs/mL).

Formulation	Samples				
	a	b	c	d	e
1	110	*	1750	100	2900
2	980	1920	210	170	190
3	140	480	1980	3100	100
4	890	1620	1890	140	9800
5	2010	1950	280	960	760
6	1940	1860	430	5900	11500
7	100	780	1590	810	110
8	1320	4900	1420	2800	4700
9	910	2600	140	970	830
10	470	160	130	210	710
11	1000	130	1680	160	1800
12	169	770	180	120	100
13	100	280	150	110	190

* Could not be counted.

Discussion and Conclusion

65 intact expectorant syrup samples of different batches, obtained from various local pharmacies in Ankara are investigated in this study. Pathogenic or potentially pathogenic bacteria and yeast growth was observed in 10 of the samples studied.

This is in contrast with the recommendations for expectorant syrups of the International Pharmaceutical Federation (FIP), USP and BP. Since this is a case of life threatening importance, its implications are evident and clear.

Table 2. Yeast - mould count in the samples (germ/mL).

Formulation	Samples				
	a	b	c	d	e
1	–	–	–	–	–
2	510	–	–	–	–
3	–	–	–	–	–
4	–	110	–	–	300
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	–	–
9	–	–	–	–	–
10	–	–	–	–	–
11	–	–	–	–	–
12	–	–	–	–	–
13	–	–	–	–	–

In a study done by Özyaral and Bozok - Johansson in 1990[22] on 100 intact syrup samples and 48 partly used samples at home, microbiological analysis was carried out and it was found out that both of the groups were mycologically contaminated. 173 moulds from used syrups and 365 moulds from intact samples were isolated in thes study.

Canefe et al.[23] in 1988 have determined that prescription liquid dosage forms prepared by pharmacies around hospitals in Ankara are contaminated.

In a study done by Devleeschouwer et al.[24] in 1980, the microbial flora, the ecological data and sensitivity to antibiotics of prescribed liquid dosage forms were investigated. The dominant flora in these preparations were found to be Gram (-) bacilli; most of them being Pseudomonas. This distribution is in agreement with the microbiological ecology of these dosage forms, since the growth of Gram(-) bacilli, which is adapted to humid media, is possible here. In contracst to that Gram (+) cocci like Staphylococcus cannot grow and keep their viability easily in these media. Antibiotic sensitivity tests done in the same study showed the presence of an antibacterial multiresistant P. aeruginosa.

Table 3. Isolated microorganisms from samples analysed.

Sample No.	MICROORGANISM TYPES and SPECIES									
	Salm.	Shig.	E.coli	Prot.	Klebs.	Pseud.	Staph.	Strep.	B.subt.	Cand.
2	–	–	+	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	+
11	–	–	–	+	–	–	–	–	–	–
17 (4b)	–	–	–	–	–	–	–	–	–	+
18 (5b)	–	–	–	+	–	–	–	–	–	–
19 (6b)	–	–	–	–	–	–	–	–	+	–
20 (7b)	–	–	–	–	–	–	–	–	–	+
29 (3c)	–	–	+	–	–	–	–	–	–	–
30 (4c)	–	–	+	–	–	–	–	–	–	–
56 (4e)	–	–	–	–	–	–	+	–	–	–

Note : (+) : Positive isolation
(–) : Negative isolation

Akın[1], in a paper in 1981, has investigated the microbiological standardisation of dosage forms and the rules set forth by International Pharmaceutical Federation (FIP) in relation to various pharmaceutical dosage forms. The author has stated his opinion on the rapid microbiological standardisation of dosage forms in our country, also taking into consideration of the state of the country.

In another paper by Akın in 1984[2], methods for investigating the microbiological purity of pharmaceutical forms such as syrups, which are not required to be sterile, are taken into consideration and views concerning the suitable microbiological methods for testing the drug products produced in the country are discussed.

Some researchers have stressed the importance of the contamination risk of the water used for the production of various pharmaceutical forms[3, 7, 25, 26]. These researchers have reported the finding, that it is not rare to find, deinoized water used for the preparation of noninjectable drugs and suggested for use by pharmacopeias, can be a massive source of contamination and on doing such work, they have found 10^5 germs (CFU) / mL[26].

It is known that, contamination during compounding arises from active subtances and adjuvants not tested during preformulation; the compounding medium; persons who are carrying out the compounding and equipment used. Factors, which have high probability for contamination should be assessed and measures taken against it. They should be standardized with microbiological tests [1, 2, 7, 25].

In a study done in the athmosphere surrounding pharmaceutical plants, 500 germs/mL in February and 800 germs/mL in July was found[5]. Buoga-Ratti also discussed sources for contamination of pharmaceutical products.

A study done by Dony in 1976, has stressed the importance of the subject, by investigating active substances and adjuvants, as well as pharmaceutical products. This reseracher has studied 1106 liquid dosage forms and found out that 87 % contain 1-100 germs/mL; 7% 10^3-10^4 germs/g; 5% 10^4 - 10^6 germs/g and 1% more than 10^6 germs/mL. Bacilli with aerobic spores in 73 %; Presudomonas and Alcaligenes in 15 %; Enterobacteriaceae in 7 %; moulds in 4.5 % and Gram (+) cocci in 0.5 % were isolated in this study[4].

A number of researchers, in order to prevent contamination from equipment, workers, atmosphere, active substances and adjuvants in production environment, have stressed the importance of working in a sterile environment and sterilization of the equipment used[1].

The first study on expectorant syrups, tonics and elixirs in Turkey has been carried out by Güven and Ötük in 1974 and various Bacilli species have been searched and found[6].

Yormaz[27], in 1983, has also microbiologically studied various syrups and drops used in therapy. In 200 samples of syrups and drops in intact enclosures, the presence of bacilli and mucor has been determined in 4.5%. The bacteria isolated were three B.subtilis and two B.mucoides.

In our study, of the 65 expectorant syrups investigated, the total aerobic bacteria count of 37% and years and mould count of 4.6 % do not conform to the recommendations of FIP. Furthermore, in 15 % of the samples, pathogenous or potentially pathogenous microorganisms were detected. The detected microorganisms were: S.epidermidis (1.5 %), B.subtilis (1.5%), E.coli (4.6%), P.vurgaris (3.1%) and C.albicans (4.6%). Therefore, no possibility of classifying the syrups was found. However, it is obvious that, various infections can occur from the administration of the contaminated syrups. This fact stresses the importance of carrying out microbiological tests, in addition to pharmaceutical quailty control in the pharmaceutical industry.

Since the microorganisms isolated belong to certain species, it follows that, such syrups have a characteristic microflora. The different batches (1, 14, 27; 6, 45, 58; 8, 21, 34, 47, 60) of formulations 1, 6 and 8 show a high count of total aerobic bacteria. In three different baches (17, 30, 56) of formulation, 4, E.coli, S.epidermidis and C.albicans were detected. C.albicans was also isolated from two different batches of formulation 7. These finding show that GMP and GLP was not followed in the production and contamination was caused by the raw materials used. It is obvious that, production procedures and packaging play a major role in the contamination of expectorant syrups.

Our investigation shows that, very little research is done domestically and internationally on this subject. For this reason, we could not compare our results. Also, for various different reasons, we could not increase the number of syrups. It is difficult with our findings, to reach to a general conclusion about all syrups produced in our country. However, 15% isolation of pathogenous and potentially pathogenous microorganisms is not a small incident.

Certainly, it is much easier to prevent the contamination of pharmaceutical dosage forms, than to cure the sicknesses resulting from them. For this reason, aseptic environments should be established during pharmaceutical manufacture. Good adherence to GMP (Good Manufacturing Practice) and GLP (Good Laboratory Practice) should be established. A suitable package for the environmental protection of the product should be selected. A quarantine adhering to the requirements should be made. Such practices will prevent both contamination and infection.

As a conclusion, in syrups, as in all pharmaceutical forms, microbiological quality controls should be carried out and the results should conform the international standards. Additionally, besides the present pathogenic microorganisms, the possibility of the count and species of saprophyte microorganisms causing infections to the druf administering patient should be taken into consideration. Another factor is the contamination during use. This contamination should not be overlooked. To prevent such a contamination. the best way is to prepare single unit dosage forms. On the other hand, it is not economical to prepare and package single dose units for certain pharmaceutical form like syrups. For this reason, adherence to GMP and GLP principles seems to be the only way.

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