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Full Length Research Paper

Microbial evaluation of some non-sterile pharmaceutical preparations commonly used at Al-khoms Market, Libya

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Pharmaceutical products of widely differing forms are susceptible to microbial contamination (Aulton,2002). The presence of microorganisms in a pharmaceutical preparations may therefore, affects the consumer, the preparation either itself or both depending on the class of contaminants. The use of contaminated pharmaceutical preparations has proved hazardous to the health of the users.To determine the type and incidence of predominant microorganisms in certainnon-sterile pharmaceuticals immediately after collection.All pharmaceutical samples were subjected to the following examinations: total bacterial count and presence of microbial pathogens, using conventional techniques.Microbial load varied among the pharmaceutical preparations. Bacterial counts ranged from 10 to more than 10^3 CFU per ml org. Several measures, including equipment automation, monitoring programs and post-marketing surveillance are required to reduce the level of microbial contamination of non-sterile pharmaceutical products.

Key words: Pharmaceuticals, Microbial evaluation, Khoms market, Streptococci

INTRODUCTION

Pharmaceuticals are used in a variety of ways in the prevention, treatment, and diagnosis of diseases. Pharmaceutical products of widely differing forms are susceptible to microbial contamination (Aulton, 2002)**.**The presence of microorganisms in a pharmaceutical preparations may therefore, affects the consumer, the preparation either itself or both depending on the class of contaminants. The use of contaminated pharmaceutical preparations has proved hazardous to the health of the users. There have been reports of drug-borne human infections world-wide (Coker, 2005)**.** Contamination of pharmaceuticals with microorganisms can also bring about changes in their physical characteristics, including breaking of emulsions, thinning of creams, fermentation of syrups, appearance of turbidity or deposit, and changes in odor and color (Shaik et al., 1988)**.** The incidence of micro flora in non-sterile preparations generally is influenced by the nature of the ingredients (whether natural or synthetic), the quality of the vehicle and the care and attitude of personnel involved in their handling (Parker, 2013)**.** Although, the presence of microorganisms whether pathogenic or not in a pharmaceutical product forces the consumer to lose faith in the manufacturing company. Thus its commercial sales would go down. In addition, changes in the stability of the products due to the activities of the contaminants may cause the company considerable financial defeat (Mugoyela and Mwambete, 2010)

OBJECTIVES OF THE STUDY

To determine the type and incidence of predominant microorganisms in certain non-sterile pharmaceuticals immediately after collection and through three weeks later after the first opening of the container.

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Table 1.List of contaminated syrup samples:

Category	Total samples tested	No. of contaminated samples	%contamination
Bronchodilator	9	3	33.3*
Mucolytic, Expectorant and Bronchodilator	9	6	66.6*
Mucolytic Antiemetic	9	0	0*
Antimycotic	27	0	0*
Antiprotozoal	9	0	0*
Vitamin B-complex	9	0	0*
Ferrous sulphate	9	0	0*
Antipyretic	9	3	33.3*
Antitussive	9	0	0*
Antiemetic	9	0	0*
Antirheumatic	9	0	0*
Laxative	9	6	66.6*
Analgesic	9	3	33.3*
For Common Cold	9	3	33.3*
Corticosteroids	9	9	100*
Total	153	33	21.6**

N.B. 1-* % was correlated to the total number of samples in each individual category.
2- ** % was correlated to the total number of syrup samples.

Table 2.List of contaminated tablet samples:

Category	Total samples tested	No. of contaminated samples	%contamination
Antiemetic	27	12	44.4*
Antibiotic	9	0	0*
Gastrointestinal tract disorder	9	3	33.3*
Folic Acid	9	0	0*
Bronchodilator	9	3	33.3*
Hypoglycemic	9	0	0*
Antihistaminic	9	3	33.3*
Analgesic	9	3	33.3*
Total	90	24	26.7**

N.B. 1-* % was correlated to the total number of samples in each individual category.
2- ** % was correlated to the total number of tablet samples.

MATERIAL AND METHODS

A total number of **369** pharmaceutical products were tested. The samples comprised of **153** syrups, **9.0** suppositories, **36**.**0**eye drops, **90.0** tablets and **81.0** skin ointments, and included locally manufactured and imported products. Samples were randomly purchased from **10** private community pharmacies in Libya.
The media used for the microbiological analysis include: casein soya bean digest agar, nutrient broth, nutrient agar, MacConkey agar, Mannitol salt agar, Blood agar, Thioglycollate medium and Sabourauddextrose agar. They were manufactured by either Oxoid (Cambridge, UK), Difco (USA) or Britannia (Buenos Aires, Argentina). The media were prepared according to the manufacturers' instructions.

Test Sample Preparation

For tablets, five units were dispersed in 10 ml of sterile

Table 3. List of contaminated eye drops samples

Category	Total samples tested	No. of contaminated samples	%contamination
Corticosteroids	18	3	16.7*
Antibiotic	18	0	0*
Total	36	3	8.3**

N.B. 1-* % was correlated to the total number of samples in each individual category.
2- ** % was correlated to the total number of eye drops samples.

Table 4. List of contaminated skin ointment samples

Category	Total samples tested	No. of contaminated samples	%contamination
Antimycotic	36	9	25*
Antibiotic	27	0	0*
Lubricating jelly	9	9	100*
Corticosteroids	9	6	66.7*
Total	81	24	29.6**

N.B. 1-* % was correlated to the total number of samples in each individual category.
2- ** % was correlated to the total number of skin ointment samples.

Table 5. List of contaminated suppositories samples

Category	Total samples tested	No. of contaminated samples	%contamination
Anti inflammatory	9	6	66.7*

N.B. *% was correlated to the total number of suppositories samples.

normal saline. The dispersion was mixed in a vortex mixer for 5 min to dislodge possible microbial cells. The solid particles were allowed to sediment and the supernatant was used for microbial test**.** For liquid samples (syrups, suspensions, oral and nasal drops), 10 ml of the product examined was diluted in sterile buffered solution with the following composition: peptone (1.0 g/L), potassium di-hydrogen phosphate (3.7 g/L), disodium hydrogen phosphate (7.2 g/L), and sodium chloride (4.3g/L). Generally, ten-fold dilutions were prepared as described in British Pharmacopoeia (British Pharmacopeia, 2003)**.**

Total Viable Aerobic Bacterial Count

This was performed according to Madigan et al.,(**1997).**

Detection, isolation and identification of potential aerobic bacteria and fungi

This was performed according to Koneman et al. (1997)**.**

RESULTS

As indicatedby the Tables (**1-5**), on the first time oftesting the pharmaceutical products, the microbial growth was observed in **11** (**21.6%)** of the syrup, **8** (**26.7%)** of the tablet, One (**8.3%)** of eye drop, **8** (**29.6%)** of skin ointment and **2 (66.7%**) of suppository samples tested. However, none of the tested five human plasma samples, were found to be contaminated.

It can be further indicated from table (**6**) that out of **33** contaminated syrups, **23 (15%)** samples showed a total aerobic count was less than **1 x 100** organism/ml of the sample. In addition, **9 (10%)** samples of tablets, **10 (12.3%)** of skin ointments, **3 (8.3)** eye drops and **six (66.7%)** of suppositories exhibited presence of total aerobic count less than **1 x 100** organism/g. However, the remainder **10 (6.6%)** of contaminated syrups, **15 (16.7)** tablets, **14 (17.3%)** skin ointments and 6 **(66.7%)** of suppositories exhibited presence of total aerobic count more than **1 x 100** organism/ml./g. The contamination is either with a single or with a mixture of microorganisms. All the batches tested found to be free from Gram-negative bacilli including *Escherichia coli, Salmonella*and*Pseudomonas* as tested by British Pharmacopoeia microbial limit tests (Table **7**).

Gram-positive cocci detected, in **54** of the total tested samples, including **21** syrup samples, **11** tablet samples, **18** skin ointment samples and **4** suppositories samples. Gram-positive bacillidetected, in **30** of the total tested

Table 6.Total bacterial count (CFU/ml or g) in the different dosage forms.

Dosage form	(Total quantity)	< 100 CFU/ml or g.		100 – 1000 CFU/ml or g.	
		No.	%*	No.	%*
Syrups	153	23.0	15.0	10.0	6.6
Tablets	90	9.0	10.0	15.0	16.7
Eye drops	36	3.0	8.3	0.0	0.0
Skin ointment	81	10.0	12.3	14.0	17.3
Suppositories	9	0.0	0.0	6.0	66.7
Blood products	5	0.0	0.0	0.0	0.0

N.B. *% was correlated to the total number of each drug dosage form.

Table 7.Microorganisms isolated from different non-sterile dosage forms.

Category	Number of contaminated dosage forms with					
Dosage form	Gram positive bacilli	Gram positive cocci	Gram negative bacilli	Gram negative cocci	Yeasts	Molds
Syrups	6	21	0	0	0	12
Tablets	9	11	0	0	0	13
Eye drops	0	0	0	0	0	3
Skin ointment	12	18	0	0	0	6
Suppositories	3	4	0	0	0	2
Blood products	0	0	0	0	0	0
Total	**03**	54	0	0	0	36

samples, including **six** syrup samples, **9** tablet samples, **12** skin ointment samples and **3** suppositories samples (Table **7**).

Further tests indicated that molds were predominant as contaminant; they were isolated from **12** syrup samples, **24** tablet samples, **6** skin ointment samples, **3** eyes drops samples and **2** suppositories samples (Table **7**).

It was clear that product might be contaminated with single microorganism or mixture of different microorganisms. Table **8** shows the microbial species isolated from the non-sterile pharmaceutical products. Again, suppositories, skin ointments, tablets and syrups, showed the highest number of microbial isolates while eye drops showed the lowest. The commonest bacterial contaminant isolated was, *Staphylococcus epidermidis* (**38**) followed by, *Bacillussubtilis* (**30**) and *Staphylococcus aureus* (**16**). However, The commonest fungal contaminant isolated was, *Penicillium sp.* (**24**) followed by, *Aspergillus sp.* (**5**), *Mucor sp.*(**5**) and *Alternaria sp.* (**2**). Values in parenthesis represent number of contaminated products.

DISCUSSION

In recent years, manufacturers of pharmaceuticals have improved the quality of non-sterile pharmaceuticals such that today such products contain only minimal bio-burden[1]**.** The occurrence of microbial contamination has been well documented, and contaminants range from true pathogens such as *Clostridium tetani*, to opportunistic pathogens such as *Pseudomonas aeruginosa (Mwambete, 2009)***.** Several reports have also been published describing clinical hazards that are attributable to microbiologically contaminated pharmaceutical United States Pharmacopeia, 2003**).** The major health concern is when such microbial contaminants exceed acceptable limits (10^2cfu/ml) Carstersen and Rhodes, 2000**).** It must be stressed, however, that the majority of cases of medicine-related infections are probably not recognized or reported as such (Denyer and Baird, 2006).

Microbial infections are not only the result of the physical presence of microorganisms, but also their metabolites/toxins that become harmful even if they are found in minute quantities (Shukla et al., 2004)**.**

Previous studies have demonstrated microbiologic quality concerns with regard to both commercially available and extemporaneously prepared pharmaceuticals, storage, and sale of expired liquid disinfectants (Denyer and Baird, 2006)**.**

This has compelled us to embark on this study in order to

Table 8. Microbial species isolated from different non-sterile dosage forms.

Dosage form	Syrups		Tablets		Eye drops		Skin ointment		Suppositories	
Microbial species	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**
Staphylococcus epidermidis	15	9.8	8	8.9	0	0	12	14.8	3	33.3
Staphylococcus aureus	6	3.9	3	3.3	0	0	6	7.4	1	11.1
Bacillus subtilis	6	3.9	9	10	0	0	12	14.8	3	33.3
Aspergillus sp.	2	1.3	2	2.2	0	0	0	0	1	11.1
Penicillium sp.	7	4.6	8	8.9	3	8.3	5	6.2	1	11.1
Mucorsp.	2	1.3	2	2.2	0	0	1	1.2	0	0
Alternariasp.	1	0.7	1	1.1	0	0	0	0	0	0
Total	39	25.5	33	36.6	3	8.3	36	44.4	9	100

N.B: -No. and % represent the number and percent of samples of the dosage form contaminated with the microbe.
- Mixture of microorganisms sometimes isolated from one product.

assess the magnitude of such microbial contaminants in non-sterile pharmaceuticals given to outpatients through community pharmacies at Alkhoms City, Libya.

The study findings have shown that reasonable number of tested samples were microbiologically contaminated.

The isolated aerobic bacteria were mainly *Bacillussp.*,*Staphylococcusaureus* and *Staphylococcus epidermidis*; while the fungal contaminants comprised *Penicillium sp.*, *Aspergillus sp.*, *Mucor sp.* and *Alternaria sp*.

The majority of the microorganisms isolated from the samples were normal human flora, which are widely distributed in nature with the exception of the *Aspergillussp.*and*Staphylococcus aureus*. This suggests that these medicines were microbiologically contaminated as a result of improper handling, poor hygienic procedures during packaging, distribution and dispensing of medicines.

All the batches were found to be free from *Escherichia coli, Salmonella* and *Pseudomonas* as tested by British Pharmacopeia microbial limit tests.

The presence of potentially pathogenic opportunistic microbes, including *Aspergillussp* and *Staphylococcus aureus,* cannot be overemphasized, because they may cause a significant deterioration in the health status of patients, particularly those who are immunologically compromised and of infants with an immature immune system (Shukla et al., 2004)**.**

Alternariasp. may be related to bakers asthma. It has been associated with hypersensitivity pneumonitis, sinusitis**,**deratomycosis, onychomycosis, subcutaneous phaeohyphomycosis, and invasive infection. Common cause of extrinsic asthma (immediate-type hypersensitivity: type I). Acute symptoms include edema and bronchospasms, chronic cases may develop pulmonary emphysema. *Penicillium sp.* may cause hypersensitivity pneumonitis and allergic alveolitis in susceptible individuals. It is reported to be allergenic (skin). Some species can produce mycotoxins. Common cause of extrinsic asthma (immediate-type hypersensitivity: type I). Acute symptoms include edema and bronchospasms, chronic cases may develop pulmonary emphysema (Pulimood et al., 2007; De Lucca, 2007)**.**

The number of isolated microorganisms in this study is smaller than that reported earlier by other authors(Coker, 2005)**.** This may be to the introduction of better adherence to 'Good Manufacturing Practices' by pharmaceutical manufacturers in recent years. Some of the preparations were contaminated by *Staphylococcus* species, suggesting contamination from the equipment and/or raw material, or poor hygiene of the factory hands during production (Shaikh et al., 1988)**.**

The proportion of the products containing viable aerobic bacterial count (100-1000 CFU per ml or g) was small (10% in syrups, 15% in tablets, 14% in skin ointments and 6.0% in suppositories) which indicates that the microbiological quality of the examined products was, in general, adequate and, in most cases, excellent. On the other hand, the presence of some molds reflects the storage quality of the preparations. The presence of certain molds is harmful since they produce metabolites that may be toxic to consumers and cause rapid deterioration of the product due to the biodegradation of the different components of formulations arising from the production of toxins, such as *Aspergillus sp. (*Parker, 2013)**.**

In a related work, Okunlolaet al (2007)investigated the microbial characteristics of 21 different herbal medicinal products of various dosage forms which were sourced from some traditional medicine sales outlets as well as retail pharmacy outlets in southwestern Nigeria. The aerobic bacterial count of the products ranged from 5.0 x 10^{2} to 2.2 x 10^{4} but their microbial load varied considerably. Ten (47.6 %) of the samples were contaminated by *E. coli*, 7 (33 % by *Salmonella,* 15 (71.4 %) by *Staphylococcusaureus*and 12 (57.1 %) by fungi. The values are considerably higher than those found in the present study, probably due to the fact that in contrast to our study, natural ingredients, which are likely to be more contaminated, were mainly used.

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