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MICROBIAL CONTAMINATION OF PHARMACEUTICAL PRODUCTS USED AT HOMES IN AMMAN-JORDAN

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ABSTRACT

Three hundred and sixty two liquid and semiliquid (emulsion in the form of lotions and creams) pharmaceutical products used at homes in Amman were examined microbiologically. Thirty six percent of these samples were contaminated. Gram staining of representative organisms isolated from the contaminated samples showed that 26% contained Gram negative rods; 38% contained Gram positive cocci and 35% Gram positive rods. The most common species of Gram negative rods were *Escherichia coli, Pseudomonas* spp., *Enterobacter* spp. *and Klebsiella* spp.

INTRODUCTION

It would be no exaggeration to state that microbial spoilage and its prevention represents the major problem of pharmaceutical microbiology. The microbiological quality of pharmaceutical products is influenced by the manufacturing environments and by the materials used in their formulation[1].

In 1965 the Royal Swedish Medical Board reported high levels of microbial contamination of non-steril drug products[2]. Since then a number of surveys have confirmed these findings[3-8]. Microbial contamination of such products is undesirable because of their spoilage and pose a health hazard to the user. Also, *Pseudomonas aeruginosa,* which cause severe ophthalmic infections, was found in inadequately designed eye drops[10].

Certainly, pharmaceutical products of widely differing forms are susceptible to contamination by a variety of microorganisms. These products, providing as inhospitable environment to invading contaminats, as well as products with more nutritious components, such as creams and lotions with carbohydrates, amino acids, vitamins and often appreciable quantities of water are known to be at risk[9,11-15].

Obviously, the majority of the reported works has been carried out in western countries with temperate climates[3,5,16,17]. Although, the isolated organisms were indigenous to those countries, there are few studies concerning numbers and types of contaminating organisms in pharmaceutical preparations used in hot climates.

In the present study, we describe the results of a microbiological examination of daily used pharmaceutical products collected from domestic environments in Amman-Jordan.

EXPERIMENTAL

Collection of samples :

Three hundred and sixty two samples were collected by volunteer students from ordinary homes within the city of Amman. Only 10 of these samples were manufactured in Jordan. Out of the remaining 352 samples, 280 were dispensed in their original imported packing and 72 were repacked into smaller containers. The nature and number of the collected samples are listed in Table 1.

Total viable counts :

Duplicate 1 ml aliquots of the preparation under investigation were aseptically added to 100 ml of sterile 0.15 M phosphate buffer saline, pH 7.2. Aliquot of 0.1 ml of the mixed solution by invertion was then added to further 100 ml of sterile phosphate buffer.

Each 100 ml aliquot of phosphate buffer was then filtered through sterile 0.45 µm membrane filters using Millipore filtration units followed by washing with a further 100 ml of sterile buffer. The membrane filters were placed onto tryptone soya agar (Oxoid) and incubated for up to 72 hrs at 35 ± 0.5°C. Following incubation, colony-forming units (CFU) were counted and representative colonies were gram stained.

Isolation of bacterial colonies :

Different bacterial colonies were isolated from the samples as required by streaking on nutrient agar, eosin-methylene-blue (EMB) and MacConkey agar (for Gram-negative bacteria), on blood agar (for haemolytic bacteria), and on *Salmonella-Shigella* (SS) agar (for *Salmonella* and *Shigella* spp.).

Identification of bacteria :

Different morphological colonies were subcultured and tested for their Gram-stain, sporulation,

capsule formation, motility, and biochemical assayes [carbohydrate fermentation(glucose, galactose, mannitol, lactose, maltose), catalase activity, acetyl methyl-carbinol production, citrate utilization, urease production, gelatin liquifaction, nitrate reduction, litmus milk reaction, and H_2S production]. Identification of bacteria was carried out according to previous protocol[18]. API-20E (Analytab products, Inc., Plainview, NY) was used to confirm the identification of the different types of bacteria isolated on EMIB agar.

RESULTS AND DISCUSSION

Three hundred and sixty two pharmaceutical samples used at home were examined. It was found that 36% of the examined samples were contaminated with ten or more organisms per ml. The number of samples contaminated in each of the selected categories is presented in Table 1. The number of items with more than 100 CFU per ml is shown in parenthesis. Nine percent of the total number of samples were highly contaminated. The percentage of contaminated samples in each category is also shwon in Table 1. The oral samples represent the highest percentage of contamination.

Table 2 shows the results of Gram staining of representative organisms isolated from the contaminated samples. Sixty eight samples (26.5%) contained Gram-positive rods; 74 samples (38.3%) contained Gram-positive cocci and 51 samples (35.2%) contained Gram-negative rods.

The Gram-negative rods were further identified using the API-20 E and these are shown in Table 3. *Escherichia coli* were found in 41% of samples and 13.7% were identified as *Pseudomonas* spp. Gram-positive occi, *Micrococcus luteus, Streptococcus faecalis* and *Staphylococcus aureus* were also isolated.

The rate of contamination (36%) (Table 1) is in agreement with previous results. It has been[16] reported that contamination rates were between 7 and 32% in non-sterile hospital pharmaceutical products, depending on the environmental conditions prevailing within the pharmacy. The Public Health Laboratory Service in England[19] reported a contamination level of 32% in pharmaceutical products examined during their use in hospital ward.

However, it was reported that a lower level of contamination (14%) of pharmaceutical products used in houses was attributed to lower levels of exposure to microorganisms in the home than in hospitals sources of contamination of non sterile pharmaceuticals in hospital and community environments were recently reported[23].

The presence of Gram-negative bacteria in pharmaceutical products have ranged from true pathogens, such as *Clostridium tetani*, to opportunist pathogens, such as *Pseudomonas aeruginosa* and other free living Ggram-negative organsims, which are capable of causing disease under special circumstances[6,10,13,20].

The Public Health Laboratory Service Report of 1971 expressed concern at the overall incidence of contamination in non-sterile products used on hospital wards (327 of 1220 samples) and the presence of *Pseudomonas aeruginosa* in 2.7% of samples mainly oral alkaline mixtures[19] some previous results have[16] recorded a much lower incidence of *Pseudomonas* spp. (0.6%) from pharmaceutical products used in the homes by the publics.

Considering that all of the tested pharmaceutical samples were liquid or semi-liquid in nature it was anticipated that *Pseudomonas* spp. would be isolated amongst the Gram-negative rods[20,21]. The fact that *Pseudomonas* spp. stands the second among the Gram-negative rods may be a reflection of the differences in natural microflora found in temperate and hot climates.

It has been considered that[9,14] pseudomonads in general and *P. aeruginosa* in particular are objectionable in eye products, as are *P. aeruginosa*, *P. multivorans* and *P. putida* in genito-urinary tract and topical products. Further work is obviously necessary with more samples.

Most of the bacteria recovered from the pharmaceutical samples namely *Enterobacter* spp., *Klebsiella* spp., *E. coli* and *Streptococcus faecalis* have been reported by other workers for their ability to cause diseases[17].

It should not be assumed that standardized western techniques and methods used in temperature climates automatically apply to hot climates. It has been proven in many instances that the level of acceptable microbial number and type of microorgaisms as indicators of contamination are very much dependent on climatic conditions[22].

Therefore, results obtained in the temperate zone cannot by simply extrapolated to those countries with hot climates.

It is hopped to further investigate the source of contamination and in particular whether the samples were contaminated prior to importation or while under domestic use.

Finally, we hope that this preliminary study will direct the attention of hospital staff and patients to the necessity for a continuous surveillance programme designed to determine sources of contamination and its control and prevention.

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Table (1): Types and the number of the tested pharmaceutical products.

Pharmaceutical product	Number of samples		Contaminated samples (%)
	Analysed	Contaminated*	
Oral liquid.	114	50 (11)+	44
Oral syrup/linctus.	96	35 (12)	36
oral suspension.	21	9 (2)	43
Oral elixir.	28	9 (1)	32
Oral emulsion.	3	1 (0)	33
Nasal drops.	40	11 (3)	28
Eye drops.	23	7 (1)	30
Ear drops.	9	2 (0)	22
External creams.	28	5 (1)	18
Total	362	129 (32)	36

* Greater than ten organisms per ml.
+ Number in parenthesis refer to samples with more than 100 organisms per ml.

Table (2): Microorganisms isolated from contaminated samples.

Pharmaceutical product	Gram +ve rods	Gram +ve cocci	Gram -ve rods
Oral liquid.	25 (37)*	22 (30)	18 (35)
Oral syrup/linctus.	20 (29)	22 (30)	15 (29)
oral suspension.	4 (6)	5 (7)	3 (6)
Oral elixir.	3 (4)	3 (4)	3 (6)
Oral emulsion.	1 (1)	0	0
Nasal drops.	4 (6)	9 (12)	6 (12)
Eye drops.	3 (4)	4 (5)	0
Ear drops.	0	1 (1)	0
External creams.	8 (12)	8 (11)	6 (12)
Total 193	68 (35)	74 (38)	51 (26)

* Number in parenthesis refer to percentage of samples in each category

Table (3): Identification of Gram negative rods found in fifty-one products.

Organism	Type of samples	Number of contaminated samples	% of contamination
Enterobacter spp.	Oral syrup Nasal drops External creams	3 1 1	9.8
Klebsiella spp.	Oral liquid Oral syrup External creams Nasal drops	2 3 1 1	13.7
Escherichia coli.	Oral liquid Oral syrup External creams	2 12 7	41.2
Serratia spp.	Oral syrup Oral elixir Nasal drops Externlal creams	2 2 1 1	11.8
Pseudomonas spp.	Oral liquid Oral syrup Oral elixir	2 3 2	13.7
Aeromonas spp.	Oral liquid	1	1.9
Elavobacterium spp.	oral liquid	1	1.9
Citrobacter spp.	Nasal drops	2	3.9
Enteromonas cloacae	Oral suspension	1	1.9

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تلوث المستحضرات الصيدلية المستخدمة بالمنازل فى عمان - الاردن

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فى هذا البحث تم إختيار ميكروبيولوجى ٣٦٢ مستحضر صيدلى سائل ونصف سائل تستخدم بالمنازل فى عمان. ولقد وجد أن ٣٦٪ من العصويات ملوثه. وبالفحص المبكروبى وجد أن ٢٦٪ ملوثه بالعصويات سالبة الجرام و٣٨٪ ملوثه بالكرويات موجبة الجرام وأن ٣٥٪ ملوثه بالعينات موجبة الجرام .