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MICROBIAL CONTAMINATION OF PHARMACEUTICAL PREPARATIONS

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Literature review

Contamination of Pharmaceuticals with micro-organisms irrespective of being harmful or objectionable or nonpathogenic, can bring about changes in their physical characteristics, including the breaking of emulsions, the thinning of creams, Fermentation of symps, and appearance of turbidity or deposit, besides producing possible off ordors and color changes.

Various reports are available describing clinical hazards that have been caused due to contaminated Pharmaceuticals.

Ayliffe et al in 1965 reported that a pot of the hand cream in use in the treatment room of St. John's hospital was contaminated with *Pseudomonas aeruginosa.*

Phillips in 1966 traced that hospital out breaks *of Salmonella cavaban* infection in both United States & United Kingdom were due to the administration of capsule products containing contaminated carmine.

White et al 1968 reported that several batches of an antifungal antibiotic powder was found grossly contaminated with *Pseudomonas aeruginaosa.*

Hirsch et al 1969 reported that 82% of 57 oral liquid products tested contained micro-organisms.

Luhdorf and Tybring 1969 performed microbiological tests on 720 batches *of* non-sterile liquid preparations for oral use. In no case was *Salmonella* detected but the presence of *E-coli* was observed in two batches.

Robinson 1971 examined 279 liquid antacid preparations for the presence of *Pseudo*monas *aeruginosa;* 85 of the finished bottles (30.5%) and two in process (33%) were positive. The aerobic plate count ranged from 100 to 9,30,000 org/gm.

Baird et al 1979 examined one thousand nine hundred and seventy seven Pharmaceutical products used in the home for microbial contamination. *Pseudomonas aeruginosa, Staphyllococeus aureus,* gram positive cocci and other bacteria were recovered from 14% of samples.

Baird et al 1985 studied the non-sterile Pharmaceuticals products made in nine hospitals in the North Thames Regional Health Authority for microbial contamination. The incidence of contamination was found to vary between hospitals. The most common isolates were aerobic spore bearers. Pseudomonas species were isolated from several samples of pippermint water.

In view of above mentioned reports, 93 samples of non-sterile liquid Pharmaceutical preparations of different manufacturers were collected from Karachi region and were analysed for their bacterial contents.

Experimental

Collection of Samples

Non sterile oral Pharmaceutical products including cough syrups, multivitamin syrups, analgesics of different manufacturers were collected from various pharmacies of Karachi city. The samples *were* subjected to quantitative and qualitative bacterial analysis according to the method proposed by British Pharmacopoeia (1980).

Test for Escheriehia coli Preparation of Enriched culture:

1.0 ml of each product (syrups, suspensions. drops) was added to 50 ml of sterile Nutrient broth in flasks. Flasks were incubated at 37°C for 24 hours. At the end of incubation period flasks showing growth were subjected to primary test.

Primary Test:

1.0 ml of enriched culture of different samples were added to 5 nil of Sterile Macconkies broth in test tubes and were incubated at 37°C for 48 hours. At the end of incubation period tubes were checked for acid and gas production. A secondary test was then carried out for tubes showing formation of add and gas.

Secondary Test:

0.1 ml of the contents of the tubes showing acid and gas was transferred to each of two sets of the tubes containing (a) 5 ml of Mac Conkey's broth and (b) 5 ml of pepton water- The tubes were incubated at 44°C ± 1 for 74 hours. At the end of incubation period, the first set of tubes(a) were examined for acid and gas formation while second set of tubes (b) for the presence of Indole.

Indole Test:

For indole test 0.5 ml of Kovac's reagent (Merck) was added into each of the tube. Development of a red colour in the reagent layer indicated the presence of indole. The presence of acid, gas and of Indole in the secondary test indicated the presence of E-coli.

Control Test:

A control test was carried out by repeating the prima*ry* and secondary tests adding one nil of enriched culture of *E-coli.*

Test for Salmonella Species:

Preparation of enriched culture

1.0 ml of each product was added separately to 100 ml of Nutrient broth in flasks. The flasks were incubated at 37°C for 24 hours. At the end of incubation period flasks showing growth were subjected to primary test.

Primary Test:

1.0 ml of the enriched culture was added to each of 2 sets of tubes containing (a) 10 ml of sterile broth and (b) 10 ml of tetrathionate broth, and incubated at 37°C for 48 hours.

At the end of the incubation period tubes showing growth were streaked separately on sterile brilliant green agar plates. The plates were incubated at 37°C for 24 hours. At the end of incubation period the colonies developed on these plates were compared with the official standard description (Bergy's manual 1974).

Secondary Test:

A single colony from the plates was transferred to tripple sugar iron agar (TSI) slope. The TSI slope was incubated at 37°C for 24 hours. The formation of acid and gas in the stab culture (with or without) concomitant blackening and the absence of acidity from the surface growth indicated the presence of *Salmonella* species.

Control Test for Salmonella Species:

A control test was carried out by repeating the primary and secondar*y* tests using one ml of enriched culture *of Salmonella typhi,* prepared from a 24 hours culture in nutrient broth.

Test for pseudomonas Species:

1.0 ml of each sample was transferred to 100 ml of *Cetrimide* broth in flasks. The flasks were incubated at 32°C for 72 hours. Each flask was then subcultured on plates containing a layer of Certimide agar and incubated at 32°C for 48 hours. At the end of incubation period the resulting growths were examined by Gram's stain and Oxidase test.

A positive Oxidase test by Gram –ve bacilli indicated the presence of *Pseudomonas* species.

Oxidase Test:

For Oxidase test few colonies from the *Centrimide* agar plates were transferred to a piece of filter paper and to this two to three drops of freshly prepared 1% w/w solution of *N N N N Tetra* methyl-p-Phenylenediammonium dichloride was added. Development of a purple colour with in 5-10 seconds indicated a positive Oxidase test.

Control Test:

A control test was carried out by repeating the test using one ml of enriched culture of *Pseudomonas*.

Table 1. List of contaminated liquid samples

Category	Total sample tested	No. of contaminated samples	Percentage
Analgesic Antipyretics	5	Nil	0%
Antidiarrhoeal	6	Nil	”
Anthelmintic	2	Nil	”
Anabolic drops		Nil	”
Antihistaminic	4	Nil	”
Antiasthmatic	4	Nil	”
Antiemetic	1	Nil	”
Antibiotics	2	Nil	”
Antimalarial	1	Nil	”
Antacids	1	Nil	”
Cough Syrvps	31	3	9.6%
Carminative mixtures	1	Nil	0%
Digestive Enzymes	9	1	11.1%
Diuretic	1	1	100%
Tranquillizer & Hypnotics	2	1	50%
Vitamins	18	1	5.5%
Miscellaneous	3	1	33.3%

Results

In this Study a total of 93 Samples of non-sterile liquid preparations from 34 different Pharmaceutical manufacturers were examined for microbial contamination.

As indicated by the Table (1) no microbial growth was observed in 85 (92%) of the batches tested seven (7) batches showed bacterial load of 1000 colonies per ml, whereas one batch showed 3000 colonies per ml. All the batches were found to be free from *E.coli, Salmonella* and *Pseudomonas* as tested by British Pharmacopocial microbial limit tests.

It can be further indicated that out of 31 cough syrups, 3 samples showed the presence of Gram positive bacilli but the total aerobic count was not more than 1 x 1000 organism/ml of the sample (Table 2). In addition one sample of digestive enzyme, one of diuretic and one of tranquillizers exhibited presence of microorganisms. Gram positive cocci were detected in three samples (Table 2). Further tests indicated that they were not co-agulase producing *Staphylococci* which are pathogenic for man.

Table 2. Bacterial Flora of Contaminated preparations

Product	Manufacture r	Total aero big count	Pseudomonas aeruginosa	Salmonella	E. coli	Gram +ve Cocci	Gram +ve Bacilli
Bliss Alkali	Bliss	1 x 1000	ve	ve	-ve	+ve	
Corex D Syrup	Pfizer	1 x 1000	-ve	-ve	-ve		+ve
Cofsed Syrup	Eros	1 x 1000	-re	ve	-ve		+ve
Diazapam	Prodes Lab.	1 x 1000	-ve	-ve	-ve	+ve	
Incremin	Lederle	1 x 1000	ve	ve	-ve		+v
Zymoplex	Hakimsans	1 x 1000	-ve	-ve	-ve		+ve
Auranti Syrup	Anglo Pak	3 x 1000	-ve	-ve	ve	+ve	

Discussion

Sterility is not a requirement in official compondia for oral Pharmaceutical dosage forms. However contamination may occur during manufacturing, packaging and handling by the consumer. This causes concern, since some dosage forms if stored in favourable environment, can serve as substrates for microorganisms. Further more the contaminated drugs can mediate infection in man and hence harmful organisms should be absent from non-sterile Pharmaceutical preparations. This exercise was carried out to obtain general information on the microbial content of non-sterile liquid preparations produced by different local and foreign Pharmaceutical firms of Pakistan.

As indicated by the results the samples of analgesics, antipyretics, antidiarrhoeal, anthelminhc, anabolic drops; antihistaminic, antiemetic, antibiotics, antimalarial, antacids

and carminative tested in the present study did not show any growth though they are supposed to be non-sterile. It can be inferred that probably an excessive quantity of preservative is used in the preparation which prevented the growth of microorganisms. The organisms which are detected in the remaining samples are not pathogenic but they are "objectionable" as they can bring about the destruction of active ingredients and thus can interfere with the function of the therapeutic product.

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