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MICROBIOLOGICAL STUDY OF BRANDS OF MULTI-VITAMIN SYRUPS MARKETED IN AURANGABAD (M.S), INDIA

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Abstract: This study investigated the microbial contamination of Five (5) brands of multivitamin syrups marketed in Aurangabad, India. The study is aimed at evaluating the microbial quality of different brands of multivitamin preparations from various manufacturers marketed in Aurangabad, India. Pour plate method was used. Preliminary investigation revealed that one out of the five different samples contains pathogenic organisms which became pertinent for further investigation to reveal the identity of the organism. Escherichia coli, Staphylococcus, Salmonella and Pseudomonas
were investigated but only salmonella was found to be present. The samples also contain Yeast & Mold organisms, and above the limit count set by microbiological quality of syrups.

Keywords: Contamination, Pathogens, Multi-vitamin Syrup.

INTRODUCTION

The microbial quality of pharmaceutical products primarily depends on the quality of raw materials, production process, production environment, hygiene of the personnel involved in manufacture and the storage conditions. Not only the presence of pathogenic microorganisms but the presence of relatively high number non-pathogenic microorganisms is also objectionable in pharmaceutical products. The presence of high number of non-pathogenic microorganisms in pharmaceutical products is objectionable for two reasons: firstly, these microorganisms can deteriorate active ingredients and can interfere with the desired activity of the product; and secondly, they can produce some metabolites that may be toxic to the consumer.

Micro-organisms cannot be seen by the naked eyes but only by the use of microscopes. They are microscopic forms of life which are ubiquitous and are present everywhere in the environment including the human body. They were first detected in 1675 by a Dutch draper Anthony Van Leeuwenhoek, who noticed tiny 'animalcules' in droplets of rain water under his microscope. He went on to discover that they where present in dental plague, feces and many other substances [1].

Most micro-organisms are harmless or even beneficial to man. Only minority cause disease in healthy humans (although many more may do so in patients with damaged immune system). After about another 12 years of Van Leeuwenhoek's discovery, it was shown that these minute creatures were also under certain circumstances the agents of disease. This was first demonstrated by Agostino Bassi in 1835 for a bacterial infection of silkworms [1].The German Robert Koch was the first to prove that a bacterium could cause a human disease, namely anthrax in 1876. Naturally the discovery aroused huge scientific and public interest, although they were those in both the lay and the scientific communities who were opposed to the new theory of infection.

The study of micro-organisms is known as microbiology and not surprisingly the much of the study is directed at those organisms which do cause human disease. It is now realized that not only are micro-organisms responsible for what are conventionally considered infectious diseases but they may also contribute to such diverse illnesses

as peptic ulcer, angina pectoris and cervical cancer. Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents [2]. There is no doubt that in the future, micro-organisms will be found to be involved in many more non-infectious diseases. However they are also essential to human life. Every square inch of our body surface is colonized by many thousands of organisms which help to protect the body from invasion by other potentially harmful organisms. If this normal 'flora' is damaged for instance by antibiotics, it leaves the way open for the harmful organisms to get a foothold and establish themselves instead. Syrups are non-sterile liquid dosage form that contain active medicaments and constitute the most convenient dosage form for babies, children and the elderly [3]. Syrups are mostly prepared for oral administration in children since tablets and capsules cannot be easily or conveniently administered to them [4]. Syrups are characterized by sweet taste and a viscous consistency. Concentrated aqueous solution of sucrose or other sugars serve as the general vehicle for all syrups Flavored and medicated syrups are the preferred dosage forms of choice for both children and adults, because they are palatable and easily absorbed by the body. Patients frequently use multidose syrups and most of them have been found to be potent. For this reason, manufacturers should use preservatives to prevent accidental contaminations to opened bottles of syrup. Preservatives are widely employed in the cosmetics and pharmaceutical industries as well as in a variety of other manufacturing industries [5]. The preservatives include both ingredients with known antimicrobials activity and ingredients that may contribute directly or indirectly to antimicrobial activity [6].Antimicrobial preservatives work by reducing the number of organisms and inhibiting the growth of micro-organisms that may be introduced during repeated use accidentally [7].The amount of a preservative varies with the proportion of water available for growth, the nature and inherent preservative activity of some formulative materials and the capability of the preservative itself. All handling and storage methods are therefore primarily concerned with minimizing microbial contamination and retarding microbial growth and activity [8]. Studied the microbiological quality of some commercially available syrups and suspensions in India, and found that 80% of the syrups did not comply with the official requirement for microbiological quality of syrups [9].The bacterial load of all the syrups except one was below 10^3 cfu/ml which is the highest permissible limit of bacteria in non sterile pharmaceuticals. *E.Coli* which was reported in this study to be the most frequent contaminant of syrup [10]. The study is aimed at evaluating the microbial quality of different brands of multivitamin preparation from various manufacturers marketed in Aurangabad, India.

MATERIALS AND METHOD:

Materials:

- Drug samples (multivitamin syrup)
- Autoclave (Osworld,India)
- Incubator (Genlab, made in Cheshire)
- Foil paper
- Disposable Petri dishes
- Colony counter (Lapiz)
- Ultra violent lamp Laminar flow
- MacConkey broth
- Soya bean Casein Degist Agar
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- Sabourauds Dextrose Agar
- Manitol Salt Agar
- Xylose Lysine Deoxycholate agar
- Centrimide Agar
- Vassiliadis Salmonella Enrichment Broth
- Buffered Sodium Chloride-Peptone Solution pH 7.0

Sample collection:

Five different brands of multivitamin syrups marketed in Aurangabad {M.S}, India were purchased from two different pharmaceutical manufacturers.

Analysis of samples:

10 ml each sample was added in flask containing 90 ml Soya bean Casein Digest Broth & sample was shaked for better mixing.

The sample were tested for following test

i) Test For Total Aerobic Microbial Count
ii) Total Combined Yeast & Molds Count
iii) Test for Specified microorganisms- *Escherichia coli, Staphylococcus, Salmonella and Pseudomonas.*

Test for Total Aerobic Microbial Count, Total Combined Yeast & Molds Count:

After addition of 10 ml of sample in 90 ml of Soya bean Casein Digest Broth Medium (SCDM) in sample testing, this was further tested for Total Aerobic Microbial Count & Total Combined Yeast & Molds Count. [13]. Pour Plate method was followed for this two test. Rehydrate and sterilize the Soya bean Casein Digest Agar (SCDA) & Sabouraud Dextrose Agar with Chloramphenicol (SDA) as directed by the manufacturer. Pour approximately 15 – 20 ml of Soya bean Casein Digest Agar for total aerobic microbial count and Sabouraud Dextrose Agar with Chloramphenicol for total combined yeast & molds count .Both media maintained at not more than 45°C and mix thoroughly by rotating the plates slowly in clockwise and anti clockwise direction. The media Plate was

kept for solidifying and finally plates were kept for Incubation for 3 to 5 days at 30° – 35°C for total aerobic microbial count and for 5 – 7 days at 20° – 25°C for total combined yeast & molds count. [14].

Test for Specified microorganisms:

The sample of the syrups were tested for four Specified Microorganisms such as *Escherichia coli*, *Salmonella*, Staphylococcus and Pseudomonas aeruginosa on Respective media and incubated[13], [14].

Results and discussion:

TABLE 1: Number of organisms recorded per 10 ml

Sample No.	Name of Test					
	Test For Total Aerobic Microbial Count	Total Combined Yeast & Molds Count	Identification of isolated microorganisms			
			Escherichia coli	*Salmonella*	*Staphylococcus*	*Pseudomonas*
1	70	Nil	P	A	A	P
2	62	2	A	A	P	A
3	64	Nil	P	P	A	A
4	76	4	P	A	A	A
5	48	1	A	A	A	A

P= Present, A= Absent

The result of the study for four pathogen on multivitamin syrup is as mention in Table-1.Contamination by *Salmonella* could also be as a result of serious microbial pollution of the factory equipment or from an infected worker working under unhygienic practices .*Escherichia coli* are not always confined to the intestine and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for faecal contamination.

Water is one of the fundamental requirements of life and any undesired addition of chemical substances leads to its contamination and makes it unfit for human utility .The major sources of contamination of pharmaceuticals have always been water, the production environment, personnel and packaging material .Therefore proper attention should be given to the prior treatment of these factors to ensure reduction in the level of microbial contamination. It can also be observed that from the results obtained, the extend of microbial contamination in the different brands of multivitamin syrup samples used was very low as only one sample was found to contain pathogenic organism above the accepted purity limit. Other non-pathogenic micro-organism including mould and yeast were within the accepted level implying that they comply with the official requirement for the microbiological quality of syrups according to FIP working committee 1975. The result of this study while complementing those of other studies have shown that non-sterile pharmaceutical mixtures such as pediatric preparations (syrups and suspensions) showed compliance with the official requirement for microbiological quality of syrups as only one (1) out of the Five (5) samples tested showed contamination greater than the accepted level. However these can serve as silent and unsuspected sources of infection to infants.

1. CONCLUSIONS:

It can be concluded that one of the multivitamin syrup was contaminated while the other four have passed the official requirement for microbiological quality of syrups. *Salmonella* was found to be the contaminant present in one of the multivitamin samples and the source of contamination could be from factory equipment, water or an infected personnel.

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