World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

MICROBIAL CONTAMINATION OF PHARMACEUTICALS

Mayank K. Dhawan*[1], Smridhi Khurana[2], Sanyam Nasa[3] and Dr. P. K. Sahoo[4]

[1,2,3]B.Pharm. (4th Year), Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sec-3, Pushp Vihar, New Delhi-110017.
[4]Professor, Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sec-3, Pushp Vihar, New Delhi-110017.

***Corresponding Author: Mayank K. Dhawan**
B.Pharm. (4th Year), Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sec-3, Pushp Vihar, New Delhi-110017.

Article Received on 14/09/2018 **Article Revised on 05/10/2018** **Article Accepted on 26/10/2018**

ABSTRACT

Contamination of Pharmaceutical Preparations by microorganisms has gained significance as it poses a health hazard in patients whose immunity is already weak and compromised by illness. Besides pharmaceutical products contaminated I[th] microbes, irrespective of their nature i.e. pathogenic or harmless; can bring about the physicochemical changes in the product. Study of the same is being considered in this article, along with the attempt to understand the various sources of contamination. Microorganisms like gram-negative pathogens – namely *Acinetobacter* species, multidrug resistant (MDR) *Pseudomonas aeruginosa* and Carbapenem resistant *Klebsiella* species and *Escherichia coli* are emerging as significant contaminants. Organisms normally found on the human skin like *Propionibacterium spp., Propionibacterium acnes, Micrococcus spp., Staphylococcus spp., Staphylococcus epidermis, Streptococcus salivarius* and *Streptococcus mutans* are all potential hazards. Sources of the contamination may be due to manufacturing or application. Contamination due to manufacturing may further be due to - Materials, which constitutes Raw material and Packaging material; Process Environment, which constitutes, improper cleaning, lack of air clearance, absence of cleaning status labeling or use of open manufacturing environment; Personnel, which may be due to direct contact, access of unauthorized personnel, malpractices like eating food etc. or Not following GMPs (Good Manufacturing Practices), SOPs (Standard operating procedures), STPs (Standard Testing Procedures) and MFCs (Manufacturing Formula Cards); Equipments , which includes, inadequate cleaning and sanitation, defective equipment or inappropriate design; Facilities, which constitutes improper filtration, inappropriate magnitude of pressure drop, improper ratio of fresh air to recirculated air; Utilities like low water activity or addition of preservatives. Contamination due to application may also be due to personnel, equipment or environmental conditions.

KEYWORDS: Multidrug Resistant (MDR), Good Manufacturing Practices (GMP), Manufacturing Formula card (MFC), Water Activity.

MICROBIAL CONTAMINATION STATISTICS OF PHARMACEUTICALS

INTRODUCTION

Microbiological contamination of pharmaceuticals is a universal problem which might cause infection and may even be life threatening. Though reputable pharmaceutical companies do their best to deliver microbiologically safe products, it would be advantageous to use an assessment system for the current risk linked with pharmaceutical batches delivered to the drug market.

Regular checking of microbiological quality in the pharmaceutical industry is an essential criterion which is used to justify safe product release and transfer to the drug market. **Good manufacturing practice** and efficient control on **bioburden level** of product components are major factors which affect the microbiological cleanliness of medicinal products.

Various highly resistant gram-negative pathogens—namely Acinetobacter species, multidrug-resistant (MDR) P. aeruginosa, and carbapenem-resistant Klebsiella species and Escherichia coli - are emerging as significant contaminants of pharmaceutical products in many countries.

MICROBIOLOGY OF PHARMACEUTICAL PRODUCTS (IN GENERAL)

Purpose
Contamination of drugs with microorganisms irrespective whether they are harmful or nonpathogenic may lead to changes in physical and chemical characteristics of the drugs. Sterility is not a requirement in official compendia for nonsterile drugs but bioburdens must be within standard limits. Therefore, this study investigated microbial contamination of 10 nonsterile pharmaceuticals, which, by identification and quantification of microbial contaminants and susceptibility pattern testing of susceptibility pattern on the isolated microbes are frequently delivered to outpatients by identifying and quantifying microbial contaminants

Results
It was shown by the results that 50% of all the products which were tested were highly contaminated, and Klebsiella, Bacillus, and Candida species were among the most common contaminants. Moreover, the results also showed that the resistance was shown by isolated Bacillus and Klebsiella species to Augmentin ® and cloxacillin.

Conclusion
It was because of the poor handling while dispensing, repackaging, and/or not following good manufacturing practice, that the non sterile pharmaceuticals were presumably microbiologically contaminated. Hence, training and educating the dispensers, and also the patients, about the proper handling and use of medicines cannot be overemphasized, as these are major aspects in controlling cross-contamination of medicines.

MICROBIOLOGY OF ORAL LIQUID DRUGS

Source: Microbiological analysis of liquid oral drugs available in Bangladesh

S Khanom, KK Das, S Banik, R Noor - Int J Pharm Pharma Sci, 2013 - researchgate.net

Objective: To determine the microbiological quality of the oral liquid drugs usually used for disease medication.

Methods: Forty (40) different types of oral liquid drugs (26 syrups and 14 suspensions) manufactured in different pharmaceutical industries were microbiologically examined making use of standard cultural and biochemical methods.

Results: Except for one syrup, all other samples were found to be contaminated with total viable bacteria and fungi with a maximum load of 10 cfu/ml out of which 4 syrup samples were found to exceed the United States Pharmacopeia (USP) limit (<10cfu/ml).

The Gram negative bacteria were observed to be completely absent in all samples, whereas the prevalence of Gram positive bacteria including.

Staphylococcus spp. and Bacillus spp. were significant in both types of samples (the former in 24 syrups and 11 suspension samples, and the later species in 7 syrup and 4 suspension samples).

Conclusion: Microorganisms presence in the oral liquid samples might explain the treatment complicacy of the diseased children. It is hence suggested to carry out a routine microbiological study of such drugs.

MICROBIOLOGY OF TOPICAL PRODUCTS

Source: Microbiological analysis of topicals available in Bangladesh.

J Rana, T Sultana, KK Das, R Noor - Int J Pharm Pharma Sci, 2014 - ijppsjournal.com.

Method
By using standard cultural and biochemical methods 30 different types of topical products (15 creams and 15 ointments) manufactured in different pharmaceutical industries were examined microbiologically.

Results
All the samples were seen to be contaminated with total active bacteria and fungi, and the bacterial load exceeded United States Pharmacopeia (USP) or British Pharmacopeia (BP) limit (<10^2 cfu/g) in half of the cases ranged between 10^3 - 10^5 cfu/g. Although fecal coliforms were absent in all samples, only in four cases, were Escherichia coli and Klebsiella spp. found to be present. Prevalence of Staphylococcus spp. and Pseudomonas spp. were scored in <50% and 80% samples, respectively.

Conclusion
This study revealed a bacterial contamination much higher than the safety limit in most of the samples, markedly more in ointments than that in samples of cream, which may impart the treatment complicacy. For such pharmaceutical medicaments, a routine microbiological assessment is thus suggested.

Microbiology of ophthalmic products
Source: Contamination of diagnostic ophthalmic solutions in primary eye care settings.

P Clark, B Ong, CB Stanley - 1995 - dtic.mil.

Objective
Pharmaceutical agents and irrigating solutions are commonly used for both optometric and ophthalmologic purposes. Contamination of such containers or solutions might possibly cause some danger to the patient. Investigation was done of the possible contamination of a reference sample of these containers in office practices.

Method
Two diagnostic pharmaceutical agents and an **irrigating solution** used in primary care optometric and ophthalmologic practices were tested to investigate the rate of contamination and to identify the types of microorganisms in the contaminated solutions.

Results
A total of sixty samples (proparacaine, tropicamide, and an irrigating solution) were randomly cultured and **11.7%** of the samples were found to have contamination. Pseudomonas cepacia, Staphylococcus epidennidis, Pseudomonas putida, and Streptococcus species were among the prevalent micro organisms isolated from the contaminated bottles. Moreover, 17 of the original 60 containers were cultured for investigation of the dried residue particles around the threads of the containers. Of these 17 investigated, 13 (76.5%) tested positive for staphylococcus and micrococcus species.

MICROBIOLOGY OF COSMETIC PRODUCTS

Source: Microbial contamination and preservative capacity of some brands of cosmetic creams.

PG Hugbo, AO Onyekweli, I Igwe - Tropical journal of Pharmaceutical …, 2003 - ajol.info.

Objective: Cosmetic and topical products need not be sterile but must contain less levels of microbial load during application. This study was conducted to check and compare the level and type of microbial contaminants in commercial cosmetic products sold in the market and an aqueous cream prepared in laboratory and their preservative capacities while in use.

Methods: Ten brands of commercially and easily available cosmetic creams and lotions were randomly purchased. Aqueous Cream was also prepared in laboratory. Their bacterial and fungal concentrations and the types were calculated. Evaluation of preservative capacity was done by challenging the creams and lotions with washed isolates of S. Aureus and viable counting was followed by the surface viable technique. This aqueous cream was similarly challenged with the experimental organism.

Results: All the products were contaminated in various degrees. Staphylococci and other gram-positive cocci were the most common; gram-negative isolates were rarely found. Fungal contaminants mainly included Asp. fumigatus, Penicillium and Microsporium species. Challenge test (re-infection) with S. aureus depicted the commercial products as having little capacity for suppressing the bacterial proliferation.

Conclusion: Commercial cosmetic creams and lotions evaluated did not usually match the standards for microbial limits as mentioned in official monographs. Such products may adversely affect health conditions of consumers and also the stability profiles of the products.

MICROBIOLOGY OF HERBAL MEDICINAL PRODUCTS

Source: Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in south western Nigeria.

A Okunlola, BA Adewoyin, OA Odeku - Tropical Journal of Pharmaceutical …, 2007 - ajol.info.

Objective: The aim of this study was to investigate the pharmaceutical and microbial qualities of 21 different (of various dosage forms) Herbal Medicinal Products (HMPs) obtained from some traditional medicine sales outlets and retail pharmacy outlets.

Method: Tablet crushing strength, friability, disintegration time; density of the solutions and suspensions; particle size and angle of repose of the powders were included in the pharmaceutical qualities which were evaluated. Phytochemical tests were carried out to estimate the category of compounds present in the formulations and the microbial quality of the preparations was also ensured.

Results: Ten (47.6%) of the samples were contaminated by E. coli, seven (33%) were contaminated by Salmonella, fifteen (71.4%) were because of Staphy. aureus and twelve (57.1%) were due to fungi.

Conclusion: Constant monitoring and control of the standards of herbal medicines available in the market is essential.

SOURCES OF MICROBIAL CONTAMINATION

This section of article mainly deals with the various sources of microbial contamination and the points from where microbes e.g. bacteria, viruses, fungi etc can gain entry in Pharmaceutical production processes. This will help us to understand the various causes and reasons of microbial contamination.

To design and establish the systems in order to control the number, survival and multiplication or proliferation of microbes in both sterile and non-sterile pharmaceutical formulations has become one of the most considered areas in pharmaceutical process controls.

To have a precise control, it is essential to have a diverse knowledge about the various sources of microbial contamination.

New advancements and techniques are being adopted from large to small scale industries so as to prevent the entry of microbes and chances of contamination in the pharmaceutical preparations.

Sources of microbial contamination can be broadly classified at the two levels:

During Manufacturing
During Application
During Manufacturing: These are those sources of contamination which exist at the time of manufacturing process of pharmaceutical preparations.

They can be further divided as

1) Materials

A) Raw Materials: Biological products such as enzymes, hormones, vitamins are generally rich in carbon sources which obviously favour microbial growth. Therefore, it is very important to have a precise control on conditions like temperature, humidity for a process so as to prevent the potential growth of microbes. Raw materials must be evaluated for their microbial quality and stored and preserved such that there are no chances of contamination or cross-contamination. **Microbial cross-contamination** may be defined as the direct or indirect transfer of microbes (bacteria, fungi, viruses) from a contaminated product to a contamination free product.

B) Packaging Materials: They are also having a great impact on the quality of the product in terms of microbiology. It has been confirmed that if cardboards are used instead of plastic then it can significantly decrease the transfer of micro-orgamisms from packaging to the final pharmaceutical formulations. Dispensing of drugs in hospital pharmacy includes the repackaging of bulk drugs purchased from a wholesaler or any other suitable supplier, into small packets or containers. This repackaging of bulk products can also increase the risk of microbial contamination.

2) Process Environment: Process environment has a direct influence and can be a potential source of the contamination in the product being processed. By reducing the number of open operations one can minimise the risk to product from external microbial contamination sources in which personnel and environmental sources are predominantly considered.

The main causes of microbial contamination from the process environment are

→ Improper clearing in between the batches so as to minimise the product changeovers.
→ Lack of an air line clearance as per the approved procedures after each clearing process and in between the batches.
→ Absence of clearing status labelling used within the manufacturing unit.
→ Use of an open manufacturing environment / system directly exposes the product to the immediate room environment.

3) Personnel: The third critical area is the personnel who are also the major source of contamination during pharmaceutical product processing. According to a research an association has been found in between the microbes commonly found in clean up-area and those present on human skin. Some of the microbes that commonly live on human skin are Propionibacterium spp., Propionibacterium acnes→ causative agent of acne, Micrococcus spp., Staphylococcus spp., such as Staphylococcus epidermidis (commonly occur in pus and boils) etc.

The flora common to human oral cavity are Streptococcus salivarius responsible for strep throat, Streptococcus mutans etc. Besides this, staff involved in manufacturing task lacking thorough and complete knowledge of the importance of personal and production hygiene can be a potential source of contamination.

The main causes of microbial contamination from personnel are

→ Direct contact between the operator's hands and raw materials and intermediate or final bulk products.
→ Access of unauthorised personnel into manufacturing, Storage, and production process control areas.
→ Malpractices like eating food, drinking beverages and cigarette smoking or using tobacco in the storage and processing areas.
→ Not following the proper rules and regulations as per GMP (Good Manufacturing Practices) norms or as given in the SOPS (Standard Operating Procedures) and MFCS (Manufacturing Formula Cards) of that Pharmaceutical industry.

4) Equipments: The equipments and machines used in manufacturing, transferring, holding and packaging are the common source of pharmaceutical contamination. The design and materials of construction for an equipment should be such that it can minimise the chances of microbial contamination by blocking the possible points from where the microbes can easily gain entry in the pharmaceutical manufacturing process.

The main causes of contamination from equipments are

→ Inadequate cleaning and sanitization.
→ Using the defective equipments deliberately.
→ Inappropriate design, size, material that can lead to the corrosion. i. e. accumulation of static material.
→ Improper calibration of equipments and irregular service.

5) Facilities: Upto some extents the facilities provided in the manufacturing or processing area can also be a source of contamination to the product under manufacturing process, especially when there is not any proper control and periodic checkings at regular intervals are made. Facilities like HVAC(Heating, Ventilation, Air-conditioning) System can be a potential source of

microbial growth if not operated carefully and can be a transportation mode for dispersing contaminants.i.e. microbes throughout the manufacturing unit.

The main causes of contamination due to HVAC issues are

→ Improper filtration of the supplied air.
→ Inappropriate magnitude of pressure drop causing flow of reversal.
→ Improper ratio of fresh air to recirculated air.
→ Accumulation of organic material in or near the air inlet point of HVAC system.

Similarly in the manufacturing of sterile products such as injections for parenteral route and ophthalmics, it is essential to maintain the sterility of the aseptic area which is the heart of sterile processings where mainly aseptic filling and sealing of the sterile products in the final containers are carried out. Hence to maintain the sterility of the product it becomes essential to maintain the sterility of the aseptic area. For this an **airflow control system** is required which is now-a-days commonly maintained by **Laminar Flow Bench.** Further, to maintain the sterility of the environment, the air is made sterilized, by irradiating it with UV ray, which are usually supplied by **UV Lamps.** In case when Laminar flow bench or UV lamps are not working properly and/or they are not periodically evaluated for their working, then they may also serve as a source of microbial contamination.

6) Utilities: The manufacturing utilities and buildings may also contribute to the contamination. Utilities like water, process gases can be attributed to the microbial contamination and spoilage of the pharmaceutical formulation either directly by serving as a carrier of microbes or by supporting their growth during manufacturing process and after packaging in final containers. Due to this reason the water activity denoted by **Aw** of the final product is kept low so as to minimising the surviving conditions for the microbes. But addition of the preservatives and their preservating action may become futile at such low level of water activity of the product. Insufficient size and improper organization of the space can result in selection errors like cross-contamination or mix-ups between consumables, raw materials, processing materials and final products.

During Application

These are those sources of microbial contamination which exist when the medicament is applied or given to the patient. Most of these contaminations occurs due to the carelessness of the medical staff such as nurses when the drug is administered to the patient and can cause severe health regarding problems in them.

It is not essential to maintain the sterility of non-sterile formulations as per official compendia but microbes need to be within acceptable limits. There is a threshold value for each pathogenic microbe .e.g. bacteria, viruses to infect but some of the microbes are having very small threshold value. e. g. Salmonella typhi having threshold value=1, it means even a single microbe can cause the infection of typhoid. Pharmaceutical products contaminated with microbes irrespective to their nature.i.e. whether they are pathogenic or harmless can bring about the physicochemical changes in the product.

They can be further divided as

1) Personnel: Again personnel and medical staff.e.g. nurses, dispensers, pharmacists involved in health care services can be a source of contamination of the medicine or product during repackaging of bulk products stored in drug store of that hospital, during dispensing and/or not following the Good Manufacturing Practices. Therefore, the dispensers must be trained about the proper handling of the pharmaceuticals and even patients should know about the proper use of medication, because these are basic aspects in controlling cross-contamination of pharmaceuticals. There can be serious health regarding issues in patients taking highly contaminated drugs, whose immunity is already weak and compromised by illness. Topical products are considered to be most at risk, as the product is most probably applied using hands and thus there are more chances of contamination from the microbial flora of the person to the product.

2) Equipments: Medical staff and even patients generally use a number of applicators such as spatulas, pads, brushes etc. to administer the medication topically. If these applicators are used again then they can easily contaminate the product and thereby enhance the chances of cross-contamination in hospital. A diverse variety of costly and complex equipments are used in hospital now-a-days during the treatment of patient such as ventilators, incubators, humidifiers etc. Such apparatus are required to be maintained properly and decontaminated after use.

3) Environment: Many microbes can gain entry in the product during its use when it is exposed to the environment.i.e. when it is to be administered to the patient. Thus environmental sources can heavily contaminate the product microbiologically. This is more prominent when the product is dispensed in multidose container and hence the chances of microbial contamination and spoilage is more in them.

Both water and airborne contaminants can spoil the product. The environment of hospital is different from that of home as the former is more contaminated with pathogenic microbes therefore hospitals are at higher risk in causing various cross-infection in patients and even in hospital staff providing medical services if proper sanitation and personal hygiene is not maintained.

Effects of microbial contamination of solid dosage forms

One thousand, nine hundred and seventy-seven pharmaceutical products used in the home were examined for microbial contamination. Viable micro-organisms were recovered from 14.0% of samples. Medicines used in the home are apparently not exposed to the same opportunities for contamination as those used in hospital.

Effects of microbial contamination of in-use ocular drugs

Microbial contamination of ocular drugs is very commom.the sterile preparations when applied to the medication site may lead to the culturing of the gram negative bacteria. Additionally, when isolated from medication sites, the gram-negative organisms were highly likely to be cultured from the conjunctiva as well. This was not true for pathogenic gram positive organisms. We conclude that a cycle of contamination between in use medications and conjunctivae may represent an important risk factor for microbial keratitis in patients with ocular surface disease.

Pseudomonas aeruginosa, Serratia marcescens and Proteus mirabilis are the commonly involved organisms involved.

Effects of microbial contamination of solid dosage forms

Contamination of pharmaceuticals with microorganisms irrespective whether they are harmful or nonpathogenic can bring about changes in physicochemical characteristics of the medicines. Although sterility is not a requirement in official compendia for nonsterile pharmaceuticals, bioburdens need to be within acceptable limits. Klebsiella, Bacillus, and Candida species are the common organisms found in these solid dosage forms. Furthermore, the isolated Bacillus and Klebsiella species were resistant to Augmentin ® and cloxacillin. 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. Solid dosage forms, mainly tablets and capsules, constitute a large proportion of medicines which are at a high risk of contamination. Most of them come in blister packs but there are some instances where they come in bulk containers. Mishandling may result in a serious health hazard following ingestion of highly contaminated drugs/solid dosage forms by patients whose immunity is already compromised by illness. The presence of microbes in drugs not only makes them hazardous from the infectious standpoint, but may also change the physical, chemical, and organoleptic properties of the drugs, alter the contents of active ingredients, or convert them to toxic products. Thus, a medicine may be considered microbiologically spoiled in this situation, depending on its intended use. The presence of even a low level of acutely pathogenic microorganisms, higher levels of opportunist pathogens, or toxic microbial metabolites that persist even after death of the original contaminants may render the product ineffective. Physicochemical deterioration as a consequence of microbial growth is a satisfactory reason to consider the product unsafe for human use.

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. Some of these toxin-related illnesses include acute gastroenteritis, abdominal discomfort, and diarrhea. Symptoms vary from mild gastric distress to death, depending on individual susceptibility to the toxin, amount of ingested toxin, and general health of the victim. Severe infections in immune-compromised persons have been attributed to **Bacillus** and **Klebsiella** spp. Several hospital acquired and some community-acquired infections, in particular pneumonia, are also ascribed to **Klebsiella** spp.

Solid dosage forms (capsules or tablets) are prone to microbial spoilage or degradation. The more serious problem arising from microbial contamination of solid dosage forms is the absence of obvious signs of spoilage. Therefore, there is a need to know the microbial content of all drugs and medicines, whether they are sterile or nonsterile. Previous studies have demonstrated microbiologic quality concerns with regard to both commercially available and extemporaneously prepared pharmaceuticals, storage, and sale of expired liquid disinfectants.

The presence of potentially pathogenic opportunistic microbes, including **Aspergillus** spp and **C. albicans,** 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.

Tropical conditions, temperature and realative humidity and inadequately stored pharmaceuticals are also prone to microbial growth. **Klebsiella** spp are found in the respiratory, intestinal, and urinogenital tracts of animals and humans. However, when **Klebsiella** moves outside the gut, it can cause a serious infection.

Because of widespread drug resistance worldwide, the observed drug-resistant microbial contaminants in the present study underscore a need for immediate and more strict measures to address the situation by adhering to rational usage of antibiotics in our communities. Ingestion of such drug-resistant microorganisms by an individual whose immune system is suppressed may result in aggravation of illness as a consequence of secondary infection. Pathogenic microorganisms become problematic when they outnumber the normal flora, and this is when they begin to create health problems. However, the **Candida** spp isolated, although found to

be susceptible to both fluconazole and ketoconazole, are opportunistic pathogens known to cause potentially fatal deterioration of health status in immune-compromised individuals.

Effects of microbial contamination of lipid emulsions

Lipid emulsions should be refrigerated between uses. When lipid emulsion contamination is suspected, endotoxin and pH determinations should be considered as possible adjunctive tests while results of bacterial cultures are pending. The results of the present study are applicable to only selected gram-negative bacteria and may not apply to gram-positive bacteria and fungi. However, measurement of pH and detection of endotoxin is quite useful when lipid emulsion contamination occurs with selected gram-negative bacteria. At lower temperatures such as 5 degrees, chances of contamination are minimum with no change in pH while at temperatures such as 25 degrees, concentration of microbial growth is greater than or equal to 10 to the power 7 CFU/ml can be observed.

To site an example, Klebsiella pneumoniae serotypes 21 and 24 and Enterobacter cloacae were responsible for an outbreak of polymicrobial bacteremia associated with the receipt of lipid emulsion.

So, it is recommended to refrigerate the lipid elusions between uses.

Effects of microbial contamination of herbal medicinal products

A WHO survey indicates that about 70–80% of the world populations depend upon non-conventional medicine consisting herbal sources in their primary healthcare. In the past few years, we have seen the increasing growth in popularity of over-the-counter (OTC) health foods, nutraceuticals, and medicinal products from plants or related sources in developed countries. This implies that the people are not satisfied with their orthodox medical (OM) treatment. This evident increase in popularity has also brought awareness over the professionalism of practitioners, and the quality, efficacy and safety of their treatment methods and products from herbal and natural sources available in the market.

Contamination of raw materials of herbal drugs by microorganisms is the prime reason for decline of India's share in such a potential market.

According to a study, Aspergillus spp. was the predominant fungi extracted and one of the most toxin-generating species. Twenty-one (65.6%) of the samples analyzed had Aspergillus flavus and Aspergillus fumigatus dominating the picture. Five (15.6%) of them had enumeration limits of more than 2x102, the enumeration limit for total fungal count as set by the USP. This high percentage of fungal isolation form herbal plants as revealed by the results may indicate the inherent capacity of these moulds to instigate deleterious effects on humans when consumed. Aside from these, microorganisms have been isolated from our samples with Bacillus cereus isolated in 14/31(45.2%) of samples. Bacillus cereus is an endemic, soil dwelling bacteria that causes food borne illness. When ingested, this microbe causes severe nausea, vomiting and diarrhea. Normally, Bacillus food-borne illnesses occur mainly due to presence of the bacterial spores in an improper cooked food. Escherichia coli can also cause serious food poisoning in humans. As a matter of fact, this species is used as an indicator of fecal contamination in water. Shigella causes dysentery by invading the colonic mucosa. They cause apoptosis and spread laterally resulting in mucosal ulceration, inflammation and bleeding. Contamination with Shigella and its subspecies also causes hemolytic-uremic syndrome, seizures, sepsis and toxic megacolon in humans. Vibrio fluvialis is thought to be associated with diarrhea although they are rarely isolated.

Acinetobacter iwoffii is known to cause severe respiratory disease. Pasteurella multocida is associated with zoonotic infection in humans and Klebsiella with chronic diseases of the upper airways. Enterobacter agglomerans and Staphylococcus epidermidis are prevalent in the surroundings and usually harmless, it does have a potential for nosocomial infection. Furthermore, the sensitivity testing results in this study suggests that most microorganisms isolated are sensitive to the more common and available antibiotics, which means that available treatment protocols exist for such bacterial colonization in humans.

The study also indicates contamination with heavy metals, some of which are toxic to humans. In general, plants do not absorb or accumulate lead but the soils with high lead content might affect its absorption. Soils with lead levels more than 100 ppm should not be used for plantation and farming. Limit of quantification for lead in herbal medicine should not be more than 2 ppm. Lead was found in all of the samples, of which 2 henna samples showed 1.2-1.5 ppm lead content. This level however did not reach considerably high to alarm for human consumption. Similarly, trace levels of mercury and cadmium were present in the samples of up to 0.1 ppm. Limit of quantification for mercury is up to 0.5 and 0.20 ppm for cadmium. The highest recorded amount of mercury was 0.102 in Artemisia herba alba whereas the highest level of cadmium was 0.025 ppm. Such levels of mercury and cadmium don’t seem to be of health concern. Aluminum levels were undesirably high in three herbs (Anastatica hierochuntica, Salvia officinalis and Zingiber officinale) with levels 14.28-19.83ppm. This is exceeding the maximum allowed level of 0.2 ppm. Aluminum, when present in our food as a metal, supply of water can induce individuals to suffer from aluminum toxicity. After years of cumulative exposure and storage, it can result in brain degeneration and skeletal deformities. It is believed that Alzheimer`s disease is related to aluminum toxicity.

Minerals such as magnesium and zinc are essential to maintain optimal health, and when taken in excess, these minerals can be toxic. Iron is required for blood cells, potassium is essential for a healthy nervous system and zinc is necessary to augment immunity and for reproductive function. However, when taken in amounts over the recommended maximum allowable range, they can be toxic to health. These effects occur in nervous system. However, this study showed that none of the samples exceeded the maximum allowable level of 5 ppm for zinc. Although 4 of the samples had iron levels exceeding the maximum limit of 15 ppm, these levels were not alarmingly high. Similarly, most of these samples have high levels of potassium and sodium. Although potassium and sodium are essential for human body, excess amounts of potassium leads to cardiac dysfunction and excess sodium can cause metabolic problems and hypertension.

The results of this study, specifically on heavy metal contents of herbal plants suggest an impending danger for consumers. Fatal consequences may appear due to accumulation of heavy metal contents due to years of frequent use of these herbal medicines. Several degenerative and life-threatening conditions were linked to accumulation of toxic metals in the body. When our body isburdened with microbial infection to an already existing toxin-filled body, the capacity of our immune system to impart protection is exhausted or impaired. Although this study showed heavy metal levels within the allowable limits, it is possible that some amounts can be taken up by the system and accumulate for years of use, thus cause serious consequences. Even if these metals found in herbal medicines are less likely than free to bind with molecules in our body and thus slower to be absorbed, the issue of safety and vigilance on its serious adverse effects be of a concern. Also, the continued practice involving safety measures from the harvest area (e.g., minimization of pesticide use) to the household (e.g., thorough cleaning and washing of herbal plants prior to use) should be practiced.

CONCLUSION

There are exorbitant amounts of microbial contamination and heavy metal concentration in commonly used herbal plants. Consumers have the right to be informed and must be warned about health hazards through proper signs, labels and brochures indicating possible dangers lurking in their food and household products.

Effects of microbial contamination of cosmetics

Contaminating micro-organisms in cosmetics may cause a spoilage of the product and, when pathogenic, they represent a serious health risk for consumers worldwide. Over the last 30 years, implementation of Good Manufacturing Practices has been the foundation for improving industrial quality control analyses. For this purpose, the United States Pharmacopoeia (USP) Microbial Limits Test provides several methods for the determination of total microbial count for bacteria, yeast and mould. In particular the USP specifies four bacterial indicators (Escherichia coli, Pseudomonas aeruginosa, Salmonella spp. and Staphylococcus aureus) while the European Pharmacopoeia establishes additional analyses for ascertaining the levels of enterobacteria. In spite of these guidelines, microbial **contamination is still one of the major causes for product recalls in the world, in particular in developing tropical** countries. Therefore**, it is important to** improve the preservative system in order to inhibit the growth of contaminating micro-organisms during manufacturing, storage and use by consumers, also by using non-invasive packaging.

Results from this study showed that bacteria isolated from various cosmetic sources were identified on the basis of growth on nutrient agar (primary identification), microscopy, growth on selective media (secondary identification) and finally biochemical analysis. Various bacteria were isolated from all cosmetics samples and no microorganisms were isolated from Lipstick and perfumes. Some of the frequently identified microorganism included **Salmonella,** which is the most common due to water contamination, followed by **Staphylococcus aureus**, which is a common bacterial skin pathogen. Cosmetics application is largely restricted to the skin. **Staphylococcus aureus** is a common skin microorganism that can cause boils, impetigo, conjunctivitis, folliculitis and food poisoning. According to the standards prescribed for cosmetic products, they must be free from high virulence microbial pathogens such as **S. aureus** and **P. aeruginosa,** however, the present study suggests that these microorganisms can be found in unused(packaged) cosmetics. Most of the samples were contaminated by **E. coli** and **Salmonella sp**. These microorganisms are known to be opportunistic, and some of them have acquired resistance to microbial agents and they easily cause infections in immunosuppressive patients. Additionally such outbreak investigations resulted in the demonstration of these opportunistic pathogens in contaminated cosmetic products. Hygienic processing and using suitable and adequate preservatives can control such microbial contamination, from manufacturer to consumer, especially **Staphylococcus aureus, Salmonella** and **E. coli** that were not allowed to be present in cosmetics.

It can be concluded from the findings of this research work that cosmetic products can be contaminated during the production process. The presence of organisms such as **Salmonella, Staphylococcus aureus** and **E. coli** in the cosmetic collected samples can serve as vehicles for the transmission of these pathogenic organisms.

CONCLUSION

It is therefore essential to have knowledge about various sources of contamination and to establish a system to control the number, survival and multiplication of microbes. Regulation of all pharmaceutical products and quality testing must be done on the samples, to ensure sterility and hence safe and efficient administration of

drugs to the patients. Therefore it is extremely important to take necessary precautions during manufacturing process in order to prevent infections due to microbial contamination. It is necessary to comply with GMP standards strictly during various production steps. Preservatives should be added to products as determined by regulation and in accordance with toxic dose limits, for consumer's health. It should also be mandatory in hospitals and other health care centers that proper training should be provided to the nursing staff and health care providers about the sources of microbial contamination that can be most probably arisen during application or administration of parental products or during the use of other medical and surgical devices and deleterious effects of these on the health of patients, especially in those who are already suffering with a weak or impaired immune response.

Abbreviations

- MDR - Multidrug-resistant.
- HVAC - Heating, Ventilation, Air-conditioning System.
- OTC- Over the counter.
- GMP- Good Manufacturing Practices.
- SOPs- Standard Operating Procedures.
- MFC- Manufacturing Formula Cards.

REFERENCES

1. Bacterial growth and endotoxin production in lipid emulsion. W R Jarvis and A K Highsmith.
2. Comparative Microbiological Study between Traditional and Modern Cosmetics in Saudi Arabia.
3. Contamination of diagnostic ophthalmic solutions in primary eye care settings. P Clark, B Ong, CB Stanley – 1995.
4. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in south western Nigeria. A Okunlola, BA Adewoyin, OA Odeku - Tropical Journal of Pharmaceutical, 2007.
5. Evaluation of the ChemScan system for rapid microbiological analysis of pharmaceutical water G Wallner, D Tillmann, K Haberer - PDA Journal of Pharmaceutical, 1999.
6. FDA, "Current Good Manufacturing Practices for Finished Pharmaceuticals," 21 CFR part 211.
7. Microbial contamination of nonsterile pharmaceuticals in public hospital settings Veronica Mugoyela and Kennedy D Mwambete.
8. Microbiological analysis of liquid oral drugs available in Bangladesh S Khanom, KK Das, S Banik, R Noor - Int J Pharm Pharma Sci, 2013.
9. Microbiological analysis of topicals available in Bangladesh J Rana, T Sultana, KK Das, R Noor - Int J Pharm Pharma Sci.
10. Microbial contamination and preservative capacity of some brands of cosmetic creams, PG Hugbo, AO Onyekweli, I Igwe - Tropical journal of Pharmaceutical, 2003.
11. Microbial contamination: Prevention, Control and Remediation: Wyndham San Jose Herradura Hotel and Convention Center, San Jose, Costa Rica.
12. Case Studies of Microbial Contamination in Biologic Product ManufacturingKalavatiSuvarna, Ph.D, Anastasia Lolas, M.S. Patricia Hughes, Ph.D. & Richard L Friedman, M.S. Biotechnology Manufacturing Team, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research, Food and Drug Administration
    Email: kalavati.suvarna@fda.hhs.gov.
13. Some aspects of toxic contaminants in herbal medicines, Author links open overlay panel KChan, Research and Development Division, School of Chinese Medicine, Hong Kong, Baptist University, Hong Kong, SAR, PR China.
14. U.S. Department of Health and Human Services, Food and Drug, Administration. Guidance for Industry: Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients. Rockville, MD, 2001.
15. U.S. Department of Health and Human Services, Food and Drug, Administration. Guidance for Industry: Q5D Guidance on Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates used for Production of Biotechnological/Biological Products. Rockville, MD; 1998. Federal Register, 1998; 63(182).
16. Baird RM, Brown WR, Shooter RA. *Pseudomonas aeruginosa* in hospital pharmacies. Br Med J, 1976 Feb 28; 1(6008): 511-512.[PMC free article] [Pubmed].
17. Kalling LO, Ringertz O, Silverstolpe L. Microbiological contamination of medical preparations. Acta Pharm Seuc, 1966 Jun; 3(3): 219-228 [PubMed].
18. Whitby JL, Rampling A. *Pseudomonas aeruginosa* contamination in domestic and hospital environments. Lancet., 1972 Jan 1; 1(7740): 15-17. [PubMed].
19. Baird RM, Brown WR, Shooter RA. *Pseudomonas aeruginosa* infection s associated with the use of contaminated medications. Br Med J., 1976 Aug 7; 2(6031): 349-350. [PMC free article] [PubMed].
20. Case Studies of Microbial Contamination in Biologic Product Manufacturing Kalavati Suvarna, Ph.D, Anastasia Lolas, M.S. Patricia Hughes, Ph.D. & Richard L Friedman, M.S. Biotechnology Manufacturing Team, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research, Food and Drug Administration Email: kalavati.suvarna@fda.hhs.gov.
21. FDA, "Current Good Manufacturing Practices for Finished Pharmaceuticals," 21 CFR part 211.
22. U.S. Department of Health and Human Services, Food and Drug Administration. Guidance for Industry: Q7A Good Manufacturing Practice

Guidance for Active Pharmaceutical Ingredients. Rockville, MD, 2001.
23. U.S. Department of Health and Human Services, Food and Drug Administration. Guidance for Industry: Q5D Guidance on Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products. Rockville, MD; 1998. Federal Register, 1998; 63(182).
24. Microbial contamination: Prevention, Control and Remediation: Wyndham San Jose Herradura Hotel and Convention Center, San Jose, Costa Rica.