MICROBIAL CONTAMINATION OF PHARMACEUTICAL PRODUCTS AND MICROBIAL ATTACKS

BY

EKWUTOSI OLIVIA ONYEKA

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NDIMELE, FUNMILOLA ESTHER
FACULTY OF EDUCATION
UNIVERSITY OF UYO

ABSTRACT

Microbial contamination of pharmaceutical products may originate from the raw materials, as microorganisms from the raw materials will invariably be transferred to the product. In addition, a lot of factors contribute to microbial load carried by a pharmaceutical preparation at every stage. These include: raw materials used, manufacturing processes or personnel, conditions of storage or from packaging materials. Most raw materials for pharmaceutical products support some forms of microbial growth, depending on the nutritive properties and moisture contents. Hence, dry powder or tablets are capable of undergoing some form of microbial spoilage or degradation. The more serious problem of microbial contamination of tablets is where there are no obvious signs of spoilage; hence, it is usually advisable to have knowledge of the microbial content of all drugs and medicines.

KEYWORDS: Spoilage, Therapeutic agent, Microbial attacks, toxins, quality control

Introduction

Pharmaceutical product used in prevention, treatment and diagnosis of disease contain wide variety of ingredients often in quite complex physicochemical stats. Such product must not only meet current pharmaceutical good manufacturing practice (GMP) requirements for quality, safety and efficacy, but also must be stable and sufficiently elegant to be acceptable to patients. Products made in the pharmaceutical industry today, must meet high microbiological specification i.e. if not sterile, they are expected to have no more than a minimal microbial population at the time of product release.

Nevertheless, from time to time, a few products with an unacceptable label and type of contamination will occasionally escape the quality assurance net. The consequences of such contamination may be serious and far reaching on several accounts, particularly if contaminants have had the opportunity to multiply to high level. Firstly, the product may be spoiled, rendering it can unfit for use through chemical and physicochemical deterioration of the formulation. Spoilage and subsequent wastage of individual batches usually result in major financial problems for the manufacturer through direct loss of faulty products (Baird, IMDX). Secondary, the threat of litigation and the unwanted damaging publicity of recalls may have serious economic implication for the manufacturer. Thirdly, inadvertent use of contaminated products may present a potential health hazard to patients, perhaps resulting in outbreaks of medicament related infection and ironically therefore contributing to the spread of disease.

According to Fraise et al (2009) deterioration of the preparation and a change in its organic properties as a consequence of the vital activity of bacteria and the fungi generated in it can be observed only in the case of high contamination of the raw-material or in the case of drying of the moistened semi-finished product at 40-50°C, as well as in the case of incorrect conditions of storage of the finished products (e.g. at elevated air humidity). The situation is different for liquid and safe drugs, in which one finds conditions that are more suitable for growth and propagation of microorganisms. Contamination from microorganisms is still big problem for all formulations containing moisture, but it can be a problem in solid dosage forms as well as if some natural polymers are used because many natural polymers are fertile sources of microorganisms. In the type of hygiene manufacture carried out today where "Quality Assurance" is a prerequisite as per the GMP producers, there are definite procedures to prevent microbial contamination in all formulations. Way back in the sixties, microbial contaminations of pharmaceutical formulations was a big problem. One case of outbreak of salmonellosis in Sweden was traced to the original defatted thyroid power, imported from Hungary, which was used to make the table. The pharmaceutical social of Great Britain set up a working party in 1955 to investigate microbial contamination of pharmaceutical preparation in manufacturing establishment and in hospital and retail pharmacies. This investigation shed light on a number of issues including microbial contents of some drugs and medicines and suggested many measure to reduce contamination (European pharmacopoeia, 2000).

Statement of the Problem

Most commonly, heavy contamination of products with opportunistic pathogens such as *pseudomonas*spp, has result in the spread of nosocomial (hospital-acquired) infection in immunocompromised patients. Less frequently, low levels of contamination with pathogenic organisms, such as *Salmonella*, have attracted considerable attention; as such products are contaminated with toxic microbial metabolites. The consequences of microbial contamination in pharmaceutical products are discussed in the course of this write-up.

Spoilage: Chemical and Physiochemical Deterioration of Pharmaceuticals

Microorganisms form a major part of natural recycling processes for biological matter in the environment. As such, they possess a wide variety of degradative capabilities, which they are able to exert under relatively mild physiochemical conditions. Mixed natural communities are often far more effective co-operative biodeteriogens than the individual species alone, and sequences of attack of complex substrates occur where initial attack by one group of microorganisms renders them susceptible to further deterioration by secondary and subsequent microorganisms. Under suitable environmental selection pressures, novel degradative pathways may emerge with the capability to attack newly introduced synthetic chemicals (xenobiotics). However, the rates of degradation of materials released into the environment can vary greatly, from half-lives of hours (phenol) to months ('hard' detergents) to years (halogenated pesticides). The overall rate of deterioration of a chemical will depend upon the following:

1. Its molecular structure
2. The physicochemical properties of a particular environment
3. The type and quantity of microbes present and
4. Whether the metabolites produced can serve as sources of usable energy and precursors for the biosynthesis of cellular components, and hence the creation of more microorganisms.

Pharmaceutical formulations may be considered as specializes microenvironments and their susceptibility to microbial attack can be ensured using convention ecological criteria. Some naturally occurring ingredients are particularly sensitive to attack, and a number of synthetic components, such as modern surfactants, have been deliberately produced to be readily degraded after disposal into the environment. Crude vegetable and animal drug extracts often contain a wide assortment of

microbial nutrients besides the therapeutic agents. This combined with frequently conducive and unstable physiochemical characteristics, leaves many formulations with a high potential for microbial attack, unless steps are taken minimize it.

How Are Therapeutic Agents (Drugs) Susceptible To Microbial Attacks?

Through spoilage, active drug constituents may be metabolized to less potent or chemically inactive forms. Under laboratory conditions, it has been shown that a variety of microorganisms can metabolize a wide assortment of drugs, resulting in loss of activity. Materials as diverse as alkaloids (morphine, strychnine, atropine), analgesics (aspirin, paracetamol), thalidomide, barbiturates, steroid esters and mandelic acid can be metabolized and serve as substrates for growth. Indeed, the use of microorganisms to carry out subtle transformations or steroid molecules forms the basis of the commercial production of potent therapeutic steroidal agents.

In practice, reports of drug destruction in medicines are less frequent. There have however, been some notable exceptions:

1. The metabolism of atropine in eye drops by contaminating fungi
2. Inactivation of penicillin infections by B-lactamase-producing bacteria.
3. Steroid metabolism in damp tablets and creams by fungi
4. Microbial hydrolysis of aspirin in suspension by esterase-producing bacteria.
5. Chloramphenicol acetylase-producing contaminant.

Table1 gives the types of organisms present in different sources.

Table1: sources of microbial contamination of drugs (Baird, 2004).

S/N	Source	Microbial contaminants
I	Water	Low demand gram-negative groups; Pseudomonas, Xanthomonas, Flavobacterium, Achromobacters
II	Air	Mould spores; Penicillin, Mucor, Aspergillus; bacteria spores: Baciilusspp; Yeasts.
III	Raw materials	Micrococci
V	Earths	Anaerobic spore formers; Clostridium spp, Salmonella
X	Pigments	Salmonella
L	Starches	Coliforms
C	Gums	Actinomyces
D	Animal products	Salmonella, coli forms
M	Personnel	Coli forms, staphylococci, streptococci, Corynebacterium

Micobial Toxins

Gram-negative bacteria contain lipopolysaccharides (endotoxins) in their outer cell membranes. These can remain in an active condition in products even after cell death and some can survive most heat sterilization. Although inactive by the oral route, endotoxins can induce a number of physiological effects if they enter the bloodstream via contaminated infusion fluids, even in Nano gram quantities or viadiffusion across membranes from contaminated haemodialysis solution. Such effects may include fever, activation of the cytokine system, endothelial cell damage, all leading to septic and often fatal febrile shock.

The acute bacterial toxins associated with food poisoning episodes are not commonly reported in drugs, although aflatoxin-producing *Aspergillus* have been defected in some vegetable ingredients. However, many of the metabolites of microbial deterioration have quite unpleasant tastes and smell even at low levels, and would defer most patients from using such a medicine.

Sources and Control of Microbial Contamination

1. In manufacture

Regardless of whether manufacture take place in industry or on a smaller scale in the hospital pharmacy, the microbiological quality of the finished drug will be determined by the formulation compounds used, the environment in which they are manufactured and the manufacturing process itself. According to Biard (1100v), quality must be built into the product at all stages of the process and not simply inspected at the end of manufacture.

i. Raw materials particularly water and those of natural origin must be of a high microbiological standard.
ii. All processing equipment should be subject to planned preventive maintenance and should be properly cleaned after use to prevent cross- contamination between batches.
iii. Cleaning equipment should be appropriate for the task on hand and should be thoroughly cleaned and properly maintained.
iv. Manufacture should take place in suitable premises, supplied with filtered airfor which the environmental requirements vary according to the type of productbeing made.
v. Staff involved in manufacture should not have good health but also a sound knowledge of the importance of personal and production hygiene and
vi. The end product requires suitable packaging, which will protect it from contamination during its shelf life and itself free from contamination.

a. Hospital manufacture

Manufacture in hospital premises raise certain additional problems with regard to contamination control.

i. Water

Main water in hospitals is frequently stored in large roof tanks some of which may be relatively inaccessible and poorly maintained. Water for drugs manufacture requires some further treatment, usually by distillation, reverse osmosis or deionization or a combination of these, depending on the intended use of water. Such processes need careful monitoring, as does the microbiological quality of the water after treatment. Storage of water requires particular care, as some Gram-negative opportunist pathogens can survive on traces of organic matter present in treated water and will readily multiply to high number at room temperature. Water should therefore be stored at a temperature in excess of 00°C and circulated in the distribution system at a flow rate of 1-11m/s to prevent the build-up of bacterial biofilms in the piping.

ii. Environment

The microbial flora of the hospital pharmacy environment is a reflection of the general hospital environment and the activities undertaken there. Free-living opportunist pathogens, such as *p. aeruginosa*, can normally be found in wet sites, such as drains, sinks and taps. Cleaning equipment, such as mops, buckets, cloth and scrubbing machines, may be responsible for distributing these organisms around the pharmacy. If stored wet, they provide convenient niche for microbial growth, resulting in heavy contamination of equipment.

Contamination levels in production environment may, however, be minimized by observing good manufacturing practices, by installing heating traps in sink U-bends, thus destroying one of the main reservoirs of contaminants, and by proper maintenance and storage of equipment, including cleaning equipment. Additionally, cleaning of production units by contractor should be carried out to a pharmaceutical specification.

iii. Packaging

Sacking, cardboard, card liners, corks and paper are unsuitable for packaging pharmaceuticals, as they are heavily contaminated for example with bacterial or fungal spores. These have now been replaced by non-biodegradable plastic. In the past, packaging in hospital has been frequently re-used for economic reason. Large numbers of containers may be returned to the pharmacy, bringing with them microbial contaminants introduced during use in the wards. Particular problems have been encountered with disinfectant solutions where residues of old stock have been ''topped up'' with fresh supplies, resulting in the issue of contaminated solutions to wards. Reusable containers must therefore be thoroughly washed and dried, and never refilled directly.
Another common practice in hospitals is the repacking of products purchased in bulk into smaller containers. Increased handing of the product inevitable increase the risk of contamination, as shown by one survey when hospital repacked items were found to be contaminated twice as often as those in the original pack (public health laboratory service report, IMC).

b. In use

Drug manufacturers may justly argue that their responsibility ends with the supply of a well-preserved product of high microbiological standard in a suitable pack and that the subsequent use, or indeed abuse, of the product is of little concern to them. Although much less is known about how products (drugs) become contaminated during use, their continued use in a contaminated state is clearly undesirable, particularly in hospitals where it could result in spread of cross-infection, all multi-dose drugs are vulnerable to contamination during use.
Regardless of whether the drugs are used in hospital or in the community environment, the sources of contamination are the same, but opportunities for observing it are greater in the former. Although the risk of contamination during product use has been much reduced in recent years, primarily through improvements in packaging and changes in nursing practices, it is nevertheless important to reflect upon past reported case histories.

i. Human source

During normal usage, patients may contaminate their medicine with their own microbial flora. Subsequent use of such drug may or may not result in self-infection. This is illustrated below in the mechanisms of contamination during use of medical products.

(i). Self-infection

Patient ⟷ Medicine

(ii). Cross-infection

Patient X ⟷ Medicine → Patient Y, Z

Nurses' hands

Topical products are considered to be most at risk, as the product will probably be applied by hand thus, introducing contaminants from the resident skin flora of *Staphylococci, Micrococcus* spp and diphtheroids but also perhaps transient contaminants, such as *Pseudomonas*, which would normally be removed with effective hand-washing. Opportunities for contamination may be reduced by using disposable applicators for topical products or by giving oral drugs by disposable spoons.
In hospitals, multi-dose drugs, once contaminated, may serve as a vehicle for cross-contamination or cross-infection between patients as illustrated above. Zinc-based products packed in large stockpots and used in the treatment and prevention of bedsores in long-stay and geriatric patients were reportedly contaminated during use

with *P. aeruginosa* and *Staphylococcus aureus*. If unpreserved, these products permit multiplication of contaminants especially if water is present either as part of the formulation, for example in oil water (o/w) emulsions or as a film in w/o emulsions, which have undergone local cracking or as a condensed film from atmospheric water. Appreciable numbers of contaminants may then be transferred to other patients when the product is re-used. Clearly, the economics and convenience of using stockpots need to be balanced against the risk of spreading cross-infection between patients and the inevitable increase in length of the patients' stay in hospital. The use of stockpots in hospitals has noticeably declined over the past two decades or so.

A further potential source of contamination in hospitals is the nursing staff responsible for medicament administration. During the course of their work, nurse's hands become contaminated with opportunist pathogens, which are not part of the normal skin flora but which are easily removed by thorough hand washing and drying. In busy wards, hand washing between attending to patients may be overlooked and contaminates may subsequently be transferred to medicaments during administration. Hand lotions and creams used to prevent chapping of nurses' hands may similarly become contaminated, especially when packaged in multi-dose containers and left at the side of the wash hand basin, frequently without lids. The importance of thorough hand washing in the control of hospital cross-infection cannot be overemphasized (Brannan, ıммx).

ii. **Environmental sources**

Small numbers of airborne contaminants may settle in drugs left open to the atmosphere. Some of these will die during storage, with the rest probably remaining at the static level of above ıoıı-ıoıı colony-forming units (CFU) per g or per ml. This problem is often encountered when drugs are stored in warm hospital wards by the patient or in hot steamy bathroom cupboards at home.

The indigenous microbial population is quite different in the home and in the hospitals. Pathogenic organisms are found much more frequently in the latter and consequently are isolated more often from medicines used in hospitals. Usually, there are fewer opportunities for contaminations in the home, as patients are generally issued with individual supplies in small quantities.

iii. **Equipment sources**

Patients and nursing staff may use a range of applications (pads, sponges, brushes and spatulas) during medicament (products applied directly to a part of the body) administration, particularly, for topical products. If re-used, these easily become contaminated and may be responsible for perpetuating contamination between fresh stocks of product, as has indeed been shown in studies of cosmetic products disposable applicator or swabs should be used preferably.

In hospitals today, a wide variety of complex equipment is used in the course of patient treatment. Humidifiers, incubators, ventilators, resuscitators and other apparatus require proper maintenance and decontamination after use. Chemical disinfectants used for this purpose if misused, may become contaminated with opportunist pathogens such as *P. aeruginosa* and ironically have contributed to rather than reduced the spread of cross infection in hospital patients. Therefore, disinfectants should be only for their intended purpose+ direction for use must be followed at all times.

Quality Assurance and the Control of Microbial Risks in medicines

Quality assurance (QA) encompasses a scheme of management, which embraces all the procedures necessary to provide a high probability that a medicine will confirm consistently to a specified description of quality (a formalized measure of fitness for its intended purpose). It includes formulation, design and development (R+D), good pharmaceutical manufacturing practice (GPMP), as well quality control (QC) and post marketing surveillance. As many microorganisms may be hazardous to patients or cause spoilage of formulations under suitable conditions, it is necessary to perform a risk assessment of contamination for each product. At each stage of its anticipated life from raw materials to administration a risk assessment should be

made and strategies should be developed and calculated to reduce the overall risk(s) to acceptable low levels (Fraise et al, 2007).

The risk of microbial infection and spoilage arising from microbial contamination during manufacture, storage and use could be eliminated by presenting all medicines in sterile, impervious, single-dosage units. However, the high cost of this strategy restricts its use to situations where there is high risk of consequent infection from any contaminants. Where the risk is assessed as much lower, less efficient but less expensive strategies are adopted. The high risk of infection by contaminants in parenteral medicines combined with concerns about the systemic toxicity of preservatives almost always demands sterile single-dosage units. With eye drops for domestic use the risks are perceived to be lower and sterile multi-dose products with preservatives to combat the anticipated in-use contamination are accepted; sterile single-dose units are more common in hospitals where these is an increased risk of infection.

Oral and topical routes of administration are generally perceived to present relatively low risks of infection and the emphasis is more on the control of microbial content during manufacture and subsequent protection of the formulation from chemical and physicochemical spoilage. As part of the design process, it is necessary to include features in the formulation and delivery system that provide as much suitable protection as possible against microbial contamination and spoilage (Baird et al, 2000).

Quality Control Procedures

While there is general agreement on the need to control total microbial levels in non-sterile medicines and to exclude certain species that have previously proved troublesome, the precision and accuracy of current methods for routing (or even detecting) some microbes in complex products are poor. Pathogens, present in low numbers and often damaged by processing can be very difficult to isolate. Products showing active spoilage can yield surprisingly low viable counts on testing. Although present in high numbers, a particular organism may be neither pathogenic nor the primary spoilage agent, but may be relatively inert e.g. un-germinated spores or a secondary contaminant, which has outgrown the initiating spoiler. Unevenly distributed growth in viscous formulations will present serious sampling problems. The type of culture medium (even different batches of the same medium) and conditions of recovering and incubation may greatly influence any viable counts obtained from products. An unresolved problem concerns the timing of sampling. Low levels of *pseudomonas* shortly after manufacture may not constitute a spoilage hazard if their growth is checked. However, it unchecked, high levels may well initiate spoilage. Thus, for a medicine to be administered orally, there should not be more than 10^3 aerobic bacteria or 10^2 fungi per gram or cm^3 of product, and there should be an absence of *Escherichia coli*. Higher levels may be permissible if the product contains raw materials of natural origin (Baird, 2007).

Conclusion

Spoilage organisms of drugs are encountered through three main sources: from raw materials for manufacturing, hospital environments and in-use process. Thus, initial stability tests should show that the proposed drug can withstand an appropriate microbial challenge, raw materials should be from an authorized supplier and should comply with in-house microbial specifications, environmental conditions appropriate to the production process should be subject to regular microbiological monitoring, and finally, end product analysis should indicate that the product is microbiological suitable for its intended use.

Recommendations

The following are recommended:

i. Efforts should be made to ensure that good manufacturing practice and aseptic procedures are maintained in handling drugs.
ii. National Agency for food, Drug Administration and Control (NAFDAC) in Nigeria should monitor this compliance.

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