Original Article

Recovery and detection of fungal contaminants in some ointments and tablets after opening of the packages in hospitals

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Abstract

Background and purpose: Tablets and ointments are used to prevent, treat, and diagnose diseases in hospitals. Although it seems that these medications are sterile in the path of the building and packaging, their mishandling or wrong application method can cause them to be contaminated. Hence, the preservation of pharmaceutical forms from contamination before and after opening the cover in hospitals is an essential measure to be taken in health care. The objective of the present study was to investigate the challenges in fungal contaminants detection and recovery in some pharmaceuticals that were high intake for patients.

Materials and Methods: This study was conducted in 4 teaching hospitals on 4 types of tablets and 3 types of ointments that were high intake for patients in hospitals before and after opening and usage in Sari, Iran. Fungi were identified by using standard mycology procedures.

Results: The results showed that among the samples of tablets after opening the cover in the delivery room and carrying them in container by trolley, and the samples of ointments after opening and usage, the contamination rates were 70.3% and 94.4-100%, respectively. *Aspergillus* species such as *A. flavus* and *A. fumigatus* were the most mold species and *Rhodotorula spp*. was the most yeast species isolated. However, it was documented that 16.7% of certain pharmaceuticals had fungal contamination ahead of opening.

***Conclusion*:** The results showed the contamination of ointments and tablets used in hospitals after opening the cover. Although the source of contamination was not investigated in the present study, the findings revealed that most of the contaminations could be due to the storage period and mishandling in pharmacies and wrong application methods after opening. Some isolated fungi can also be harmful to patients who have a weakened immune system.

***Key words*:** Pharmaceuticals; Ointment; Tablet; Fungal contamination; Health care; Drug contamination hazard

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1. Introduction

Pharmaceutical products such as oral dosage or ointments are used in a variety of ways for prevention, treatment, and diagnosis of diseases in hospitals (1). The manufacturers have improved the quality of these products by sterilizing procedures (2, 3). Although these medications are supposed to be sterile in the process of development and packaging, their mishandling or wrong method of use can cause them to become contaminated (4). It is necessary to know the common sources of microbial contaminants in manufacturing or storing environment and the typical organisms that might arise from each source (5). The environment influences the microbial quality of pharmaceuticals and quality of the raw materials used during formulation (6). All natural organic compounds are at the risk of degradation, and even synthetic compounds could be attacked, though in a less amount (7, 8). It is also useful to know about the rate of contamination and frequency of those organisms in pharmaceutical materials. Some infectious occurrences have been associated with the use of contaminated raw materials of natural origin (9). There are a large number of studies proving the incidence of mycotic contamination of pharmaceutical products, and referring to the fact that contaminants vary in their form of true pathogens and opportunistic pathogens. Despite this research background, few accounts of fungal degradation of pharmaceuticals and cosmetics have been published (10, 11, 12). Several studies have been published describing clinical hazards due to microbiologically contaminated pharmaceuticals (13-17). Contamination of pharmaceuticals with fungi can change physicochemical characteristics of the medicines and may be harmful or pathogenic (18). Spores of fungi can be found in dust particles in the atmosphere or on floors, work surfaces or equipment. Modern pharmaceutical factories or pharmacies are supplied with filtered air, so the level of particulate contamination in the atmosphere in a room is usually very low (19). Several species of fungi specially *Aspergilus flavus* produce toxic molecules and may render a product dangerous if they grow in it under conditions supporting toxin production (20). Aflatoxins are heat-stable compounds which exhibit potent toxic and carcinogenic properties in human and animals, which could also be produced by some fungi particularly Aspergillus species (21). The growth of these fungi occurs under poor storage conditions, and it is observed that toxic doses of aflatoxin accumulate in the contaminated materials (22). In tropical areas, pharmaceutical preparations may be kept under uncontrolled conditions and be dispensed in non-protective packaging or even with no packaging at all, where the average temperature is 31°C and the average relative humidity is 75% (23). In hospital pharmacies, clinics and nursing homes, tablets and capsules are usually stored in large packs. Hence, if pharmaceutical products are contaminated with potential pathogens, they are not obviously fit for use (24). However, in the present study, the main focus was on the type of contamination caused by fungi and the fungal degradation of pharmaceuticals or cosmetics. This emphasis was mainly because the preservation of pharmaceutical forms from contamination before and after opening the

cover in hospital is a necessity in health care about which relatively few accounts have so far been published.

Microbial contamination of medicines arises from three principal sources: 1) raw materials including water, from which the product is manufactured; 2) environment including the atmosphere, equipment and work surfaces; 3) manufacturing, pharmacy, and healthcare personnel or patients.

Raw materials may vary in their extent of microbial contamination if their origins are different (25, 26). Materials with natural origin such as gelatin, starch, talc, kaolin, and bentonite may show a little higher contamination than those for synthesized chemicals. Despite the application of cleaning and purification procedures such as heat, extremes of pH or organic solvents, pharmaceuticals may be contaminated by high levels of microorganisms to be found in the atmosphere, equipment and work surfaces, spores of fungi attached to dust particles, suspended in the atmosphere, or settled onto floors, work surfaces or equipment. Modern pharmaceutical factories or pharmacies are supplied with filtered air, so the level of particulate contamination in the atmosphere in a room is usually very low (27). Operators' skin scales are constantly shedding particles with attached skin fungi; these are typically about 20 µm in size and so cannot be seen with naked eyes. Many factors such as the design and coverage of protective clothing, personal hygiene and, in particular, levels of activity or motion can influence the extent to which skin scales are shed (5, 28). Washing with disinfection solution reduces the number of microorganisms on the skin, but is by no means totally effective (29). If fungal spores influence the pharmaceutical products, factors such as nutrient availability, temperature, pH, redox potential and the presence and concentration of antimicrobial chemicals can affect the growth and development of fungi.

2. Material and Methods

The study was carried out at four teaching hospitals in Sari, Iran. This research was conducted on four types of tablets and three types of ointments that were high intake for patients in hospitals. For indicating different manufacturers, each sample was given a code. Four high intake tablets included Acetaminophen (N=27), Ranitidine (N=36), Acetylsalicylic acid (ASA) 80 (N=18), and vitamin C (N=12). Three high intake ointments were Zinc oxide (N=18), Tetracycline (N=18), and Betamethasone (N=6). In the present research, for comparing the fungal contamination of tablets and ointments, before and after opening the coverage and transporting from delivery rooms to patients' rooms in unsterile container or procedure, the data were collected from different pharmacies in the city. Identifying the isolated fungi on mycological culture media was also done by using standard mycological procedures.

3. Results

165 samples (60 ointments and 105 tablets) produced by three different manufacturers were purchased from three teaching hospitals and pharmacies of Sari. Table 1 shows the percentage of fungal contamination of different pharmaceuticals products before and after opening the coverage. Among tablets, ASA and among ointments, Betamethasone had the most fungal contamination before opening the coverage. However, among tablets, Acetaminophen and among ointments, Betamethasone and Tetracycline showed the most fungal contamination after opening the coverage.

Table 1. percentage of fungal contamination of different pharmaceuticals products before and after opening coverage

Type of Pharmaceuticals products		Fungal contamination before opening of coverage	Fungal contamination after opening of coverage
tablet	Acetaminophen	0%	92.6%
	Ranitidine	0%	66.7%
	ASA	16.7%	50.0%
	Vitamin C	8.3%	66.7%
ointment	Zinc oxide	0%	94.4%
	Tetracycline	0%	100%
	Betamethasone	16.7%	100%

In ointments group opened and used to patients in hospitals, among mold fungi, *Aspergillus flavus* (88.1%), *Aspergillus fumigatus* (52.4%) and among yeast, *Rhodotrola spp*. (52.4%) were the most common contaminants. Candida, that has ability to cause pathogenic yeast infection, was isolated from 23.8% of ointment samples used in hospital after opening (Chart 1). 16.7% of Betamethasone ointment (code B) obtained from different pharmacies have shown fungal contamination with Aspergillus fumigatus before opening coverage prior to expiration date.

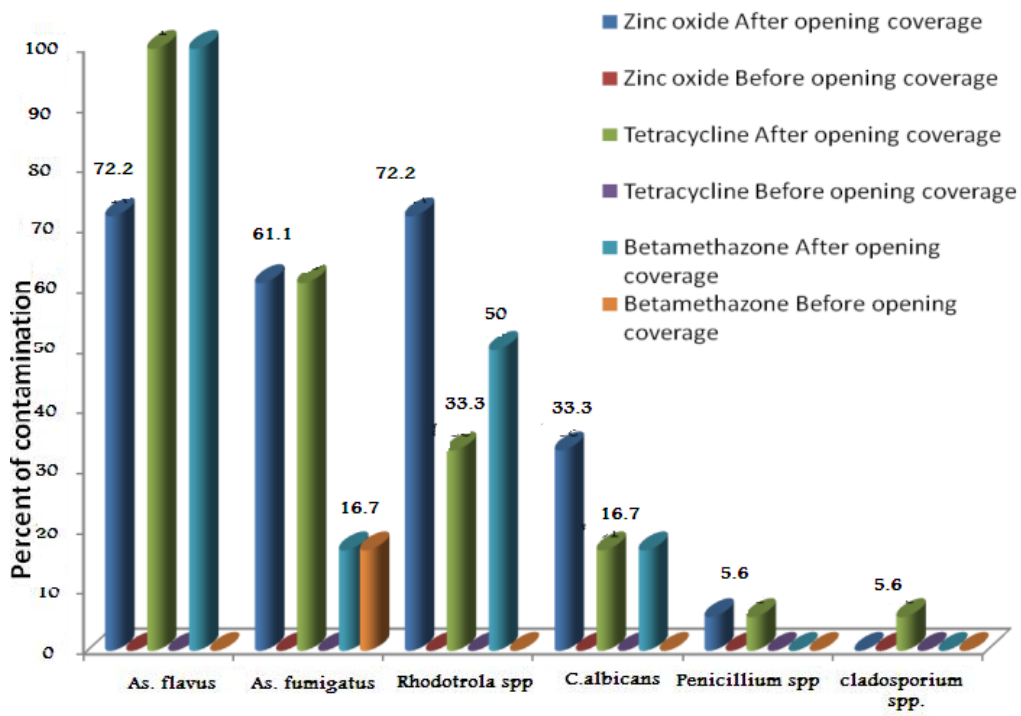

Figure 1. Percentage of contamination to a variety of fungal species in three ointments (before and after opening the coverage)

In tablets group, after opening the coverage and transporting from delivery rooms to patients rooms in unsterile container or procedure in hospitals, among mold fungi, *Aspergillus flavus* (34.4%), *Aspergillus niger* (24.7%) and among yeast, *Rhodotrola spp*. (8.3%) were the most common contaminants. *Candida albicans* was not isolated from tablet samples that were used in the hospital after opening. *Penicillium spp.* and *Cladosporium spp.* were also isolated from some samples (both ointments and tablets) in less degrees (Table 2).

Table 2. Percentage of contamination to a variety of fungal species in 4 tablets (before and after opening coverage)

Type of tablets	After / Before opening coverage	A. flavus	A. fumigatus	A. niger	Rhodotrola spp.	Candida albicans	Penicillium spp.	Cladosporium spp.
Acetaminophen	after	72.2%	61.1%	7.4%	72.2%	33.3%	5.6%	0%
	before	0	0	0	0	0	0	0
Ranitidine	after	100%	61.1%	27.7%	33.3%	16.7%	5.6%	5.6%
	before	0	0	0	0	0	0	0
ASA	after	100%	16.7%	22.2%	50%	16.7%	0	0
	before	0	16.7%	0	0	0	0	0
Vitamin C	after	29.6%	18.5%	41.7%	0	0	25.9%	7.4%
	before	8.3%	8.3%	0	0	0	0	0

4. Discussion

Pharmaceutical contamination is a health hazard to a patient, although the extent of the hazard depends on the types and numbers of organisms present, the route of administration, and the resistance of the patient to infection (25). Invasive fungal infections with high mortality rates can be found in hospital settings, especially in intensive care units where patients may be immune-compromised, due to invasive procedures and treated by antibiotics. Usually the fungi are passed on ointments or tablets from the hands of medical personnel, patients or the general hospital environment, and occasionally pharmaceutical drug products.

The number of fungal species isolated in the study was higher than that reported earlier by other authors (7, 30, 31). This may be due to the application of standard mycological procedure to isolate and distinguish fungal species in the current study. The most common nosocomial fungal infections are due to the genera Candida and Aspergillus and other less frequently isolated moulds (32). It was also observed that some of the ointments and tablets were contaminated by *Aspergillus fumigatus* and *Aspergillus flavus* before and after opening the coverage. The presence of some fungi in pharmaceutical products before opening the coverage reflects the equipment and raw material quality, poor hygiene of the personnel during production, and the storage quality of the preparations. Degradation of pharmaceutical products could affect therapeutic properties of the product and may discourage the patient from taking the medication (33, 34). Some fungi can also be harmful by producing metabolites that may be toxic to consumers (21), and some mycotoxines produced by *Aspergillus flavus* and some *Penicellium spp.* cause rapid deterioration of the product (11, 35). An opportunist fungus such as *Aspergillus species* causes a wide range of human diseases depending on the immune

status of the host (36). Among the pathogenic species of Aspergillus, *A. fumigatus* is the primary causative agent of human infections, followed by *A. flavus* and *A. niger* (37). Profoundly immunocompromised patients, particularly those with hematological malignancies or who have undergone transplantation, are at the risk of most severe cases of Aspergillus-caused infections (38, 39). In the present study, it was also documented that other molds such as *Penicillium spp*. and *Cladosporium spp.* were drug contaminant after opening the coverage of some pharmaceuticals. In Iran, several studies have investigated the presence of fungi in the air and equipment of hospitals (40-45). In these studies, *Cladosporium spp*., *Aspergillus spp*. and *Penicillium spp.* were identified as the most frequent fungi in the air and equipment of hospital operating rooms and different wards. In the world, *Penicillium spp*. has been isolated from patients with keratitis (46-49), ear infections (50–53), pneumonia (54-56), endocarditis (57, 58), peritonitis (59, 60) and urinary tract infections (61, 62). Penicillium infections are most commonly exhibited in immunosuppressed individuals (63, 64), while *Cladosporium spp.* are the causative agents of skin lesions (65, 66), keratitis (67), nail fungus (68), sinusitis (69), asthma (70) and pulmonary infections (71,72) in human. The most common symptoms of exposure to *Cladosporium* are edema and bronchio-spasms, which may lead to pulmonary emphysema (73). *Candida albicans* that can be human borne and *Rhodotrola* spp., were the frequent yeast contaminants of pharmaceuticals. During the study, these yeasts were isolated from some pharmaceuticals after opening the coverage, too. Oxidative yeasts in acidic product can also cause a rise in pH level by utilizing organic acids causing bacterial growth. A typical of spoilage by yeast is an alcoholic odor produced from fermentable substrates (7). Some researchers found that *Candida albicans* as the most important nosocomial fungal pathogen can survive up to 4 months on surfaces (74). *Candida albicans* is an opportunistic fungal pathogen found as part of the normal microflora on the human skin and digestive tract. However, if the host defense system is weakened, or host ecological environment is changed, it can cause the transformation of *C. albicans* into a pathogen capable of causing infections that may be fatal (75). Although recent studies revealed that some nosocomial Candida infections may act like minor epidemics through the selection of more virulent species (76), it is often the endogenous organisms that are the main sources of infection. However, it should be noted that *C. albicans* is able live in harmony with the host within the resident complex microflora on body surfaces (77).

Rhodotorula spp. is as emerging yeast pathogens in humans in recent years that can be recovered from some environmental sources and may be found in pharmaceutical products (78, 79). Most infection due to Rhodotorula in humans is found to be fungemia associated with central venous catheter (CVC) use (80, 81). In addition, *Rhodotorula spp*. have the ability to cause diseases such as meningeal, skin, ocular, peritoneal, and prosthetic joint infections and they are not necessarily linked to the use of CVCs or immunosuppression (82). So, the application of drug contaminated by

Rhodotorula in patients admitted to hospital due to debilitating diseases may result in the emergence of infection. In the current research, it was also found that the rate of fungal contamination in ointments was bigger than tablets after opening the coverage. This may be due to the fact that ointments are fatty base or emulsions of water-in-oil and fungal elements have better growth in these pharmaceutical products (7). On the other hand, fungi require water activity levels at around 0.7 for the growth to occur (83). Dry tablets often have lower water activities which leads to the prevention of proliferation of fungi. However, unlike a preservative, it does not kill the microorganisms which could be found in the tablet. Typically, fungi and fungal spores can survive at extremely low water activity levels (84). Hence, any pharmaceutical ointment, even manufactured in the industrial environment has the potential to be contaminated by fungi. Furthermore, microbial contamination in sterile products before opening will be an unacceptable risk the application of which can cause a harm to a patient. Most reports related to the contamination of pharmaceutical products are concerned with bacterial contamination rather than fungi. This may be due to the fact that there are few trained mycologists in microbiology laboratories in pharmaceutical organizations. In a related work, Adenike Okunlola et al. in 2007 investigated the microbial characteristics of twenty different pharmaceutical products which were produced in southwestern Nigeria (85).

5. Conclusion

The findings of the present study revealed that the contamination risk posed by fungi to pharmaceuticals is greater than when they are opened and transported from delivery rooms to patients' rooms in unsterile container or procedure. In addition to this investigation, further microbial examination of the other creams and ointments will definitely increase the actual setting of microbial safety. Microbiological safety is one of the most vital of pharmaceutical products quality parameters. The results of the current research also showed that microorganisms such as *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Penicillium spp*., *Cladosporium spp*., *Rhodotrola spp.* and *Candida albicans* were contaminant of ointment and tablet products. As these pharmaceutical products should be produced under sterile conditions, appropriate control of many factors involved in the microbiology of the products is necessary. These factors include the quality of raw materials, training of manufacturing personnel, application of standard cleaning and sanitization processes, application of general chemical /physical factors including heat, time temperature, pH, and the use of appropriate barrier packaging. Thus, the current study was highly suggestive of randomized microbiological testing of topical or oral dosage products sold in the delivery rooms of pharmacies and hospitals in order to ensure consumer safety.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

SRA designed, developed the original idea and the protocol, designed training program, re-analyzed statistical data in collaboration, drafted and approved the manuscript. AHN and MRJA participated in the search of databases, sampling, examination and data extract. MA collaborated in re-evaluated the data, read and approving of the final manuscript.

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