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The Effect of Gamma Radiation on Cephalosporin and Amino glycosides Antibiotics

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Abstract: The microbial contamination of pharmaceutical preparations is considered to be a common problem which has been reported for several medicaments. Thus, many of the ingredients used in pharmaceutical formulations can become substrates for microorganisms when the right conditions are present**.** In this regard, one hundred antibiotic samples were collected from the Egyptian pharmacies. These samples were tested for their microbial load. Then, the isolated organisms were identified. After that, the active ingredients of the drugs were irradiated in order to determine the sterilization dose. The efficiency of the sterilization was tested using organoleptic properties (e.g., color, solubility, pH measurement, HPLC (High Performance Liquid Chromatography)), which were used in order to determine the effect of sterilization on the drug structure and the microbial load. The results revealed that the fungal isolates were more predominant than bacteria, with the percentage of 88.3% and 11.7% respectively. The dose response curve for the two fungal isolates MAM-F15 and MAM-F48 showed that 6.0 kGy and 5.0 kGy reduced the viable count of the two fungal isolates completely. Thus, the dose of 5.0 kGy reduced the viable count of MAM-F15 by 4.48log cycles, while 4.0 kGy reduced the viable count of MAM-F48 by 3.58 log cycles. The doses, tested for the sterilization of active ingredients of Amikacin, Ceftazidime and Cefotaxime, were 3.0, 5.0, 7.0, 10.0 and 25.0 kGy. The dose of 3.0kGy was sufficient to reduce the microbial load. On the other hand, the higher gamma radiation doses could not degrade the chemical structure of the active ingredients as determined by HPLC, indicating the stability of the drugs for sterilization by the gamma radiation.

Keywords: Cephalosporin, Amikacin, Gamma Radiation, Sterilization, Fungi.

1 Introduction

The microbial contamination of pharmaceutical preparations is considered as a common problem, which has been reported for several medicaments [1,2].The contaminants may evolve from the raw materials, manufacturing machines, production atmosphere, water used in product development, the persons conducting the process, the container into which it is finally filled and sealed, poor preservative system, and even during storage and use [3,4]. In addition, the environmental parameters including temperature, relative humidity, differential air pressures, air turbulence and the status of high efficiency particulate air filters may also have a significant impact on the microbial access into the pharmaceutical products and premises[4,5]. That is to say, the microbial contamination has a huge impact on drug manufacturing in light of the fact that it can reduce or even eliminate the therapeutic effect of drugs, or even cause drug-induced infections. The microbes presented in drugs, not only make them hazardous from the infectious point of view, but may also change the chemical, physical and organoleptic properties of the drugs, or even change the contents of the active ingredients. Furthermore, the microorganisms can convert the drugs into toxic products [6,7]. Most of the microorganisms contaminating the non-sterile pharmaceutical products may be nonpathogenic commensals of environmental origin; they pose significant problems as agents of spoilage [8], although the presence of even a low level of pathogenic microorganisms, higher levels of opportunistic pathogens, or bacterial toxic metabolites - which persist even after the death of the primary contaminants - can lead to the fact of

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the product being ineffective. Not only the presence of micro organisms causing undesirable bacterial infections is harmful, but the presence of metabolites/toxins may also cause bad symptoms, even if they are included in small amounts. Some of these toxin-related diseases include abdominal pain. However, the symptoms vary from mild gastric distress to death[6,7]. In addition, the microbial contamination may also affect the odor, color, taste, etc. of the products, thus rendering them unacceptable for use by patients [3,9]. Many of the nonpathogenic organisms may become opportunistic pathogens with the compromised individuals [8]. Furthermore, the cross-contamination of medicines contributes to most of the nosocomial infections occurring in hospitals [10,11], as there have been reports of drug-borne human infections worldwide [12,13]. Several different cases of infections caused by the use of contaminated medicaments have been reported [7,14,15]. Thus, the microbial contamination of non-sterile products has become one of the major reasons for product recalls and production shutdown at the beginning of the 21st century [16,17]. In the United States, the biopharmaceutical research companies invested approximately 48.5 billion USD in new Research and Development (R&D),and the Food and Drug Administration (FDA) approved 44 new medicines in 2012[4,18]. Therefore, there is a need to know the microbial content of all drugs and medicines, whether they are sterile or non-sterile [6,19],so that rapid corrective actions could be taken in order to prevent any further contamination of production samples, huge financial losses, and release of contaminated product that can cause disease to consumers [16,20].In addition, the pharmaceutical products can also be contaminated with pathogens that may include bacteria, fungi, yeasts or molds[3]. The *in vitro* studies revealed that a variety of microorganisms can metabolize a wide range of drugs as their substrates under the right conditions, including water activity, pH and temperature, which in turn results in the loss of the drug potency [4,20,21]. Molds can produce mycotoxins which can be carcinogenic and mutagenic. They can also cause acute and chronic poisoning, allergies, diseases of the respiratory and digestive systems, and liver damage [7,22,23].

In modern medicine, a number of sterilization methods are applied, including tempering, cauterization, hot air sterilization, steam sterilization, sterile filtration, radiation sterilization (e.g., by ionizing radiation or UV light), gas sterilization (e.g., by ethylene oxide or formaldehyde), and chemical sterilization [24,25]. Radiation sterilization - as a physical treatment - has gained much interest in the sanitization/sterilization processes for food, drugs, pharmaceutical systems and medical supplies[26]. The necessity of studying the effect of radiation on each drug molecule arises from the fact that the radiation effect cannot be generalized for a class of compounds. Therefore, there is a real need to look into the various degradation products formed on irradiation in different drugs, and into the factors which could minimize such degradation in order for the successful employing of the radiation sterilization [27,28].

On the foregoing, the main aim of the current study is to determine the microbial load of some antibiotics present in the Egyptian pharmacies, and to evaluate the efficiency of gamma radiation on the sterilization of these antibiotic active ingredients and its effects on the drug structures.

2 The Experimental Section

2.1 Materials

2.1.1. Antibiotics Collection

A total number of 100 cephalosporin and amino glycoside antibiotics samples were collected from the different Egyptian pharmacies. They comprised sixty (60) of third generation of cephalosporin, twenty (20) of fourth generation of cephalosporin and twenty (20) of amino glycoside. In addition, these antibiotics included both locally manufactured and imported products by five different pharmaceutical companies. Thus, the samples were randomly purchased from twenty-one private pharmacies in three different locations in Cairo, Egypt; and that is at the period from January 2013 to March 2013, then from January 2014 to March 2014. The average number of samples per one pharmacy was approximately four. On the other hand, the active ingredients of the tested drugs were kindly supplied by EIPICO (Egyptian International Pharmaceutical Industries Company). That is to say, the selected drug samples are representative products that are already available and commonly used in the community; and all of them were vial for I.V injection.

2.1.2. Used Media

The media used for the microbiological evaluation were: Luria Bertani Agar (LB), Sabouraud's Dextrose Agar and Malt Agar Media [29].

2.1.3. Gamma Irradiation

The source of the used irradiation was Indian Chamber of Cobalt-60, as an ionizing radiation source at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Nasr City, Cairo, Egypt; and the used dose rate was (1.0 kGy/25 min) at the time of the experiment.

2.1.4. Preparation of Spore Suspension

According to Abu- State [30], 250 ml Erlenmeyer flasks containing 100 ml of SabouraudAgar Medium were inoculated by the fungal isolates. Then, the inoculated media were incubated at 28°C for 7 days, and then the spores were collected by adding 30 ml of sterile saline containing 0.1% (v/v) Tween-80. After that, the spore suspension of each flask was collected in a new sterile flask to form a pool as stock for inoculation.

2.1.5. HPLC Analysis

The analytical determination for the irradiated (10 and 25 kGy) and non-irradiated control have been determined by High Performance Liquid Chromatography at the Regional Center for Food & Feed, Agricultural Research Center, Giza, Egypt. Thus, the conditions of the experiment were as follows: the system of HPLC (Agilent technologies 1200 series), LC-MS/MS 4000 Q-trap (Applied bio-systems), MDS SCIEX,LC Column (Agilent C18 150x4.6mmx5 um). In addition, the mobile phases were as follows: Mobile Phase A: (Water +0.1% formic acid), and Mobile Phase B: (Methanol +0.1% formic acid) or (Acetonitrile + 0.1% formic acid). Finally, the separation was performed at the ambient temperature at a flow rate of 500µl/min.

2.2 Method

2.2.1. Microbial Load Determination

First, a half gram was diluted with 4.5 ml of sterile saline and vortex for 1 min, and then serially diluted with sterile saline as described in the British Pharmacopoeia [31]; thus, the sterile saline was used as a negative control [6]. Then, the viable count was evaluated using the surface plate method on LB Agar (for bacteria) and Sabouraud's dextrose agar (for fungi). According to Abo-State *et al.***[**1], the aliquot of 100µlfor each sample was directly spread on the surface of sterile LB and Sabouraud media. In addition, duplicate plates were used for each sample. The inoculated plates were subsequently incubated at 37°C for 48 hours for bacteria, and at 28 °C for 7 days for fungi. Then, the viable microbial counts were determined. Thus, the frequency of contamination by each group (%) = number of contaminated samples / total number of samples x100 **[**13,32].

2.2.2. Isolation and Identification of the Microbial Contaminants

The separated single microbial colonies were isolated from the plates and sub-cultured on LB agar and Sabouraud agar media for purification. Then, the pure separated single colonies were finally preserved for identification purpose and further use [6]. The identification of the selected fungal isolates was performed according to the Database Identification Program of the Regional Center for Mycology and Biotechnology (RCMB)**,** Al-Azhar University**,** Nasr City, Cairo, Egypt; and that is on the basis of cultural characters (color, shape, surface and reverse pigmentation), as well as the microscopic structure (septate or non-septate hyphae, structure of hyphae, and conidia).

2.2.3. Determination of the Dose Response Curve

The spore suspensions of isolates were distributed in clean sterile screw cap test tubes; thus, each tube contained 5.0 ml of spore suspension. Then, the spore suspensions of each isolate were exposed to different doses of gamma radiation (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 kGy) from a Cobalt-60 gamma source; while the control (0 kGy) was left without irradiation. After that, two replicates were used for each dose as well as for the non-irradiated control [1]. The irradiated suspensions and the non-irradiated control were serially diluted and inoculated on the surface of Sabouraud agar plates (surface plate technique). Then, the plates were incubated at 28°C for 7 days before counting. After that, the count (spore/ml) was determined; thus, the log number of survivors was plotted against the absorbed radiation dose in kGy (the dose response curve) [1,23].

2.2.4. Sterilization of Antibiotics by Gamma Radiation

The three selected active ingredients were Ceftazidime Pent hydrate and Cefotaxime Sodium which belong to the third generation cephalosporin antibiotics, and Amikacin Sulphate which belong to the amino glycoside antibiotic. They were all in the powder form; thus, approximately 0.5 g of each substance was directly transferred to the Eppendorf tube for irradiation treatment. Then, they were exposed to different doses of gamma irradiation (0, 3.0, 5.0, 7.0, 10.0 and 25.0 kGy), using a Cobalt-60 gamma source. After that, the irradiated and non-irradiated samples were tested for organoleptic analysis, chemical and microbiological tests. The non-irradiated samples were used as controls in order to detect the physicochemical and microbial activity changes resulting from the action of ionizing radiation on the studied samples [33].

2.2.4.1. Organoleptic Analysis

The irradiated samples were subjected to organoleptic analysis in order to determine the change in its color, form, odor, solubility and clarity of solution, compared to those of the non-irradiated sample [33, 34].

2.2.4.2. Measurements of pH

The measurements of the pH values for the irradiated and control (non-irradiated) active ingredients were performed using the pH meter (Thermo Scientific, Singapore).

2.2.4.3. Microbiological Sterility Test

A half gram of each sample (irradiated and non-irradiated samples) was added under aseptic condition to 4.5 ml sterile saline in the test tubes. Then, the tubes were shaken, and then 100 µl suspended samples were plated on the surface of the Sabourad agar plates; thus, two plates were used for each dose. Then, they were incubated for 7 days, at 28 °C, and the microbial count as (CFU/gm) was determined [1].

3 Results and Discussion

3.1. Determination of the Microbial Load of the Samples

The microbial load of samples was counted through the surface plate method in terms of CFU/gm. Thus, the antibiotic samples of the third generation cephalosporin generally had microbial counts that ranged from $0.5x10^2$ to $13.5x10^2$ CFU/gm. However, in case of Cefotaxime, out of

20 samples, only seven samples did not contain any bacterial and fungal contaminants, i.e. they were completely sterilized. On the other hand, (11) samples were contaminated with fungi, as the fungal count varied from $0.5x10^2$ to $7.5x10^2$ CFU/gm; and (2) samples contained bacterial contaminants with $0.5x10^2$ and$1.0x10^2$ CFU/gm. As for Ceftazidime, out of 20 samples, only (14) samples were sterile, while the fungi were isolated from (5) samples with a count ranging from $0.5x10^2$ to $1.0x10^2$ CFU/gm; and the highest number was recorded from one sample of them, which was $6.5x10^2$ CFU/gm. In addition, one sample was contaminated with bacteria with the count of $0.5x10^2$ CFU/gm. As for Ceftriaxone, out of 20 samples, only (13) samples were completely sterile; thus,(6) samples contained fungal contaminates varying from $0.5x10^2$ to $13.5x10^2$ CFU/gm, while (1) sample contained bacterial and fungal contaminants with the count of $1.5x10^2$ CFU/gm for both of them. On the other hand, the samples of the fourth generation cephalosporin antibiotics were also tested for the microbial load. Out of 20 samples of Cefepime, only seven samples were completely sterile; thus, ten samples were contaminated with fungi, and their count ranged from $0.5x10^2$ to $2.0x10^2$ CFU/gm; where (3) samples were contaminated with bacteria and their count ranged from $0.5x10^2$ to $1.0x10^2$ CFU/gm. As for the 20 samples of Amikacin (Aminoglycoside) antibiotics, all samples did not contain bacterial contaminates, and (13) samples were completely sterilized as they did not contain any bacterial and fungal contaminants; while (7) samples had fungal contaminates ranging from $2.0x10^2$ to $8.0x10^2$ CFU/gm as indicated in Tables (1 & 2).

According to the number of the contaminated samples and the number of isolates for the third generation cephalosporin antibiotics, out of 20 samples of Cefotaxime, only(13) samples were contaminated with a percentage of (65%), and they gave twenty-nine isolates of bacteria and fungi. In addition, out of 20 samples of Ceftazidime, only six samples were contaminated with a percentage of (30%), and the total number of isolates was eight; and out of 20 samples of Ceftriaxone, only(7) samples were contaminated with a percentage of (35%), and they gave (12) isolates. As for the fourth generation cephalosporin antibiotics, in case of Cefepime, out of 20 samples, there were (13) contaminated samples with a percentage of (65%) and the total number of isolates was (20). As for the amino glycosides antibiotics, out of 20 samples of Amikacin, only(7) samples were contaminated with a percentage of (35%), and the total number of isolates was (8) isolates of fungi as indicated in Table (1).

Overall -according to the type of isolates- the fungi were the common isolates, which contaminate thirty-nine samples of one hundred with a percentage of (39.0%), and the bacteria was next to the fungi, as it contaminated (6) samples of one hundred with a percentage of (6.0%);in addition, a mixed growth of both bacteria and fungi was observed in one sample only with a percentage of (1.0%). Therefore, the total number of contaminated samples with bacteria and fungi were (46) samples with a percentage of (46.0%), where (68) isolates were fungi and (9) isolates were bacteria; consequently, the total number of isolates were (77) isolates as indicated in Table (1).

The distribution of fungal counts in the tested samples is summarized in Table (2). As for the fungi, about (54%) of the tested samples had no fungal count, while about (18%) of the tested samples contained fewer than or equal to $0.5x10^2$ CFU/gm. In addition, about (9%) of the contaminated samples contained fewer than or equal to $1.0x10^2$ CFU/gm, while about (3%) of the contaminated samples contained fewer than or equal to $1.5x10^2$ CFU/gm. Furthermore, about (16%) of the contaminated samples contained more than $1.5x10^2$ to $13.5x10^2$ CFU/gm.

On the foregoing, the total numbers of isolates were (77) isolates. Thus, the diversity of the isolates shows that there are (66) isolates of fungi with a percentage of (85.7%), and (2) isolates of yeast with a percentage of (2.6%). On the other hand, there are (4) bacterial isolates with a percentage of (5.2%), and (5) isolates of actinomycetes with a percentage of (6.5%).

Table (1): Microbial Contamination of the Collected Antibiotic Samples

Groups of Antibiotics	Active ingredients	Total No. of samples	No. of samples contaminated with					No. of isolates		
			Fungi	Bacteria	Fungi and Bacteria	Total		Fungi	Bacteria	Total
						No.	%			
Third generation Cephalosporin Antibiotics	Cefotaxime	20	11	2	0	13	65.0%	26	3	29
	Ceftazidime	20	5	1	0	6	30.0%	7	1	8
	Ceftriaxone	20	6	0	1	7	35.0%	11	1	12
	Total	**60**	22	3	1	26	43.3%	44	5	49
Fourth generation Cephalosporin Antibiotics	Cefepime	20	10	3	0	13	65.0%	16	4	20
	Total	**20**	10	3	0	13	65.0%	16	4	20
Aminoglycosides Antibiotics	Amikacin	20	7	0	0	7	35.0%	8	0	8
	Total	**20**	7	0	0	7	35.0%	8	0	8
Total		100	39	6	1	46	46%	68	9	77

Table (2): Distribution of Fungal Counts in Different Tested Antibiotics

Antibiotics	Total No. of samples	Number of items with fungal counts (CFU/ gm) of									
		0.00		≤0.5x10²		≤1.0x10²		≤ 1.5 x10²		>1.5 x10²-13.5x10²	
		No.	%	No.	%	No.	%	No.	%	No.	%
Cefotaxime	20	7	35%	5	25%	4	20%	1	5%	3	15%
Ceftazidime	20	14	70%	4	20%	1	5%	0	0%	1	5%
Ceftriaxone	20	13	65%	2	10%	1	5%	1	5%	3	15%
Cefepime	20	7	35%	7	35%	3	15%	1	5%	2	10%
Amikacin	20	13	65%	0	0%	0	0%	0	0%	7	35%
Total	100	54	54%	18	18%	9	9%	3	3%	16	16%

The findings of the current study show that 46.0% of the total samples were contaminated. This result agree with Mugoyela and Mwambete [6]who studied the microbial contamination of drugs, as they found that all of the tested products were contaminated, and 50% of them were heavily contaminated by *Bacillus* sp., *Candida* sp. and *Aspergillus* sp. The reasons for the high microbial contamination were due to the improper handling and poor hygienic procedures during dispensing or repackaging. In addition, the climate conditions also play an important role in the higher microbial contamination; thus, high temperature and relative high humidity enhance the microbial growth, especially in the sub-tropical regions as the case is in Egypt, as well as the under tropical conditions as in Tanzania, which has an average temperature of 31°C and a high average relative humidity in combination with the inadequately stored pharmaceuticals, which became more prone to microbial spoilage [6,35].

Interestingly, in the current study, it has been observed that more than one isolate was recovered from the same sample, resulting in a total recovery of 9 bacterial and 68 fungal isolates out of 46 contaminated samples. Similar observations have been reported previously [13,36];and this provides an explanation for why the total number of isolates was greater than the number of contaminated samples.

The current study showed that the level of fungal contamination was the most prevalent, as it compromised about 88.3%, followed by bacteria 11.7%. There are some explanations for these results, which are summarized as higher fungal contamination and low bacterial contamination may be related to the nature of the samples, where they are vial antibiotics which have generally antibacterial activity. Gad *et al.*[13]found out that the spoilage of the solid raw material itself by mold growth on the surface is due to the improper storage with inadequate coverings in a damp environment or under conditions of fluctuating temperature. They also evaluated the microbial contamination on some non-sterile pharmaceutical preparations in Egypt; and that is over a storage period of one year at the room temperature, as they found out that the presence of some molds reflects the storage quality of the preparations. Moreover, Mwambete *et al.*[37] reported that it was observed in a previous study that such a long shelf-life in poor storage conditions may lead to the decomposition of some ingredients in the formulation, resulting in a pH change and ultimately microbial contaminations.Furthermore, moderate to heavy contamination by microbes in the liquid preparation of 75% of the studied samples indicates a poor hygienic condition in the manufacturing process. The inadequate preservation may lead to the microbes being exposed to sub-lethal concentration of preservatives, developing resistance variants. However, a high concentration may prove to be toxic for the consumer's health [38].

In addition, the fungal contamination may take place during the manufacturing process, due to the poorly ventilated clean rooms with insufficient air changes, where areas are damp, and where there are ridges or cracks in the finishes [39]. Moreover, unclean equipment is another reason for fungal contamination as molds like *Aspergillus* spp. And *Penicillium*spp, which is commonly found in walls and ceilings [40]. Since fungi were found on the heel, toes and areas related to the head, neck and eyebrows of workers as *Pencillium*spp. And *Aspergillus*spp.[39,41], so the later easily contaminates products during the handling and processing by personnel. The fact remains that some fungal spores could have been accidentally introduced in the products during the manufacturing or packaging processes, and then upon the inoculation onto appropriate media, they have germinated [35]. Hence, the failure of strict observation of good manufacturing practice at any stage of production may greatly affect the microbiologic quality of the end products [6]. This was also explained by Nwakile *et al.*[42], as they reported that there was a microbial contamination on the tested syrups and suspensions due to the inappropriate Current Good Manufacturing Practice (CGMP) on the part of the pharmaceutical company. In addition, the pharmaceutical products can be also contaminated during handling and storage. The presence of fungi in some of brands, however, suggests a contamination from air and packaging materials.

In the current study, a mixed growth was observed as a combination of different organisms of fungi and/or bacteria was isolated from one sample. In addition, the spoilage of medicines involved basically, initially or early pioneer invaders of biodegrading microorganisms, whose microbial by-products prepare the way for later invaders, by degrading complex nutrients, altering the surrounding pH and making more moisture available, thus creating a more favorable condition for growth of other kinds of microorganisms (later invaders) [43].

3.2. Identification of the Fungal Isolates

In the current study, out of 100 samples, only(46) samples were contaminated; as (40) samples were contaminated with multicellular fungi, giving (66) isolates. According to the macroscopic examination, these isolates belonged to *Penicillium* spp. which were the common isolates, showing (42) isolates with a percentage of (63.64%), (8) isolates of *Aspergillus* spp. with a percentage of (12.12%), (8) isolates of white fungi with a percentage of (12.12%), and (8) isolates of other species with a percentage of (12.12%). In addition, two isolates were selected and identified as representative isolates for studying the effect of gamma radiation. These two isolates were MAM-F15and MAM-F48; as MAM-F15 was *Aspergillus Niveus* and MAM-F48 was *Aspergillus Japonicas*. The contamination of antibiotics with *Aspergillus* spp. represents a great threat to the patient's in life, since these contaminants were injected intravenously, and entered the blood stream directly.

In this regard, the microorganisms that contaminate medicines and cause diseases in patients are either classified as true pathogens or as opportunistic pathogens. *Aspergillus* and *Penicillium*spp. fall under the category of opportunistic pathogens [44]. This is

consistent with Mwambete [35], who revealed the presence of viable fungal contaminants on tablet samples, namely A*spergillus Niger* and *Penicillium*spp. that have been implicated with opportunistic infections. Both fungi are ubiquitous in soil and environment, though they rarely occur in products; however, when present in large quantities, they may cause serious health problems. On the other hand, the presence of certain molds is harmful, since they produce metabolites that may be toxic to consumers [13], and cause rapid deterioration of the product due to the biodegradation of the different components of formulations arising from the production of toxins, such as *Aspergillus Flavus*and*A.Parasiticus*[13]. 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 infants with an immature immune system[6,45].

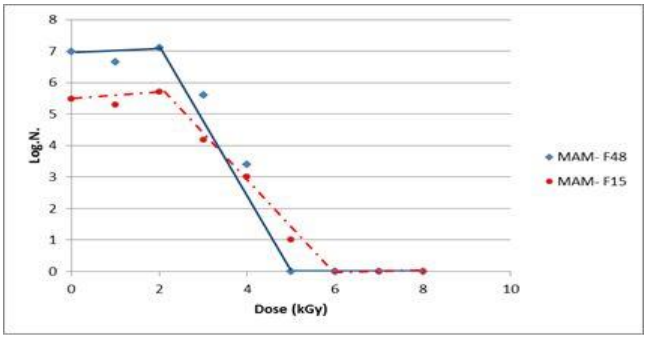
Despite of most microbial contaminants being non-pathogenic commensals of environmental origin, yet they pose problems as agents of spoilage [35,46]. In addition, many of these microorganisms may also become opportunistic pathogens in already ill or compromised individuals [35,46]. Moreover, in the absence of viable cells, the microbial metabolites may be toxic, and the cell wall fractions may be pyrogenic [8]. In this regard, the available information on medicine-borne contamination is scanty; and such reports are portrayed as episodic, being associated with specific isolated incidents [35,46].

3.3. The Effect of Gamma Radiation on the Viable Count of the Isolated Fungi

The two isolates (MAM-F15 and MAM-F48) were selected and identified as representative isolates in order to investigate the effect of gamma radiation on their viable count. Then, the dose response curve for each isolate was determined as indicated in Figure (1).The results of the isolates' exposure to gamma radiation revealed that as the dose of gamma radiation increased, the viable count decreased gradually.

The results of gamma irradiation on the viability of MAM-F15 revealed that 6.0 kGy reduced the viable count completely. Therefore, 5.0 kGy reduced the viable count of this isolate by 4.48 log cycles. As for the MAM-F48 isolate, the lethal dose was 5.0 kGy, which reduced the viability completely;

consequently, 4.0 kGy reduced the viable count by 3.58 log cycles.



Figure(1): The Dose Response Curve for MAM-F15 and MAM-F48

The above results were confirmed by the results of other investigators; Shathele [47]reported that the minimum lethal effective dose of 6.0 kGy (600 krad) was established for the *Aspergillus* spp. mold. The same results were obtained by Paun *et al.* [48] when testing the sterilization of 30 types of molds, as they found the minimum effective dose to be 7.0 kGy. Furthermore, Urban [49]tested four types of molds- *Aspergillus Flavus, Penicillium Spinulosum, Chhartonium Globosum and Aspergillus Niger* – and he found out that the dose of 6.0 kGy eliminated all of the tested cultures. Thus, it was found that 8.0 kGy is the minimum effective radiation dose. On the other hand, **Al-**Abdalall [23]demonstrated that the fungi differed in their response to different doses of gamma radiation; and a decline was noticed in the ability of fungi on the production of mycotoxins. These results proved the variable decrease in the inhibitory action of fungi after being exposed to different doses of γ-rays. As for *A.Melleus, A.Flavus* and *A. Niger*, after being exposed to 3.0 kGy, they stopped the inhibitory activity and the toxin production of these fungi, although they can give mycelia. In all of the fungi exposed to a dose of 10.0 kGy, the fungal cells died and were not able to grow or produce my cotoxins, except for *Fusarium Solani* with an increased toxin production. Shathele [47]found out that the radiation dose of 10.0 kGy completely inhibited the growth of *Aspergillus Fumegatous*. In addition, Abo-State *et al.* [50] found that 5.0 and 4.0 kGy reduced the viability of *Aspergillus* MAM-F23 and MAM-F35 completely.

The results of the current study revealed that the MAM-F15 isolate was more sensitive to gamma radiation than the MAM-F48isolate; as the D_{10} values for the MAM-F15 and MAM-F48 isolates were 1.11kGy and 1.18kGy respectively. The microorganisms differ greatly in their resistance to the ionizing radiation. Thus, the relative sensitivity of different microorganisms to the ionizing radiation is based on their respective D_{10} values (which is the dose required to reduce the population by 90%). Hence, lower D_{10} values indicate greater sensitivity of the organism in question [51].

3.4. The Effect of the Sterilization of Active Ingredients by Gamma Radiation

In the current study, three active ingredients commonly used in medical therapy were studied. One of them was used in the form of salt **(**Cefotaxime as Sodium Salt), another in the form of hydrates (Ceftazidime as Pentahydrate), and the third one was amino glycoside (Amikacin as Sulphate).

3.4.1. The Organoleptic Properties

Before being exposed to the gamma irradiation, the substances were white and pale yellow powders. After the irradiation, no changes were observed in their odor, form, clarity, solubility in water. However, there were some changes in their color. As for

Ceftazidime pentahydrate and Amikacin Sulphate, changes from white to light cream and cream were observed, and to dark cream and beige at 25 kGy respectively. As for Cefotaxime sodium, changes from pale yellow to light yellow and yellow were observed and to dark yellow at 25 kGy as shown in Table (3).

Table (3): Changes in Color as a Result of the Irradiationof Active Ingredients

Active ingredient Name	Colour					
	Dose (kGy)					
	Control	3	5	7	10	25
Ceftazidime pentahydrate	White	Cream	Cream	Cream	Light cream	Dark cream
Cefotaxime sodium	Pale yellow	Light yellow	Light yellow	Light yellow	Yellow	Dark yellow
Amikacin sulphate	White	Light cream	Light cream	Cream	Cream	Beige

A simple and helpful observation to get information about the possible radiolytic intermediates produced in these substances upon irradiation [33]. In this regard, the findings of this study showed that the change in color was observed in all samples,as the color changed from white to varied degree of creamy in Ceftazidime Pentahydrate, and from yellow to dark yellow in Cefotaxime Sodium. It was also noted that the discoloration was more intense with the increase of the dose of irradiation for the three active ingredients.

In a similar study, Singh *et al.*[28] studied the effect of gamma radiation and electron beam on two thermo labile cephalosporin antibiotics, namelyCefdinir and Cefiximeina solid state; and they demonstrated that the color change is not visually perceptible in case of Cefdinir, but the difference reflectance spectra indicate that slight discoloration has actually occurred. As for Cefixime, the "whiteness index" was calculated and observed, thus, the radiolytic degradation is more pronounced for it because there is a significant decrease in the whiteness index at a dose of 25 kGy. This result was confirmed by **Singh *et al.***[52], as they studied the effect of gamma radiation and electron beam on two photolabile and the rmolabile fluoroquinolone antibiotics, namely Norfloxacin(NFX) and Gatifloxacin(GFX) in a solid state; and they noticed that the first observable change in the irradiated samples is slight yellowing. In this regard, a suitable explanation for the color change was reported by Varshney and Dodke [27]. They related the change in color to that both trapped free radicals and degradation products are responsible for the discoloration in the irradiated samples. This study indicates that the radiation degradation products are not formed in significant concentration to be detected in the IR spectrum.

3.4.2. The Measurement of pH

The values of pH for the active ingredients before irradiation ranged from 3.0 to 6.7 pH. In addition, no or little change was found for all samples. The results are shown in Table (4).

Table (4): The Measured pH Values for Control and Irradiated Samples

Active ingredient Name	pH					
	Dose (kGy)					
	Control	3	5	7	10	25
Ceftazidime pentahydrate	6.71	6.67	6.70	6.68	6.70	6.66
Cefotaxime sodium	5.30	5.34	5.28	5.19	5.22	5.81
Amikacin sulphate	3.06	3.10	3.00	3.01	3.00	3.06

The findings of this study were similar to Özer *et al.*[33] who studied the change of the pH values of raw materials of Diclofenac Sodium (DFNa),Phospholipids and Surfactants after irradiation at four different dose levels. The results reported that no significant change was found for all samples after the irradiation with different radiation doses.

3.4.3. The Microbiological Sterility Test

As indicated in Table (5),the results of the current study showed that the dose of 3.0 kGy was the effective dose, which prevented the viable count completely and sterilized the three substances (Ceftazidime Pentahydrate, Cefotaxime Sodium and Amikacin Sulphate). Similar

Table (5): The Effect of Gamma Radiation on theMicrobial Count of Irradiated Antibiotics

Dose (kGy)	Total Count CFU/gm					
	Ceftazidime pentahydrate		Cefotaxime sodium		Amikacin sulphate	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
Control	$3.0x10^1$	0.0	$3.0x10^1$	0.0	$1.0x10^1$	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0

results have been recorded by Özer *et al.*[33]and El-Ridy *et al.*[53]. Thus, gamma radiation sterilization is a reliable

sterilization method for a wide range of raw materials[33].

3.4.4. The HPLC Analysis

The doses of gamma radiation cannot degrade the chemical structure of the active ingredients as determined by HPLC, indicating the stability of the drugs for sterilization by gamma radiation. In addition, the formation of new peaks was not observed as indicated in Figures (2, 3 &4).

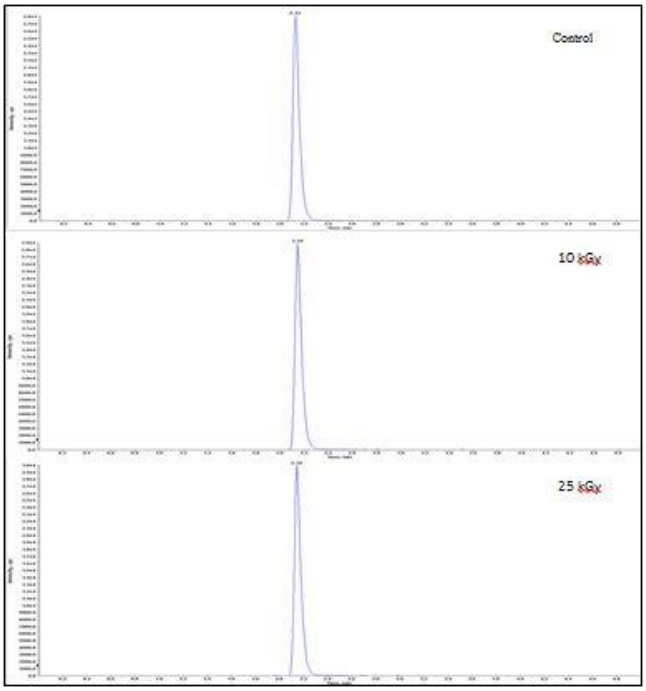


Figure (2): The HPLC Chromatogram of Ceftazidime Pentahydrate before (Control) and after Radio-Sterilization (10 and 25 kGy)

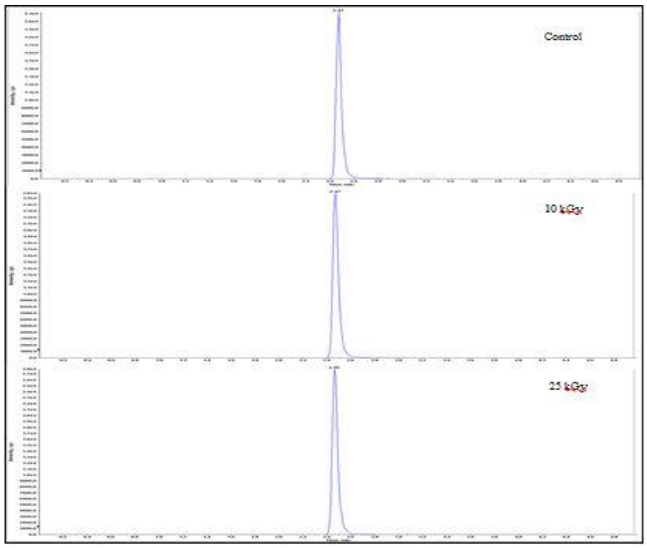


Figure (3): The HPLC Chromatogram of Cefotaxime Sodium before (Control) and after Radio-Sterilization (10 and 25 kGy)

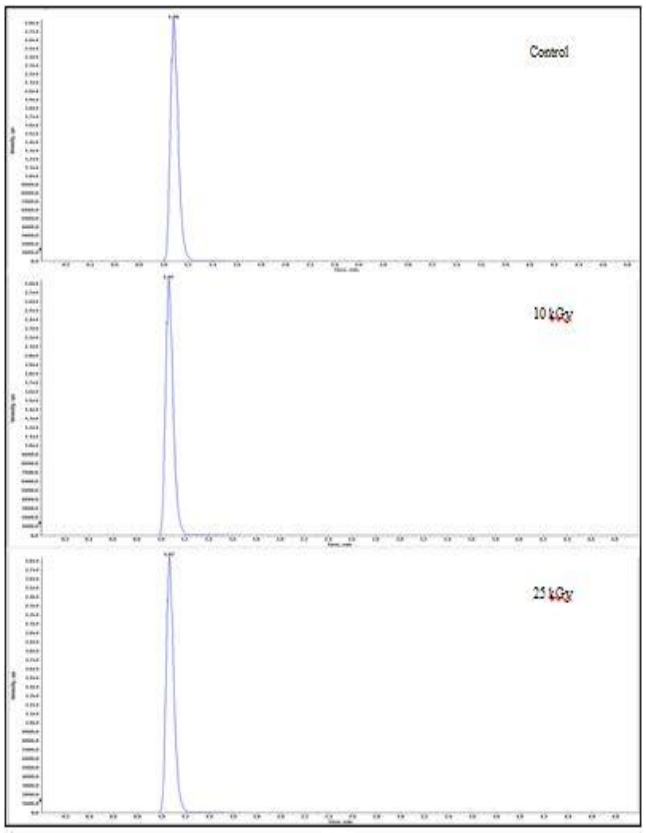


Figure (4): The HPLC Chromatogram of Amikacin Sulphatebefore (Control) and after Radio-Sterilization (10 and 25 kGy)

In this regard, similar results have been recorded by Varshney and Dodke[27] who found out that the samples of Doxorubicin Hydrochloride (DOXO) did not show significant differences in their HPLC profile, indicating the stability of the drug at 30 kGy. Hence, DOXO can be sterilized by radiation at normal 25 kGy, which is the maximum dose recommended for the sterilization of medical devices and pharmaceutical packaging [54]. In addition, Singh *et al.*[52] studied the results of the HPLC content assay and demonstrated that loss of content for the samples irradiated at a dose of 25 kGy was less than 1% for Norfloxacin(NFX) and 2% for Gatifloxacin(GFX). Therefore, the results for them both confirm the radiation resistant nature of GFX and NFX, as the loss percentage of the content is not significant atthedose of25 kGy, and it is within the pharmacopoeia limit. Moreover, the loss of content for the irradiated samples at a four-time higher dose of 100 kGy was always less than 3%. Thus, they concluded the concentration of the calculated impurities, using ICH guidelines [55] in favor of a radiation sterilization of both NFX and GFX, and more for NFX since the radiolytic impurities of NFX are not unique. In case of GFX, five new impurities have emerged, but their percentage was always less than 0.1% threshold level.

4 Conclusions

In this current study, it was found that third and fourth generations of cephalosporins and aminoglycosides antibiotic were contaminated with molds. Thus, the gamma radiation could sterilize the active ingredient of these antibiotics by killing the microbial contaminants without degradation in the active ingredients. Therefore, gamma radiation could be used safely for the sterilization of Ceftazidime, Cefotaxime and Amikacin.

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