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Selection of Gamma Radiation Dose for Decontamination of Some Personal Care Products

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IN THIS STUDY, the bacterial evaluation of 74 personal care products representing 16 brands revealed that 43 samples representing 10 brands were contaminated with bacteria. The isolated bacterial contaminants from the samples of each brand were prepared in mixed populations to determine the gamma irradiation lethal doses (kGy). The range of the lethal doses was found to be from 10 to 25 kGy. The radiation-resistant bacteria isolates from hair gel brands were *Bacillus megaterium, Bacillus brevis and Micrococcus luteus*. From hair cream brands, they were *Bacillus megaterium, Bacillus subtilis,* and *Streptococcus mutans*. While, from wet wipes brands they were *Bacillus megaterium, Bacillus brevis, Micrococcus luteus, Staphylococcus epidermidis* and *Streptococcus mutans*. The radiation-resistant bacteria were selected to study their response to gamma radiation and the D_{10} values were calculated from the dose response curves and they ranged from 0.65 to 2.2 kGy. The highest lethal doses were applied to the selected contaminated personal care products to ensure the success of the process of decontamination of the tested personal care products using gamma irradiation.

Keywords: Personal care products, Bacterial, Decontamination.

Personal care products contain variable amounts of natural materials that may be nutrients that support microbial growth (Mukund *et al.*, 2015), in addition to the warm and humid weather in tropical countries that supports survival of many microorganisms (Hugbo *et al.,* 2003). Microbiological contamination incidents resulted in an increasing awareness and concern over the safety of cosmetics and led to the implementation of good manufacturing practices (GMPs) and more stringent legislation. These changes had a direct impact on the contamination incidents, which decreased in number following their introduction. However, product recalls still occur, and an increase in the numbers of microbiologically contaminated products between 2005 and 2008 was reported in Europe (Lundov & Zachariae, 2008).

Decontamination by gamma radiation is gaining increasing attention in

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cosmetic production. There is no certain radiation dose level in pharmacopoeia and guidelines for decontaminating cosmetic preparations and cosmetic raw materials. However, acceptable microbiological limits are recommended in guidelines for a variety of cosmetic preparations (Naki Sivri *et al.,* 2006 and Ribeiro *et al.,* 2012).

Co^{60} source, which is commonly used for gamma irradiation, can be used for cosmetic raw materials and finished products. The method does not leave any residues that may be harmful to the consumers. Gamma radiation can penetrate the packaging materials and sealed packages containing the finished products, thus destroying the existing microorganisms (Naki Sivri *et al.,* 2006).

The effectiveness of decontamination by gamma radiation is dependent upon factors like the radiation dose, the dose rate for the process and the type of microorganisms present (Geba *et al.,* 2014). The aim of the present study is to determine the gamma radiation lethal dose of bacterial contaminants in personal care products sold in the Egyptian market and to study the response of radiation-resistant bacteria to gamma radiation.

Materials and Methods

Materials

Personal care product samples

A total of 74 personal care product samples (25 hair gel, 25 hair cream and 24 wet wipes) were implied in the study and were given code numbers from (1 to 74). The samples represent 16 different brands from local market.

Bacterial isolates

Twenty nine bacterial isolates from personal care product samples were isolated. Bacterial cultures were maintained on slants of tryptic soya agar and stored at 4ºC.

Media

Tryptic soya agar (Oxoid) was used for isolation and purification of the bacterial isolates. Tryptic soya agar (Oxoid) supplemented with (0.07%w/v) lecithin (Defico) and (0.1%v/v) tween 80 (Oxoid) for isolation of bacteria from personal care samples(Geis ,2006 and USP, 2009).

Chemicals

Neutralizing solution: It was used for inactivation of preservatives; it contains L-Histidine (0.1%w/v), monopotassium phosphate (0.36%w/v), disodium phosphate (0.72%w/v), sodium lauryl sulphate (0.4%w/v), tween 80 (3.0%v/v), sodium thiosulphate (0.5%w/v), lecithin (0.3%w/v) and glycine (0.5%w/v), according to Geis (2006) and USP (2009). Other chemicals used during the present work were of the reagents grade.

Gamma irradiation facility

Cobalt-60, 220 Gamma Cell, Canada Co. Ltd. (located at the National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt) has been

utilized as radiation resources. The dose rate at the time of experiments was 0.048 kGy/min.

Automated identification system

Microscan® Positive Identification (PID) panel type 20 (Dade, Behring, West Sacramento, CA) National Cancer Institute, Cairo, Egypt.

Methods

Total aerobic bacterial counts

The spread plate technique was used, for each personal care product sample. One ml of each of the hair gel and hair cream samples was suspended in test tubes containing 9 ml sterile neutralizing solution (Geis, 2006) and one ply of wet wipes samples was added to 9ml and 18ml sterile neutralizing solution for small wipes and big wipes, respectively. Tenfold serial dilution was made in the same diluents. Aliquots of 0.1 ml was taken from each suitable dilution and spread on triplicate sterile plates containing tryptic soya agar supplemented with (0.07%w/v) lecithin and (0.1%v/v) tween 80 using a pre-sterilized bent glass rod for each dilution. The medium was left to absorb the inoculums before inversion. Inverted plates were incubated at 37ºC±1 and examined daily up to 72 hr, Then, suitable dilutions were counted and the results were recorded (FDA, 2001 and Geis, 2006).

Determination of the Gamma irradiation lethal dose (kGy)

According to El-Bazza *et al.* (2009) with some modifications; for each tested brand, the bacterial contaminants isolated from the contaminated samples of same brand were used to prepare each mixed population. A 24 h of each bacterial culture was surface inoculated on tryptic soya agar in sterile duplicate plates. The plates were incubated at 37ºC±1 for 48 h. After incubation, all the heavy bacterial growth were scrapped off by a sterile glass slide and all together were transferred into small porcelain mortar in a laminar air flow cabinet. Sterile saline (containing 0.1% tween 80) was added dropwise to the mortar and the mixture was homogenized using sterile pestle in order to have a well mixed population in a thick suspension.

Aliquots of 20 µl of the resulting dense suspension of mixed populations of the bacteria isolated from the selected contaminated samples of each brand were withdrawn using a sterile micropipette, and each aliquot was transferred into test tubes. The test tubes were exposed at room temperature to the different gamma radiation dose levels(0.5, 1.0, 3.0, 5.0, 7.0, 10.0, 15.0, 20.0, 25.0 and 30.0 kGy), the test was performed in triplicate for each mixture of bacteria, at each radiation dose. As soon as possible after irradiation, the gamma radiation dose at which no detected survivors upon growing on tryptic soya agar plates (incubated at 37ºC±1 and examined daily up to 72 h) was taken as the lethal dose and the dose at which the radiation-resistant organisms were isolated, is the sublethal dose.

Isolation of the radiation-resistant bacteria

The bacteria which could survive the highest radiation doses, under treatment, were isolated on tryptic soya agar. They were taken as the radiation-resistant isolates, identified and then subjected for studying their response to gamma radiation.

Identification of the radiation-resistant bacteria

Identification of the radio resistant gram positive bacteria using the PID system, which is an *in vitro* diagnostic product that uses fluorescence technology to detect bacterial growth or metabolic activity and thus can automatically identify Gram-positive to species level (Ashour & El-Sherif, 2007).

Study the response of the most radiation-resistant bacteria of each brand to gamma radiation

This study was carried out according to El-Bazza *et al.* (2009) with some modifications as follows: The most radiation-resistant microorganisms were selected, they were prepared separately as mentioned before. Aliquots of 20 µl of the resulting dense suspension of each microorganism were exposed at room temperature to gamma radiation doses in the range of 0.0 kGy to 25 kGy. After irradiation, the mean of the bacterial counts were recorded as cfu/ml at each irradiation dose level and then, log number of survivors was determined, in triplicate.

Construction of the dose response curves of the microbial isolates

The survival curves were obtained by plotting the logarithm of the number of microbial survivors versus the radiation doses in kGy.

The D_{10} values which are the measure for the radiation resistance of the microorganisms to gamma radiation were read directly from the curves by finding the gamma radiation dose which reduces the microbial population by one logarithmic cycle (Aquino, 2012).

Applicability of the radiation lethal doses

According to El-Bazza *et al.* (2010), the highest radiation lethal doses of the isolated microorganisms of personal care products were applied on 5ml of selected contaminated hair gel and hair cream samples. While, for wet wipes one ply of each selected contaminated sample was used. The probability of sample contamination was studied by determination of the viable count on the samples after application of the lethal doses, as mentioned before.

Results and Discussion

The data in Table 1. show that, from 25 hair gel, 25 hair cream and 24 wet wipes samples examined, it was found that 10, 10 and 9 samples, respectively, were contaminated with bacteria at levels more than 10^3cfu/ml.

Onurdag *et al.* (2010) found among 73 personal care products samples examined, 5 samples contaminated with bacteria at level more than 10^3 cfu/ml for non -eye area products. Mwambete & Simon (2010) found out of 10 cosmetic products investigated, 7 samples yielded bacterial contaminants more than 10^3 cfu/ml. But El-Bazza *et al.* (2011), evaluated the microbial count in 50 cosmetic cream samples, and they found that 20 samples were contaminated with bacteria at level more than 10^5 cfu/ml.

TABLE 1. Survey on the bacterial contamination of the examined personal care products.

Type	Hair gel	Hair cream	Wet wipes
No. of brands	5	5	8
No. of samples	25	25	24
C.S.B.	13	18	12
% C.S.B.	52%	72%	50%
L.C.B.(cfu/ml)	$4.0x10^2$ to $2.5x10^5$	$1.3x10^2$ to $2.3x10^3$	$1.0x10^2$ to $6.5x10^3$
C.S.B < 10^3 (cfu/ml)	3	8	3
C.S.B > 10^3 (cfu/ml)	10	10	9

(C.S.B.): Contaminated samples with bacteria, (L.C.B.): Level of contamination with bacteria.

In the present study, the mixed populations of bacteria were exposed to gamma irradiation doses in the range of 0.5 to 30 kGy. It was observed that the gamma radiation lethal doses at which all bacteria were not detected for the tested brands of hair gel samples, hair cream samples and wet wipes were between 10 and 25kGy and the identified radioresistant bacteria are illustrated in Table 2.

TABLE 2. The gamma radiation lethal doses (kGy) for bacteria isolated from tested personal care products of different brands and identification of radioresistant bacteria.

Type	Brand code	No. of S.	No. of isolates in M.P.	SLD (kGy)	LD (kGy)	Radioresistant bacteria
Hair gel	G-C	5	14	15	20	*Bacillus megaterium*
	G-D	5	10	10	15	*Bacillus brevis*
	G-E	3	5	7	10	*Micrococcus luteus*
Hair cream	C-C*	5	7	20	25	*Bacillus subtilis*
	C-F	5	11	10	10	*Streptococcus mutans*
	C-D	5	7	20	25	*Bacillus subtilis*
	C-G	3	6	10	10	*Bacillus megaterium*
Wet wipes	W-H	2	9	15	15	*Bacillus megaterium*
	W-I	3	8	7	10	*Staphylococcus epidermidis*
	W-J	3	9	7	10	*Micrococcus luteus*
	W-K	2	6	10	15	*Streptococcus mutans*
	W-L	2	6	10	15	*Bacillus brevis*

(S): Samples, (G): Hair gel, (C): Hair cream, (W): Wt wipes, (M.P.): Mixed population, (LD): Lethal dose, (SLD): sublethal dose.

El-Bazza *et al.* (2010) studied the effect of gamma irradiation on microorganisms isolated from contaminated personal care products, the study showed that the lethal doses level of bacteria were ranging between 2 and 25 kGy.

Atique *et al.* (2013) studied the effect of gamma radiation doses on 41 bacterial isolates (17 *Staphylococcus sp.*, 14 *Streptococcus sp.*, *5 Pseudomonas sp.*, 5 *Bacillus sp.*). Among the 41 bacterial isolates, 40 isolates were capable of surviving at 5 kGy, while, 26 isolates survived up to 6 kGy, 18 isolates survived up to 7 kGy, and only 7 isolates were able to survive at 8 kGy and 2 isolates were found to survive at 9 kGy. Gamma irradiation doses equal or greater than 9 kGy were sufficient for the total elimination of the growth and multiplication of bacterial isolates.

In the present study, the bacteria that survived the gamma radiation at ≥ 10 kGy were considered as the most radioresistant bacteria. Three different bacterial isolates from the radioresistant bacteria were selected for the dose response study**,** where it was found that the Calculated D_{10} values for the studied bacterial isolates were; 2.2 kGy for *Bacillus megaterium* isolated from hair gel of brand G-C , 0.75 kGy for *Bacillus subtilis* isolated from hair cream of brand C-D, and 0.65 kGy for *Streptococcus mutans* isolated from wet wipes of brand W-K. The three curves showed exponential rate of death manifested by the straight line curves as illustrated in Fig. 1(a-c).

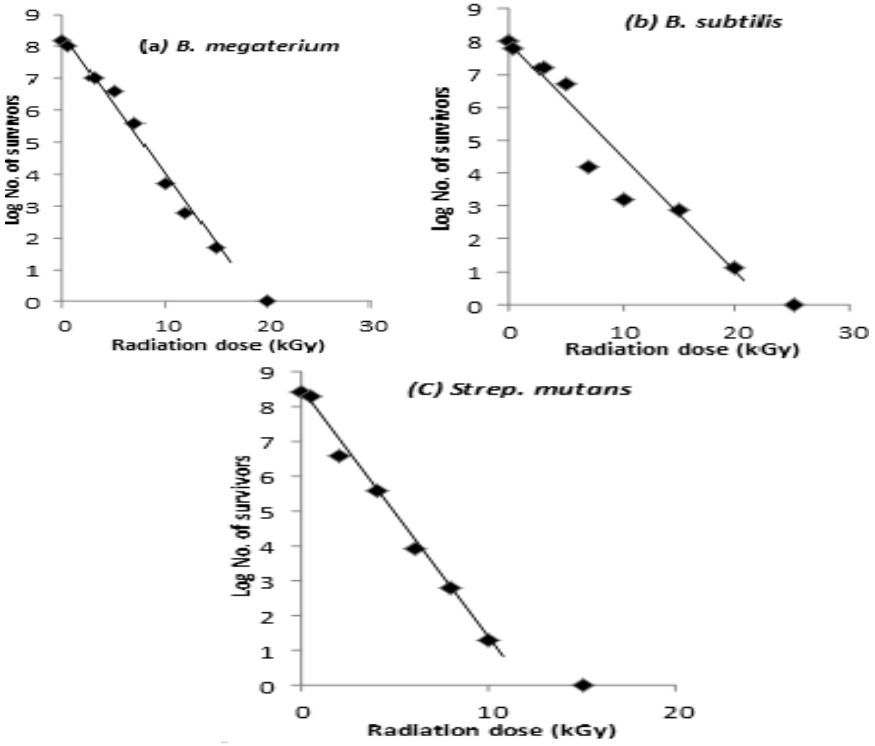

Fig. 1a-c. The dose response curves of the selected most radioresistant bacteria isolates isolated from hair gel (a), hair cream (b) and wet wipes (c).

In this study, *Bacillus megaterium* was considered to be the most radiation-resistant in comparable with the other isolated microorganisms with the highest D_{10} 2.2 kGy. *Bacillus megaterium* is a rod-like, Gram-positive, mainly aerobic

spore forming bacterium found in widely diverse habitats, which is ubiquitous in the environment around us. It grows at temperatures from 3°C to 45 °C, with the optimum around 30 °C. (Vos *et al.,* 2009).

In consistence with the present investigations, other studies carried out by El-Fouly *et al.* (2000) Who found the D_{10} values of *Bacillus cereus* and *Staphylococcus aureus* were to be 1.02 and 0.37 kGy, respectively. Farrag *et al. (*2000) found the D_{10} values of *Bacillus cereus, Micrococcus luteus* and *Bacillus sphaericus* were to be 1.0, 1.4 and 1.4 kGy, respectively. Bashandy & Hassan (2005) found the D_{10} values of four different *Bacillus* sps were found to range from 2.3-2.9 kGy, also Abostate *et al.* (2006) found that *Bacillus cereus* strains exhibited exponential rate of death and the D_{10} values were calculated to be 1.9 and 2.2 kGy.

As well, El-Bazza *et al.* (2009) found that, the dose response curves of all the isolated gram positive cocci showed an exponential rate of death and the D_{10} values of *Staphylococcus aureus* were found to range from 0.7 kGy to 1 kGy, while, the D_{10} values of *Staphylococcus epidermidis* were found to range from 0.7 kGy to 0.8 kGy, the dose response curves of two gram positive spore forming *Bacillus megaterium* showed an exponential rate of death and the D_{10} values were found to be 1.7 and 1.8 kGy.

El-Bazza *et al.* (2010) studied the dose response curve of radioresistant microorganisms isolated from cosmetic samples. The D_{10} values were calculated from the dose response curve for the most redioresistant bacteria and fungi. The D_{10} values of *Acenatobacter baumann/haem*, was found to be (1.1 kGy), three strains of *Micrococcus* sp. were (0.4, 0.75, 2.5 kGy), *Staphylococcus hominis-novo* was 0.85 kGy, *Staphylococcus haemolyticus* was 0.47 kGy, two strains of *Bacillus sphaericus* were 0.85 and 1.25 kGy, two strains of *Bacillus pantothenticus* were 0.4 and 0.5 kGy. Also, the microbial strains exhibited exponential response towards gamma radiation except one *Micrococcus sp*. and one *Bacillus pantotheticus*, which exhibited non exponential response towards gamma radiation.

Atique *et al.* (2013) mentioned that, the D_{10}values range for 17 *Staphylococcus* sp. strains, 14 *Streptococcus sp.* strains, 5 *Pseudomonas* sp. strains and 5 *Bacillus* sp. strains, were from 1.06 to 1.27 kGy, 0.06 to 0.74 kGy and 0.83 to 0.99 kGy, respectively.

The use of gamma radiation for decontamination is advantageous for finished cosmetic products as well as raw materials. Sterilization is not an obligation for personal care products. However, they have to be protected from any contamination or deterioration (Naki Siviri *et al.,* 2006).

As illustrated in Table 3, after applying the highest lethal doses to the selected contaminated samples representing each brand of the 3 types of the studied personal care products, no microbial growth was detected in any of the samples after irradiation. This suggests the success of the process of microbial decontamination of the personal care products by gamma irradiation.

The maximum lethal doses concerning the present investigations are 20, 25 and 20 kGy for decontamination of the hair gel, hair cream and wet wipes, respectively.

TABLE 3. Irradiation of the selected contaminated personal care products samples with the highest lethal doses.

Type	Brand code	LD (kGy)	Microbial contamination (cfu/ml)	
			B.ir	A.ir
Hair gel	G-C	**20**	$3.0x10^3$	-
	G-D	15	$1.4x10^3$	-
	G-E	10	$1.5x10^5$	-
Hair cream	C-C	**25**	$4.0x10^2$	-
	C-F	15	$1.0x10^3$	-
	C-D	25	$1.6x10^3$	-
	C-G	15	$4.0x10^2$	-
Wet wipes	W-H	**20**	$2.2x10^3$	-
	W-I	10	$2.1x10^3$	-
	W-J	10	$3.3x10^3$	-
	W-K	15	$6.5x10^3$	-
	W-L	15	$2.4x10^2$	-

(LD): Lethal dose, (B.ir): before irradiation, (A.ir): after irradiation, (-): No detected growth.

In other studies, El-Bazza *et al.* (2010) found that the doses of decontamination of different eye area cosmetic preparations were 8.3, 12.3 and 16 kGy.

Also, Oliveira *et al.* (2010) found that 10 kGy and 100 kGy were efficient irradiation doses to treat microbial contamination of cosmetic samples without affecting the properties of the formulations. It was found that gamma radiation dose level of 10 kGy was optimal to cause less than 10 cfu/ml count when the initial irradiated counts for *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Candida albicans*, and *Aspergillus niger*, were $5.4x10^4$ cfu/ml, $7.6x10^4$ cfu/ml, $5.8x10^4$ cfu/ml, $1x10^6$ cfu/ml, respectively.

Concerning radiation-resistant microorganisms, other investigators have suggested that prokaryotic DNA repair mechanisms may have evolved not to counter the damage of ionizing radiation but rather to compensate for desiccation. The process of desiccation is inherently DNA damaging and results in DNA double-strand breaks, the primary lethal lesions resulting from exposure to ionizing radiation, and it is assumed that desiccation-tolerant species, as well as ionizing-radiation-resistant species, can avoid or effectively repair these lesions (Rainey *et al.*, 2005).

Our results and that obtained by other investigators show that the resistance and response of microorganisms towards gamma radiation differ between the different microorganisms and the different strains of the same microorganisms. This may be attributed to species of microorganisms, number of microorganisms, medium suspending the microorganisms, temperature, water activity, oxygen effect and the use

of radiation sensitizing compounds during the irradiation process (Goldblith, 1971 and Geba *et al.,* 2014).

As a result, it can be concluded that decontamination using gamma radiation is an alternative method for decontamination of cosmetic products and raw materials, so gamma radiation can also be used much more extensively in the cosmetic field (Naki Sivri *et al.,* 2006 and El-Bazza *et al.,* 2011).

The gamma radiation technology can offer the process of decontamination of personal care products as a mean of approaching a higher standard of microbiological safety limits, decreasing the bioburden and elimination of pathogenic microorganisms. Furthermore, gamma irradiation is a clean and non-residual technology that is environmentally friendly and safe for both employee and the community.

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اختيار جرعة من الاشعاع الجامي لإزالة التلوث الميكروبي من بعض منتجات العنايه الشخصية

زينب الدمرداش البزه ،عبير خيري عبد العال* ومها احمد شفيق
قسم البحوث الدوائية الاشعاعيه- المركز القومي لبحوث و تكنولجيا الاشعاع -هيئه الطاقه الذرية - مصر و*قسم الميكروبيولوجيا والمناعة- كليه الصيدلة (بنات)- جامعه الازهر-القاهرة - مصر.

في هذه الدراسة تم تقييم التلوث الميكروبي في عدد٧٤ عينه ممثله لعدد ١٦ ماركه من منتجات العناية الشخصية وقد أوضحت الدراسة أن عدد ٤٣ عينه ممثله لعشرة ماركات كانت ملوثة بالبكتيريا. وقد تم عمل خليط من المستعمرات لعينات كل ماركه على حده و ذلك لتعيين الجرعات المميتة للبكترية وقد وجد أن مستوي الجرعات يتراوح بين ١٠ و٢٥ kGy. و كانت العزلات البكترية المقاومة للإشعاع المعزولة من ماركات جل الشعر هي *بسيليس ميجاتيريم وبسيليس بريفيس وميكروكوكس لوتيس*، ومن كريم الشعر *كانت بسيليس ميجاتيريم، بسيليس ستليس، ميكروكوكس روسيس، ستافيلوكوكس أوريس، وستربتوكوكس ميوتنس* بينما من المناديل الرطبة كانت *بسيليس ميجاتيريم، بسيليس بريفيس، ميكروكوكس لوتيس, ستافيلوكوكس ايبيدرميديس و ستربتوكوكس ميوتنس* وقد تم اختيار أكثر الميكروبات مقاومه للإشعاع لدراسة مدى استجابتها لأشعة جاما وقد تم حساب قيمة ال D_{10} لتكون بين ٠,٦٥ إلي ٢,٢ kGy .كذلك تم تطبيق أعلى جرعات أشعاعيه مميتة لمنتجات عناية شخصية ملوثة للتأكد من نجاح عمليه إزالة التلوث الميكروبي بها باستخدام التشعيع الجامي.