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Contamination of Eye Drops with *Bacillus* Species and Evaluation of Their Virulence Factors

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Abstract: Seventy five eye drops samples were collected from different pharmacies in Cairo, Egypt. Out of 75 tested samples, 58 (77.3%) are contaminated with bacterial growth when cultured on MYP medium. The bacterial count ranging from 1.0 CFU/ml to $6.0x10^2$ CFU/ml. The microscopic examination indicated that 57 out of 133 isolates are Gram positive, spore forming bacilli. From 58 positive samples 32 samples were found to be contaminated with *Bacillus* spp. (55.1%). So, the contaminated samples with *Bacillus* spp. represent 42.6% of the total eye drops samples. Out of 57 *Bacillus* isolates, 41 (71.9%) isolates produced different levels of hemolysins. Two *Bacillus* isolates (MAM-9 and MAM-40) produced large quantity of phospholipace C around their colonies in addition to hemolysin production. These two isolates were also lethal to mice. *Bacillus* spp are resistant to gamma radiation, ceftazidime, cefuroxime and colistin. Ten kGy reduced the viable count of *Bacillus* strain MAM-40 by 4.17 log cycles.

Key words: *Bacillus* spp · Hemolysin · Toxin · Gamma radiation · Antibiotics

INTRODUCTION

The genus *Bacillus* consists of heterogenic group of Gram positive, endospore-forming, facultative anaerobic bacteria that are widely distributed in nature. Due to their endospore forming abilities, these bacteria tolerate adverse conditions more than the non sporulating bacteria [1, 2, 3]. *Bacillus* spp. Produces many toxins and causes a serious case of infections [3, 4, 5, 6]. *Bacillus* species can cause out- breaks and systematic infections and they well known cause of food poisoning [7-11]. Many cosmetic formulations are now available in the form of wet wipes packaged in sealed sachets or packets. Like the majority of cosmetic products having an aqueous phase, wipes are susceptible to microbial contamination and require the addition of preservatives [12]. *Bacillus cereus* contaminate non sterile disposable gloves [13]. Bacteria also contaminated contact lenses. The restriction in the use of contact lenses is associated with the cost of acquisition and maintenance of the lenses especially of the disinfecting solutions [14]. Also, Donzis *et al.* [15] reported a case of *B. subtilis* eye infection related to contamination of contact lenses. The most group of drugs contaminants is members of the genus *Bacillus* (34.4%) [16]. The disinfectant 75% alcohol was found to be contaminated with *Bacillus amyloliquefacin* [17]. Gamma radiation and electron beam can be used to reduce the spores of the *Bacillus* species and sterilize the third generation of cephalosporins [18, 19, 20]. Also radiation can be used for sterilization of antibiotic liposome formulations [21]. Radiation can be used for sterilization of medical and pharmaceutical products, but in case of wet products, addition of preservative or antibiotics is the choice, especially for a product sensitive like eye drops. Contamination of eye drops with the genus *Bacillus* may cause severe eye infection and could develop complete panophthalmitis or endophthalmitis [22, 23]. Endophthalmitis caused by *Bacillus* spp often result in poor outcome [24].

Therefore, the aim of this study is to investigate the ability of eye drops to be contaminated with bacteria in general and the genus *Bacillus* in particular. Also to evaluate the virulence factors of the *Bacillus* isolate and effect of gamma radiation and antibiotics on these isolates. Then determining the most efficient antibiotics against the isolated *Bacillus* spp. to be added to eye drops as a solution for *Bacillus* spp. contamination.

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MATERIALS AND METHODS

Samples: Seventy five samples of eye drops samples were purchased from different pharmacies in Cairo, Egypt. They collected at random and marketed as pharmaceutical products, contained antimicrobial agents.

Determination of the Microbial Load of the Samples: The collected samples were used from the original vials and serially diluted with sterile saline solution. Three successive dilutions were plated using (100µl) on the surface of MYP medium [25] with the omission of polymyxin B-sulphate. The inoculated plates were incubated at 37°C for 24 hours. The microbial load was determined.

Isolation and Characterization of Microorganisms: The bacterial colonies were isolated from the MYP plates as separated single colonies and subcultured on L.B agar slants [26]. The bacteria isolates were examined by Gram and spore staining and the isolates of *Bacillus* spp. were identified by the criteria of Cowan and Steel [27] and Kramer *et al.* [28]. Each strain was examined by light microscope Leica LEITZ, LABOR, LUX, Germany.

Production of Hemolysin: Each Gram positive endospore forming *Bacillus* strain was streaked on the surface of blood agar plates [29]. The positive control is *Bacillus cereus* ATCC 11778 (American Type Culture Collection, USA).

Determination of Phospholipase C Production: Each Gram positive endospore forming *Bacillus* strain was streaked on the surface of Tris-buffered saline medium [30]. The positive control was *B. cereus* ATCC 11778.

Induction of Lethal Toxin: According to Shinagawa *et al.* [31], the bacterial isolates under study were inoculated on slants of brain heart infusion agar (BHIA, Difco) and incubated at 32°C for 14-16 hours. From each slant inoculate 10 ml brain heart infusion broth BHIB in 100 ml conical flask and incubated for 16 hours at 32°C on shaking water bath at 100 cycles/minute. One ml from 16 hours culture of each strain was inoculated into 100 ml BHIB supplemented with 0.1% glucose (BHIG) in 500 ml conical flask for 6 hours at 32°C with continuous shaking at 90 cycles/minute. Bacterial cells were removed by centrifugation at 8000 rpm for 20 minutes at 4°C, followed by filtration of the supernatants through disposable Millipore (0.45µ) filter. The resulted filtrates were used as crude toxins.

Mouse Lethal Test: One half (0.5 ml) volume of culture filtrate was injected into the veinus tail of adult mouse (20-22 gram). Five mice are used for each filtrate. Positive control is *B. cereus* ATCCII778 filtrate and negative control is BHIG. The mice were observed for lethal effects for one hour at least [2].

Antibiotic Susceptibility Test: The isolated *Bacillus* spp. that have virulence factors were used to determine their susceptibility against 15 different antibiotics. One ml ($1x10^5$ CFU/ml) of culture in L.B broth was spreaded on the surface of Muller-Hinton agar medium [29] and incubated for one hour before antibiotic discs were placed on the surface of inoculated plates. The following antibiotics were used azithromycin (AZM) 15µg, amikacin (AK) 30 µg, cefuroxime (CXM) 30 µg, ciprofloxacin (CIP) 5 µg, ceftazidime (CAZ) 30 µg, clarithromycin (CLR) 15 µg, clindamycin (DA) 2µg, chloramphenicol (C), 30 µg, colistin (CT) 10 µg, Gentamicin (GN) 10 µg, levofloxacin (LEV) 5 µg, Imipenem (Imp) 10 µg, Ofloxacin (OFX) 5 µg, amoxicillin/clavulanic acid (AMC) 20/10 µg and vancomycin 30 µg. They were obtained from Bioanalyse, LTD, Tibbi Malzemeler San. Ve Tic. Ltd Sti, Ankara, Turkey.

Effect of Gamma Radiation on Isolated *Bacillus* spp.: According to Abo-State [2] the selected *Bacillus* sp. isolate were grown in L.B. broth for 24 hours on shaker (150 rpm) at 30°C. The grown culture was centrifuged (8000 rpm for 15 min). The supernatants were decanted and the pellets were suspended in sterile saline. The suspended *Bacillus* cells were collected in a sterile clean flask to form a pool. The bacterial suspension was distributed in clean sterile screw cap test tubes. Each tube contains 5 ml of bacterial suspension, the tubes were exposed to different doses of gamma radiation (0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 kGy) form Indian cell Co-60 located at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The used dose rate is 1 kGy/12.5 min. The non irradiated control and the irradiated cultures were serially diluted and plated on the surface of L.B agar plates to determine their viable count (dose response curve).

RESULTS AND DISCUSSION

Determination of the Bacterial Load Contaminating Eye Drops Samples: Seventy five eye drops were investigated to determine their bacterial contamination and microbial load. The results in Table 1 revealed that 58 samples (77.3%) out of the 75 eye drops samples were

Table 1: Bacterial count of eye drops samples on MYP medium

Sample No.	Count	Sample No.	Count	Sample No.	Count
1	1x10[1]	27	5x10°	54	3x10°
2	1x10[1]	28	1x10[1]	55	- ev
3	- ev	29	3x10°	56	4x10[1]
4	2 x 10[1]	30	- ev	57	3x10°
5	2x10[1]	31	- ev	58	2x10°
6	2x10[1]	32	-ev	59	1x10°
7	1x10[1]	33	- ev	60	5x10[1]
8	- ev	34	1x10[1]	61	5x10°
9	36x10[1]	35	- ev	62	- ev
10	1x10[1]	36	- ev	63	7x10°
11	1x10[1]	37	- ev	-	-
12	1x10[1]	38	15x10°	64	- ev
13	2x10[1]	39	1x10°	65	3x10°
14	1x10[1]	40	4x10[1]	66	2x10°
15	1x10[1]	41	1x10°	67	3x10[1]
16	60x10[1]	42	2x10°	68	2x10°
17	6x10[1]	43	2x10°	69	1x10°
18	3x10[1]	44	3x10°	70	3x10°
19	25x10[1]	45	1x10[1]	71	120x10[1]
20	20x10[1]	46	2x10°	72	1x10[1]
21	2x10°	47	10x10°	73	3x10°
22	-ev	48	2x10°	74	- ev
23	2x10°	49	- ev	75	- ev
24	2x10°	50	1x10°	-	-
25	2x10[1]	51	12x10[1]	-	-
26	- ev	52	8x10°	-	-
-	-	53	6x10°	-	-

contaminated with bacteria. The bacterial count of the positive sample ranging from 1.0 CFU/ml to $6.0x10^2$ CFU/ml.

Identity of the *Bacillus* spp. Contaminating the Samples: The microscopic examination revealed that, 133 bacterial isolates have been isolated from the fifty eight positive samples of eye drops. From these 133 isolates, 57 isolates are Gram positive, spore forming, Long chain, large bacilli (42.85%) as indicated in Fig. 1 where the results revealed that out of 58 positive eye drop samples, 32 samples were contaminated with *Bacillus* spp. (55.1%). Sample No. 24 is the highest sample contaminated with *Bacillus* spp. five isolates were picked up from this eye drop sample (No. 24). Little have been recorded about contamination of pharmaceutical products especially eye drops. So, in this study evaluation of the contamination of this critical product with *Bacillus* spp was conducted. Eye drops used to cure patient's eyes, when they were contaminated with *Bacillus* spp. which cause serious eye infections, the problem became very complicated. Gram-positive rods and cocci are the most frequent bacterial contaminants in pharmaceutical production environments and in raw materials because of their resistance to a wide variety of

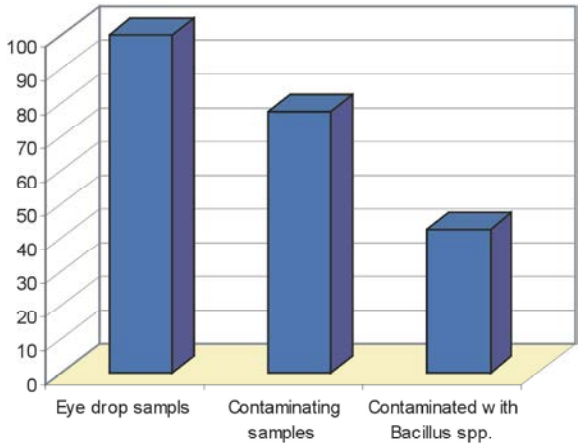

Fig. 1: Percentage of contaminating samples with bacteria and with *Bacillus* spp.

destructive factors. It has been reported that the genus *Bacillus* is the most frequent contaminant in oral drugs with *Bacillus cereus* as common contaminant [32, 33]. This finding confirmed the present results and was confirmed with the results of Garcia-Arribas *et al.* [16]. The microbial contamination of 68 samples of topical and 324 samples of oral medicaments has been studied. The most common group of contaminants is members of the genus *Bacillus* (34.4%). Because of the pathogenic significance of *B. cereus* 39 strains could be characterized by morphology and biochemical properties [16].

The Virulence Factors: The results of testing the ability of *Bacillus* spp. isolated from eye drops samples to produce hemolysin on blood agar medium indicated that out of 57 isolates 41 isolates exhibited positive hemolysin production (71.9%), while 16 isolates (28.1%) failed to produce hemolysin (negative) as indicated in Fig. 2. Among the strains isolated from eye drops, *Bacillus* spp., strains which produced hemolysin have been streaked on Tris-buffered saline. Strains MAM-9 and MAM-40 isolated from eye drop samples produced very large quantity of phsopholipase C around these colonies on MYP medium and on Tris-Buffered saline medium. This means that isolated strains MAM-9 and MAM-40 produced hemolysin and phospholipase C in a large quantity. The other strains produced hemolysin in addition to phospholipase C but beneath the colonies tested only not around the colonies as in case of MAM-9 and MAM-40. For this reason, these two isolates were used for induction of their toxin and injection into the tail vain of the mice. The injected mice with these two strains were die after one hour of injection, while the control

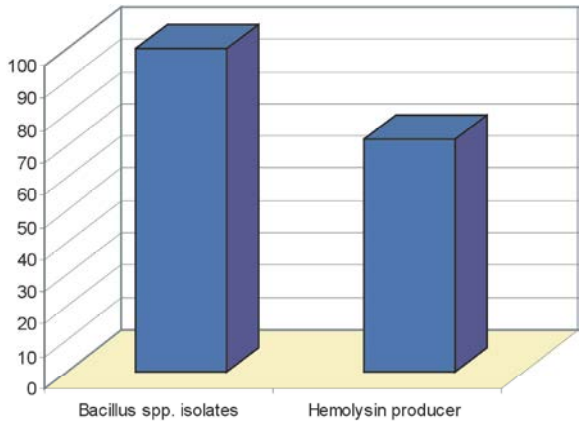

Fig. 2: Percentage of hemolysin production among the contaminating *Bacillus* spp. isolates

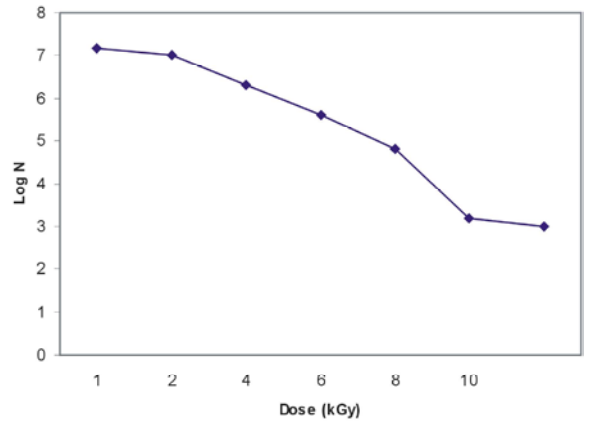

Fig. 3: Effect of different doses of gamma radiation on the viable count of the isolated *Bacillus* strain MAM-40 isolated from eye drops

group which injected with BHIG broth exhibited no affect (not die). The above results were confirmed by Abo-State [2] and Shinagawa *et al.* [34]. The results revealed that 71.9% of the eye drops *Bacillus* spp., isolates were hemolysin producer. Two isolated strains from eye drops are hemolysin producer, phospholipase C producer and lethal to mice. These results are in agreement with those obtained by Garcia Arribas *et al.* [16], Beattle and Williams [35], Taylor *et al.* [36] and Sergeev *et al.* [37].

Determination of the Antibiotic Profile of Representative Isolates: In this study MYP medium which was used as selective medium for *B. cereus* and related strains (*B. anthracis, B. mycoides, B. thuringiensis* …etc), with the omission of the antibiotic polymyxin B-sulphate for two reasons was conducted. The first one, because some strains of *B. megaterium* can not grow on medium containing polymyxin. The second reason because some of the eye drop samples contain already antibiotics which may be interfered with the presence of another antibiotic in the medium (polymyxin). Samples of eye drops can not sterilize by gamma irradiation, another solution for this problem must be achieved. Determination of the antibiotic profile of representative isolates from eye drop sample has been conducted. The results presented in Table 2 revealed that, the most virulent *Bacillus* strains isolated from eye drops (MAM-9, MAM-21, MAM-28 and MAM-40) were tested against 15 different antibiotics. The results of *Bacillus* isolate MAM-40, which was isolated from eye drops and produce hemolysin and phospholipase C and was found to be lethal to mice was found to be highly sensitive to imipenem, amikacin and azithromycin and was resistant to ceftazidime cefuroxime and colistine. *Bacillus* isolate MAM-9 which also possessed two virulent factors (hemolysin and phospholipase C) and was found to be lethal to mice was found to be susceptible to imipenem, ciprofloxacin, levofloxacin, azithromycin, ofloxacin and clarithromycin, while it is resistant to gentamycin, vancomycin, ceftazidine (cefuroxime, chloramphenicol and colistin. It is intermediate in its susceptibility to amikacin. *Bacillus* isolate MAM-21 isolated from eye drop is highly susceptible to imipenem and amikacin and resistant to azithromycin, ceftazidime, amoxicillin, cefuroxime and colistin and intermediate in its susceptibility to gentamycin, vancomycin, ciprofloxacin, levofloxacin, clindamycin, ofloxacin and clarithomycin as recorded in Table 2. However, isolate MAM-28 isolated from eye drops is susceptible to imipenem, amikacin, ciprofloxacin, gentamycin and levofloxacin and is resistance to azithromycin, ceftazidime, amoxicillin + clavulanic acid, cefuroxime and colistin. This isolate was intermediate in its susceptibility to vancomycin, clindamycin, chloramphenicol and clarithromycin.

Also the presence of some aminoglycosides, Gentamycin, tobramycin and neomycin are classified as aminoglycosides which containing 2-deoxystreptamine in a diglycoslyated form and inhibiting protein synthesis in a triphasic way [38]. Ciprofloxacin, levofloxacin and ofloxacin are classified as a broad spectrum Quinolones [39] and as second generation quinolones which inhibit DNA gyrase and DNA topoisomerase IV according to Yao and Moellering [40]. The other antibiotics sulfamide, oxytetracyclines and chloramphenicol are classes of antibiotics, sulfonamides, tetracycliens and chloramphenicols respectively according to Kayser *et al.* [39]. These antibiotics are broad spectrum antibacterial antibiotics. Tetrayclines and chloramphenicol antibiotics

Table 2: Susceptibility of different isolates to different antibiotics

Item	Isolate code	CN	VA	IPM	AK	CIP	LEV	AZM	CAZ	AMC	CXM	DA	C	OFX	CLR	CT
Eye drops	MAM-9	13	17	41	23	33	36	39	-ev	11	-ev	29	13	33	40	-ev
	MAM-21	24	15	50	34	28	31	12	-ev	9	-ev	23	16	25	19	-ev
	MAM-28	22	16	25	34	33	35	11	-ev	-ev	-ev	19	18	28	13	-ev
	MAM-40	27	17	55	35	34	30	42	-ev	ND	-ev	20	31	30	38	-ev

inhibit protein synthesis of bactieral ribosomes. The bacteriostatic or bactericidal effect depends on the antibiotic concentrations [40]. Also, Rezk [41] isolated 14 antibiotic resistant bacterial isolates out of 45 bacterial isolates which have been isolated from 117 pharmaceutical products.

The results revealed that imipenem and amikacin are the most effective antibiotics against the majority of *Bacillus* spp. isolated from eye drops. The results revealed also that each of the tested *Bacillus* isolated strains had its own pattern for antibiotic resistance. The levels of susceptibility to antibiotics varied widely for different species of the genus *Bacillus* and even among strains of the same species as indicated from the above results and confirmed by the results of other investigators. Laflamme *et al.* [42] reported that the majority of antibiotic resistance genes has a plasmidic or chromosomal origin and is present in low copy numbers in the cell. All strains of *Bacillus cereus* isolated from open fractures in traumatology-orthopaedy were 100% resistant to penicillin, ampicillin, amoxicillin, cefalotine, cefotaxime and imipenem, while they are 100% susceptible to gentamicin, petimicin, tobramycin and 33% resistant to minocycin, 6% to erythromycin, 3% to pristinamycin, 12% to rifampicin. Also, they are sensitive to ciprofloxacin and vancomycin [43]. *B. cereus* strains isolated from topical and medicaments drugs are highly resistant to lincomycin, polyxin B and penicillin-G, cephalosporin and are susceptible to streptomycin erythromycin and chloramphenicol [16]. These antibiotics (imipenem and amikacin) were not only effective against Gram positive but also against Gram negative bacteria. Itokazu *et al.* [44] revealed that amikacin and imipenem are the most efficient against 33.869 Gram negative *Bacilli* (GNB). These antibiotics (Imipenem and amikacin) are not only effective against Gram positive but also Gram negative, carbapenem found to be the most active against Gram negative bacilli (GNB) in US. ICUs. Imipenem resistance rate with the Enterobacteriacea remained at the level of 1% or less. Also amikacin is broadly active against the enterobacteriacea and *P. aeurginosa* [45]. Tetracycline resistance was attributed to a small plasmid isolated from *B. cereus* Gp7 and this plasmid could be subsequently transformed in *B. subtilis* [46]. *Bacillus* spp. caused serious infections were found to be sensitive to clindamycin, erythromycin, chloramphenicol, kanamycin sulfate and tetracycline hydrochloride and resistant to oxacillin and cephalothin sodium and penicillin G [47, 48].

Bacillus spp are nearly always sensitive to tetracycline, kanamycin, gentamycin and chloramphenicol. Sensitivity to penicillin and cephalosporins is high for *B. subtilis*, intermediate for *B. pumilus* and *B. licheniformis* and low for *B. cereus* [49]. *B. cereus* is the cause of fulminating panophthlamitis infection. All *B. cereus* isolates are resistant to penicillin, semisynthetic penicillins and cephalosporins but sensitive to gentamicin, clindamycin, vancomycin and chloramaphenicol [50]. *Bacillus* species are sensitive to aminoglycoside, tetracycline and chloramphenicol. *B. cereus* as rule is resistant to penicillin G, ampicillin, methicillin and cephalothin with other species of *Bacillus* having varying levels of sensitivity [51]. *Bacillus cereus* isolates are highly resistant to lincomycin, polymyxin B and penicillin G. cephalosporin and are susceptible to streptomycin, erythromycin and chloramphenicol [16]. *B. cereus* isolated from valvular heart disease endocarditis is susceptible to imipenem, vancomycin, chloramphenicol, gentamycin and ciprofloxacin while clindamycin is variably effective [52]. Santini *et al.* [53] found that *Bacillus licheniformis* taken intraoperatively from the aortic area is susceptible to cephalothin, gentamicin, clindamycin, vancomycin and trimethoprim-sulfamethoxazole; it is resistant to penicillin, methicillin, tetracycline and erythromycin. Seventy five *Bacillus* strains were grouped in six species, *B. subtilis*, *B. licheniformis, B. pumilus, B. cereus, B. megaterium* and *B. polymixa* are tested against different antibiotics. Most of these strains are resistant to lincomycin; low susceptibility to polymyxin could be detected with strains related to *B. megaterium* while other species are completely resistant to this antibiotic. The levels of susceptibility to methicillin and streptomycin varied widely for different strains considerable interspecific differences in susceptibility could be detected with oleandomycinacillin, chloramphenicol, ampicillin, carbenicillin, ristomycin, tetracycline and benzyl penicillin [54]. *B. popilliae* may have a precursor to or have ancestral gene in common with vancomycin resistance

genes in enterococci [55]. *B. popilliae*, the causative organism of endocarditis is susceptible to penicillin but not to gentamycin [56]. Eye drop can be treated by the addition of imipenem or amikacin as a suitable antibiotic to confirm protection against contamination with serious pathogens of the genus *Bacillus*.

Effect of Gamma Radiation on the Representative *Bacillus* sp. Isolated From Eye Drops: Also, eye drops can not sterilized by gamma radiation, it is interested to determine the effect of gamma radiation on the *Bacillus* sp. isolated from eye drops, in general, *Bacillus* sp. were resistant to gamma radiation and adverse conditions, because *Bacillus* spp. were spore forming bacteria. The tested *Bacillus* strain MAM-40 was exposed to different doses of gamma radiation. As the gamma radiation increased, the viable count decreased gradually. Ten kGy reduced the viable count by 4.17 log cycles. The sterilization of thermolabile medical devices, such as catheters or syringes with ionizing radiation is successfully practized in many countries. The advantages of sterilization by irradiation include high penetrating power, low chemical reactivity, low measurable residues, small temperature rise and the fact that there are fewer variable to control [57]. The compounds on which radiation sterilization studies have been reported include vitamins [58], antiprotozoals [59], antibiotics [60-64], steroids [65], bronchodilators [66], anti-hypertensive [67]. So, with the application of Co-60 radiation sterilization in pharmaceutical industry, attention should be paid to the possibilities of sterilizing traditional medicine drugs produced in Vietnam [68]. Abo-State [1, 2] found that 10.0 kGy Co-60 gamma radiations reduced the viable count of *Bacillus cereus* by 1.97 log cycles. Abo-State and Khalil [69] reported that 10 kGy gamma irradiation reduced the viable count of *B. cereus* NRRL 569 and ATCC11778 by 5.5 and 2.7 log cycles respectively. The most promising Gram +ve spore forming *Bacillus* isolate MAM-96 which can on 8 mM of chloroniated compounds is highly resistant to gamma radiation. Ten kGy reduced its viability by 2.5 log cycles [70]. D_{10} value of the two highly toxigenic strains of *B. cereus* after exposure to gamma radiation is 2.2 and 1.9 kGy respectively. Gamma radiation failed to exhibit any effect on *B. cereus* toxin lethality till 2.0 Mega [71]. De Lara *et al.* [18], studied the effect of irradiation with accelerated electron beam on *B. cereus* and *B. subtilis* spore counts. Spore counts reduced approximately two log cycles for *B. cereus* and up to five log cycles for *B. subtilis* after a radiation dose of 7.6 kGy with D_{10} values ranging from 1.5 to 3.8 kGy. Two anticancer drugs were exposed to 30 kGy gamma and electron beam radiation to study the effect of irradiation on the sterilization of drugs. Cyclophosphamide (CPH) undergoes less than 2% degradation at 30 kGy. However, Doxorubicin (DOXO) was found to be quiet radiation resistant failed to undergo significant changes [72]. Valero *et al.* [73] studied the effect of electron beam irradiation (EBI) on *B. cereus* spores at doses 1.3, 3.1, 5.7 kGy.

REFERENCES

1. Abo-State, M.A.M., 1991.Control of *Bacillus cereus* isolated from certain foods. M.Sc. Thesis, Faculty of Science, Cairo University. Egypt.
2. Abo-State, M.A.M., 1996. Study of genetic background and effect of radiation on toxin production by *Bacillus cereus*. Ph.D. Thesis, Fac. Sci. Cairo University.
3. From, C., R. Pukall, P. Schumann, V. Hormazabal and P.E. Granum, 2005. Toxin producing ability among *Bacillus* spp outside the *Bacillus cereus* group. Appl. Environ. Microbiol., 71(3): 1178-1183.
4. Andersson, M.A., P. Hakulinen, U. Honkalampi-Hamalainen, D. Hoornsta, J.C. Lhuguenot, J. Maki-Paakkanon, M. Savalainen, I. Severin, A.O. Stammati, L. Turco, A. Weber, A. Von Wright, F. Zucco and M. Salkinoja-Salonen, 2007. Toxicological profile of cereulide, the *Bacillus cereus* emetic toxin, in functional assays with human, animal and bacterial cells. Toxicon, 49: 351-367.
5. Raymond, B., D. Davis and M.B. Bsonsall, 2007. Competition and reproduction in mixed infections of pathogenic and non pathogenic *Bacillus spp*. J. Invert. Pathol., 96: 151-155.
6. Hong, H.A., R. Khaneja, N.M.K. Tam, A. Cazzato, S. Tan, M. Urdaci, A. Brisson, A. Gasbarrini, I. Barnes and S.M. Cutting, 2009. *Bacillus subtilis* isolated from human gastrointestinal tract. Res. Microbiol., 160: 134-143.
7. Minnaard, J., M. Humen and P.F. Perez, 2001. Effect of *Bacillus cereus* exocellular factors on human intestinal epithelial cells. J. Food Prot., 64(10): 1535-1541.
8. Minnaard, J., V. Lievin-Le Moal, M.H. Concominer, A.L. Servin and P.F. Perez, 2004. Disaadembly of F-actin cytoskeleton after interaction of *Bacillus cereus* with fully differentiated human intestinal Caco-2 cells. Infect. Immun., 72(5): 3106-3112.

9. Minnaard, J., L. Delfederico, V. Vasseur, A. Hollmann, I. Rolny, L. Semorile and P.F. Perez, 2007. Virulence of *Bacillus cereus*: a multivariate analysis. Int. J. Food Microbiol., 116: 197-206.
10. Claudio, T.S.L., E. Velasco, C.A.S. Martins, L.M.D. De Faria, N.P. de Fonseca, L.M.C. Dias and V.M.S.C. Gonclaves, 2005. An outbreak of *Bacillus* species in a cancer hospital. Infect. Cont. Hosp. Epidemiol., 19: 856-858.
11. Sasahara, T., S. Hayashi, Y. Morisawa, T. Sakihama, A. Yoshimura and Y. Hirai, 2011. *Bacillus cereus* bacteremia outbreak due to contaminated hospital linens. Eur. J. Clin. Microbiol. Infect. Dis., 30: 219-226.
12. Cremieux, A., S. Cupferman and C. Lens, 2005. Method for evaluation of the efficacy of antimicrobial preservatives in cosmetic wet wipes. Int. J. Cosmetic. Sci., 27: 223-236.
13. Berthelot, P., J. Dietermann, P. Fascia, A. Ros, F.O. Mallaval, F. Lucht, B. Pozzetto and F. Grattard, 2006. Bacterial contamination of non sterile disposable gloves before use. Am. J. Infect. Cont., 34: 128-130.
14. Ajayi, O.B., C. Obuekwe and A.D. Ighoroje, 1996. Antibacterial efficacy of three soft contact lens disinfectants in the tropics (Nigeria). Clin. Exp. Optom., 79(3): 112-118.
15. Donzis, P.B., B.J. Mondino and B.A. Wiessman, 1988. *Bacillus* keratitis associated with contaminated contact lens care systems. Am. J. Ophthalmol., 105: 195-197.
16. Garcia-Arribas, M.L., C.J. Plaza, M.C. Dela Rosa and M.A. Mosso, 1988. Characterization of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. J. Appl. Bacteriol., 64: 257-264.
17. Zhang, X., T. Kodama and Y. Ohashi, 2008. Eyeball luxation in *Bacillus cereus*. Induced panophthalmitis following a double penetrating Ocular Injury. Jpn. J. Ophthalmol., 52: 407-425.
18. De Lara, J., P.S. Fernandzez, P.M. Periago and A. Palop, 2002. Irradiation of spores of *Bacillus cereus* and *Bacillus subtilis* with electron beams. Innovative Food Sci. Emerging Technol., 3: 379-384.
19. Zacarias, S.M., M.C. Vaccari, O.M. Alfano, H.A. Irazoqui and G.E. Imoberdorf, 2010. Effect of the radiation flux on eth photocatalytic inactivation of spores of *Bacillus subtilis*. J. Photochemist. Photobiol. A., Chem., 214: 171-180.
20. Singh, B.K., D.V. Parwate, I.B. Das Sarma and S.K. Shukla, 2010. Study on gamma and electron beam sterilization of third generation cephalosprons cefdinir and cefixime in solid state. Rad. Phys. Chem., 79: 1079-1087.
21. Botelho, M.L., S.C. Verde, L. Alves, A. Belchior, J. Reymao, S. Trabulo, M.M. Gaspar, M.E.M. Cruz and S. Simoes, 2007. Radiation sterilization of antibiotic liposome formulations: A case study. Radiat. Phys. Chem., 76: 1542-1546.
22. Kotiranta, A., K. Lounatmaa and M. Haapasalo, 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. Microb. Infect., 2: 189-198.
23. Gerri, S. and G.L.W. Hall, 2006. Medical bacteriology, in McPherson, P. (Ed) Henry's Clinical Diagnosis and Management by Laboratory Methods. W.B. Saunders Company, ed. 21.
24. Miller, J.J., I.U. Scott, H.W. Flynn, W.E. Smddy, T.G. Murray, A. Berrocal, D. Miller, 2008. Endophthalmitis caused by *Bacillus* species, Am. J. Ophthalmol., 145: 883-888.
25. Mossel, D.A., M.J., Koopman and E. Jongerius, 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol., 15: 650-653.
26. Martin, P.A.W., J.R. Lohr and D.H. Dean, 1981. Transformation of *Bacillus thuringensis* protoplasts by plasmd deoxyribonucleic acid. J. Bacteriol., 145: 980-983.
27. Cowan, S.T. and K.J. Steel, 1974. Identification of Medical Bacteria, 2nd Ed. Cambridge: Cambridge University Press.
28. Kramer, J.M., P.C.B. Turnbull, G. Muntshi and R.J. Gilbert, 1982. Identification and characterization of *Bacillus* and other *Bacillus* species associated with foods and food poisoning. In Isolation and Identification Methods for Food poisoning organisms ed. Carry, J.F.L., D. Roberts and Skinner, F.A., pp: 261-286, London: Academic Press.
29. Oxoid, Ltd., 1980. The Oxoid Manual of Culture Media, Ingredients and Other Laboratory Services. 4th Edition.
30. Beecher, D.J. and MacMillan, 1990. A novel bicomponent hemolysin from *Bacillus cereus*. Infect. Immun., 58: 2220-2227.
31. Shinagawa, K., K. Ichikawa, N. Matsusaka and S. Sugii, 1991. Purification and some properties of *Bacillus cereus* mouse lethal toxin. J. Vet. Med. Sci., 53(3): 469-474.

32. Willames-Collinet, M.F., G.T. Hosperg, M.P. Tro and H. Visingat, 1978. Identification of *Bacillus* species isolated from antacid liquids. Journal of Applied Bacteriology, 45: 71-79.
33. Gil, M.C., M.C. De La Rosa, M.A. Mosso and M.L. Garcia, Arribas, 1986. Numerical taxonomy of *Bacillus* isolated from orally administrated drugs. Journal of Applied Bacteriology, 61: 347-356.
34. Shinagawa, H., H. Iwasaki, Y. Ishino and A. Nakata, 1991. SOS-inducible DNA polymerase II of *E. coli* is homologous to replicative DNA polymerase of eukaryotes. Biochimie, 73: 433-435.
35. Beattie, S.H. and A.G. Williams, 1999. Detection of toxigenic strains of *Bacillus cereus* and other *Bacillus* spp. with an improved cytotoxicity assay. Lett. Appl. Microbiol., 28: 221-225.
36. Taylor, J.M.W., A.D. Sutherland, K.E. Aidoo and N.A. Logan, 2005. Heat stable toxin production by strains of *Bacillus cereus*, *Bacillus firmus, Bacillus megaterium, Bacillus simplex and Bacillus licheniformis.* FEMS Microbiol. Lett., 242: 313-317.
37. Sergeev, N., M. Distler, M. Vargas, V. Chizhikov, K.E. Herold and A. Rasooly, 2006. Microarray analysis of *Bacillus cereus* group virulence factors. J. Microbiol. Methods., 65: 488-502.
38. Davey, R.T. and W.B. Tauber, 1987. Post traumatic endophthalmitis: the Emerging role of *Bacillus cereus*. Rev. Infect. Dis., 9(1): 110-123.
39. Kayser, B., A. Mauron and A. Miah, 2005. Viewpoint: Legalisation of performance-enhancing drugs. Lancet, 366(Suppl 1): S21.
40. Yao, J. and R. Moellering 2007. Antibacterial agents, In: P. Murray, E. Baron, J. Jorgensen, A. Landry and M. Pfaller (Eds.), Manual of clinical microbiology, 9th edition, ASM Press, Washington, DC., 1: 1077-1113.
41. Rezk, 2011. Environmental contamination with antibiotic resistance bacteria from drugs and hospital waste water in Cairo. MSc. Thesis. Faculty of Science Al Azhar University.
42. Laflamme, C., L. Gendron, N. Turgeon, G. Filion, J. Ho and C. Duchaine, 2009. In situ detection of antibiotic resistance elements in single *Bacillus cereus* spores. Syst. Appl. Microbiol., 32: 323-333.
43. Dubouix, A., E. Bonnet, M. Alvarez, H. Bensafi, M. Archambaud, B. Chaminade, G. Chabanon and N. Marty, 2005. *Bacillus cereus* infections in traumatology. Orthopaedics Department: Retrospective investigation and improvement of healthcare practices. J. Infect., 50: 22-30.
44. Itokazu, G.S., J.P. Quinn, C. Bell-Dixon, F.M. Kahan and R.A. Weinstein, 1996. Antimicrobial resistance rates among aerobic gram negative bacilli recovered from patients in intensive care units: evaluation of a national post marketing surveillance program. Clin. Infect. Dis., 23: 779-784.
45. Lockhart, S.R., M.A. Abramson, S.E. Beekmann, G. Gallagher, S. Riedel, D.J. Diekma, J.P. Quinn and G.V. Doerin, 2007. Antimicrobial resistance among gram–negative bacilli causing infection in intensive care unit patients in the United States between 1993 and 2004. J. Clin. Microbiol., 45(10): 3352-3359.
46. Bernhard, K., H. Schrempf and W. Goebel, 1978. Bacteriocin and antibiotic resistance plasmids in *Bacillus* cereus and *Bacillus* subtilis. J. Bacteriol., 33: 897-903.
47. Ihde, D.C. and D. Armstrong, 1973. Clinical spectrum of infection due to *Bacillus species.* Am. J. Med., 55: 839-845.
48. Tuazon, C.U., H.W. Murray, C. Levy, M.N. Solney, J.A. Curtin and J.N. Sheagren, 1979. Serious infections from *Bacillus* species. J. Am. Med. Assoc., 241: 1137-1140.
49. Colpin, G.G.D., H.F.L. Guiot, R.F.A. Simonis and F.E. Zwaan, 1981. *Bacillus cereus* meningitis in a patient under gnotobiotic care. Lancet., 126: 694-695.
50. Ho, P.C., M.O. Denis and W. Stevenhead, 1982. Fulminating panophthalmitis due to exogenous infection with *Bacillus cereus*: Report of 4 cases. Br. J. Ophthalmol., 66: 205-208.
51. Johnson, D.A., P.L. Aulicinoand, J.G. Newby, 1984. *Bacillus cereus*. Induced Myonecrosis. J. Trauma., 24(3): 267-270.
52. Steen, M.K., L.A. Bruno-Murtha, G. Chaux, H. Lazar, S. Bernard and C. Sulis, 1992. *Bacillus cereus* endocarditis: Report of a case and review. Clin. Infect. Dis., 14: 945-946.
53. Santini, F., V. Borghetti, G. Amalfitano and A. Mazzucco, 1995. *Bacillus licheniformis* prosthetic aortic valve endocarditis. J. Clin. Microbiol., 38: 3070-3073.
54. Reva, O.N., V.A. Vyuntiskaya, S.R. Reznik, I.A. Kozachko and V.V. Smirov, 1995. Antibiotic susceptibility as a taxonomic characteristic of the genus *Bacillus*. Int. J. Syst. Bacteriol., 45(2): 409-411.
55. Rippere, K., R. Patel, J.R. Uhl, K.E. Piper, J.M. Steckelbrg, B.C. Kline, F.R. Cockerill and A.A. Yousten, 1998. DNA sequence resembling *van A* and *van B* in the vancomycin-resistant biopesticide *Bacillus papilliae*. J. Infect. Dis., 178: 584-588.

56. Wu, Y.J, T.C. Hong, C.J.Y. Hou, Y.S. Chou, C.H. Tsai and D.I. Yang, 1999. *Bacillus popilliae* endocarditis with prolonged complete heart block. Amer. J. Med. Sci., 317: 263-265.
57. Basly, J.P., I. Longy and M. Bernard, 1997c. Influence of radiation treatment on: ESR and HPLC study.
58. Basly, J.P., I. Basly and M. Bernard, 1998. Radiosterilization dosimetry of vitamin: An ESR study. Int. J. Radiat. Biol., 74: 521-528.
59. Basly, J.P., I. Basly and M. Bernard, 1997a. Influence of radiation treatment on two antibacterial agent and four antiprotozoal agents ESR dosometry Int. J. Pharm., 154: 109-113.
60. Marciniec, B., Z. Plotkowiak, L. Wachowski, M. Kozak and M. Popietarz-Brzezimska, 2002. Analytical study of β-irradiated antibiotics in the solid state. J. Therm. Anal. Calorim., 68: 423-436.
61. Marciniec, B., M. Stawny, M. Kozak and M. Naskrent, 2008. The influence of radiation sterilization on thiamphenicol. Spectrochem. Acta. Part A, 69: 865-870.
62. Onori, S., M. Pantaloni, P. Fattibene, E. Ciranni Signoretti, L. Valvo and M. Santucci, 1996. ESR identification of irradiated antibiotics: Cephalosporins. Appl. Radiat. Isot., 47: 1569-1572.
63. Gibella, M. and B. Tilquin, 1999. Detection of the radiolysis of solid ampicillin by UV-spectroscopy. Analysis, 27: 657-662.
64. Crueq, A.S., C. Slegers, V. Deridder and B. Tilquin, 2000. Radiosensitivity study of Cefazolin sodium. Talanta, 52: 873-877.
65. Marciniec, B., M. Ogrodowczyk and K. Dettlaff, 2005. Search for the effect of E-beam irradiation on some steroids. Radiat. Phys. Chem., 72: 517-254.
66. Basly, J.P., J.L. Duroux and M. Bernard, 1997b. The effect of gamma radiation on the degradation of salbutamol. J. Pharm. Biomed. Anal., 15: 1137-1141.
67. Engalytcheff, A., M. Kolberg, A.L. Barra, K.K. Andersson and B. Tilquin, 2004. The use of multi-frequency ESR techniques to identify the radicals produced in irradiated β-blockers Free Radical Res., 38: 59-66.
68. Hang, N.D., T.T. Cahn and T.T. Thuy, 1995. Radiation sterilization of traditional medicine drugs in Vietnam. Rad. Phys. Chem., 46: 4-6.
69. Abo-State, M.A.M. and M.S. Khalil, 2001. Effect of gamma radiation on protein finger-printing and enzymes of *Bacillus cereus* NRRL 569 and *Bacillus cereus* ATCC 11778. Egypt. J. Genet. Cytol., 29: 159-173.
70. Abo-State, M.A.M., M. Swelm, N.H. Aziz, N.M. Aly and O.A. Khalil, 2005. Characterization and effect of gamma irradiation on indigenous chloroaromatic degradation bacteria. Isotope Rad. Res., 37: 1139-1157.
71. Abo-State, M.A.M., D.A. Zahran and H.N. El-Hifnawy, 2006. Incidence of *B. cereus* in some meat products and the effect of gamma radiation on its toxin. Int. J. Agric. Biol., 8: 1-4.
72. Varshney, L. and P.B. Dodke, 2004. Radiation effect studies on anticancer drugs, Cyclophosphamide and doxorubicin for radiation sterilization. Radiat. Phys. Chem., 71: 1103-1111.
73. Valero, M., J.A. Sarrias, D. Alveraz and M.C. Salmerson, 2006. Modeling the influence of electron beam irradiation on the heat resistance of *Bacillus cereus* spores. Food Microbiol., 23: 367-371.