ISSN: 1110-7219; e-ISSN: 2682-2512 (Online) Journal homepage: http://vetj.mans.edu.eg/

Prevalence and Antimicrobial Resistance of *Bacillus cereus* in Milk and Dairy Products

Rowayda Osama, Marwa F. E. Ahmed, Amir Abdulmawjood, Maha Al-Ashmawy

To cite this article: Rowayda Osama, Marwa F. E. Ahmed, Amir Abdulmawjood, Maha Al-Ashmawy. Prevalence and Antimicrobial Resistance of *Bacillus cereus* in Milk and Dairy Products. Mansoura Veterinary Medical Journal 2020; 21, 2: 11-18.

To link to this article: https://doi.org/10.35943/mvmj.2020.2.202

Published online: 25 June 2020

Mansoura Veterinary Medical Journal 21:2(2020) 11-18

https://doi.org/10.35943/mvmj.2020.2.202

Original Article *Milk Hygiene*

Prevalence and Antimicrobial Resistance of *Bacillus cereus* in Milk and Dairy Products

Rowayda Osama[1], Marwa Ahmed[2], Amir Abdulmawjood [3], Maha Al-Ashmawy[1]

[1]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt
[2]Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University
[3]Institute of Food Quality and Food Safety, Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Foundation, Bünteweg 17, D-30559 Hannover, Germany

ARTICLE HISTORY

Received: 10.02.2020

Revised: 07.04.2020

Accepted: 31.05.2020

Address correspondence Rowayda osama; Tel: + 01000285377; E-mail: rody_osama84@yahoo.com

ABSTRACT

***Objective*:** To investigate the prevalence of *Bacillus cereus* in milk and dairy products along with detection of its antibiotic sensitivity.
***Design*:** Descriptive study.
Samples: One hundred and fifty samples of market milk, ultra high temperature milk packs (UHT), condensed milk, Milk powder, Damietta cheese, Kariesh cheese and Ras cheese.
***Procedures*:** Samples were examined for isolation and identification of *Bacillus* spp. via direct and indirect isolation, molecular examination and antimicrobial resistance. Further molecular examination was carried out in 46 isolates to detect hblA*, hblC, hblD, nheA, nheB* and *nheC* genes
Results: The prevalence of *B. cereus* by direct isolation was 52%, 13.3 %, 10%, 8%,44%, 0 % and 16% in market milk, ultra high temperature milk packs (UHT) , condensed milk , Milk powder, Damietta cheese, Kariesh cheese and Ras cheese, respectively, whereas its prevalence by indirect isolation was 64%, 20%, 20%, 48%, 52%, 40% and 36% in market milk, ultra high temperature milk packs (UHT) , condensed milk , Milk powder, Damietta cheese, Kariesh cheese and Ras cheese, respectively. *B. cereus* isolates were 100% resistant to colistin (CT), ampicillin (AM) and amoxicillin (AML). However, 83.01% were resistant to ampicillin-sulbactum (SAM), 67.9% resistant to streptomycin (S), 45.2% resistant to spiramycin (SP), 35.8% resistant to lincomysin (MY), 22.6% resistant to tetracyclin (TE), and 5.6% resistant to erythromycin (E). A prevalence of 58.6% for hblA*, hblC* and *hblD* was recorded, while a prevalence of 86.9%, 93.4% and 89.1% for *nheA, nheB* and *nheC was recorded.*
***Conclusion and clinical relevance*:** This study provides data on prevalence, contamination level and antibiotic sensitivity of *B. cereus* in milk and its products, suggesting a potential risk to health and the dairy industry.

Keywords: *Bacillus cereus*, milk, Dairy product, Antimicrobial susceptibility, Prevalence.

1. INTRODUCTION

The milk and its products are the most important source of food for human as they contain most of the nutrients required [1] . However, they represent a potential source of many organisms, including *B. cereus* that adversely impacts both the public health as well as the economy of the dairy industry. Environment plays an important role in milk contamination including soil, bedding, air, feed and faeces of animal and human [2]. Furthermore, poor hygiene during milking and the subsequent handling of the milk increases the risk of contamination with bacteria [3]. Different kinds of bacteria including aerobic psychrotrophic, Gram-negative bacteria, heterofermentative lactobacilli, and spore forming bacteria are considered to be the most frequent pathogens contaminating the milk [4].

Spore formation of some sort of bacteria is a method of withstand unfavorable conditions as sever dryness, subzero temperatures and boiling. Because of these facts, spores are very problematic aspects of spore forming pathogens such as *Bacillus cereus,* especially in food production and technology [5].

B. cereus is Gram-positive, motile, aerobic-to-facultative, spore-forming rod that is widely found in food and the environment. It produces spores, enterotoxins and lecithinase enzyme. It is mainly present in soil, milk, cereals, spices and other dried foodstuffs [6, 7].

As one of widely existing bacteria in the environment, *B. cereus* is a causative agent of food poisoning [8]. It has been also found that *Bacillus cereus* is widely spread in soil, food and in the human intestine [9]. Moreover, *B. cereus* has been

related food poisoning [10]. According to the European Food Safety Authority report on food-borne outbreaks, *B. cereus* has been found the causative agent in 77 outbreaks and 17.1 % of the cases due to bacterial toxins [11] .

Consequently, the current work aims to investigate the prevalence of *B. cereus* in milk and dairy products by using both conventional and molecular techniques along with detection of antibiotic sensitivity in order to select the appropriate antibiotics for outbreak control.

2. MATERIALS AND METHODS

2.1. Sample Collection

One hundred and fifty samples were randomly obtained including: market milk, Milk powder, Damietta cheese, Kariesh cheese and Ras cheese (25 each). In addition, 15 ultra-high temperature milk (UHT) and 10 condensed milk packs. These samples were aseptically collected from different localities of Dakahlia province, Egypt in clean, dry and sterile containers, then immediately shipped in ice box at 4ºC to the laboratory for t analysis at the same day of collection

2.2. Quantitative enumeration of Bacillus species

Quantitative enumeration of Bacillus species was performed according to standard method [12]. Briefly, each sample was thoroughly mixed prior to examination, then 25 ml (or g) from each sample was aseptically added to 225 ml of nutrient broth (Oxoid, UK). From this homogenate, (10^{-1}) first dilution, 1 ml aliquot was taken to prepare serial dilutions till 10^{-6}.

From each previously prepared dilution, 0.1 ml aliquot was aseptically inoculated onto *B. cereus* selective agar base (Oxoid, UK), supplemented with polymyxin B (50,000 IU/500 ml medium) and egg yolk emulsion (25 ml/500 ml medium) in duplicates and then the inoculated plates were incubated at 35ºC for 48h. The plates were examined for characteristic *B. cereus* colonies characterized by being large (3-7mm diameter), dull and turquoise to peacock blue surrounded by a good egg yolk precipitation of the same color due to lecithinase production. Other members of the Bacillus group are mannitol positive and appeared as green or yellow colonies with no lecithinase production. Subsequently, the average numbers of colony forming units (cfu) from the presumptive plates with 25-250 colonies were used for calculating the total cultural bacteria per gm or ml of the sample.

2.3. Qualitative detection of B. cereus

For indirect isolation of *B. cereus* previously prepared homogenate were incubated at 35ºC for 24h then streaked on *B. cereus* selective agar base plates, incubated as mentioned above and examined for *B. cereus* colonies characteristic.

2.4. Identification of Bacillus species

The suspected *Bacillus* spp. colonies were purified and identified via biochemical tests such as sugar fermentation tests, Nitrate reduction test and anaerobic growth on blood agar [12].

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility patterns of the recovered *B. cereus* isolates were determined by disc diffusion method using Mueller-Hinton agar [13]. Overnight-grown cultures in nutrient broth were prepared and swapped across Mueller-Hinton agar. The antibiotic discs were placed aseptically on it and incubated at 37ºC for 24h. Strains were evaluated as susceptible, intermediate or resistance based on Clinical and Laboratory Standards Institute (CLSI) guidelines [14] .

The following antimicrobials (manufactured by Oxoid) were used: colistin (CT) 25µg, lincomysin (MY) 10µg, ampicillin (AM) 25µg, pefloxacin (PEF) 5 µg, norfloxacin (NOR) 5µg, neomycin (N) 10µg, ampicillin-sulbactum (SAM) 30µg, tetracyclin (TE) 30 µg, amoxicillin (AML) 10µg, gentamycin (GN) 30µg, cephradine (CE)30 µg, spiramycin (SP) 100µg, vancomycin (VA) 30µg, erythromycin (E) 15µg, clindamycin (DA) 10µg and streptomycin (S) 25µg.

2.6. Detection of hbl and nhe toxine genes

2.6.1. DNA extraction

Pure Colonies from the overnight culture on Columbia agar plates containing sheep blood (Oxoid, Wesel, Germany) were used for DNA extraction guided by the manufacturer's instructions for Gram-positive bacteria with the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacture guidelines. Finally, DNA concentration was measured with nanodrop 2000C (Thermo Fisher Scientific, Germany) at 260 nm and stored at -20°C until used for PCR amplification.

Table1. Oligonucleotide primers used in DNA-based PCR of *Bacillus* isolates.

	Gene Name	Primer Name	Primer sequences (5'–3')	Gene Size (bp)
HBL complex	*hblA*	hblA-F	CAAGGTGCAGATGTTGATGC	352
		hblA-R	GAACGCCCGAATATTGAG	
	hblC	hblC-F	AATGGTCATCGGAACTCTAT	750
		hblC-R	CTCGCTGTTCTGCTGTTAAT	
	hblD	hblD-F	AATCAAGAGCTGTCACGAAT	410
		hblD-R	CACCAATTGACCATGCTAAT	
NHE complex	*nheA*	nheA-F	TACGCTAAGGAGGGGCA	500
		nheA-R	GTTTTTATTGCTTCATCGGCT	
	nheB	nheB-F	CTATCAGCACTTATGGCAG	770
		nheB-R	ACTCCTAGCGGTGTTCC	
	nheC	nheC-F	CGGTAGTGATTGCTGGG	580
		nheC-R	CAGCATTCGTACTTGCCAA	

2.6.2. Toxin-genotyping

All primers in the current study were utilized according to Melnick et al. [15], and were added to the reaction mixture at a concentration of 10 pmol/µl (Table 1). Each PCR reaction mixtures (25 µl) consisted of 1 µl primer 1 (10 pmol / µl), 1 µl primer 2 (10 pmol / µl), 12.5 µl PCR master mix (Red'y'Gold Mix, Eurogentec, Köln Germany) and 8.5 µl of nuclease free water. Finally, 2 µl DNA were added to each reaction tube. The PCR was carried out in a thermal cycler (T3000 Thermocycler, Biometra, Goettingen, Germany) started with an initial denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s and 45 sec at 72°C followed by a final extension incubation of 72°C for 5 min. The presence of amplification products was determined by loading of 10 µl of the reaction product in a 2% agarose gel (Pequlab, Erlangen, Germany) and electrophoresis was performed for 120 min at 10 volt/cm with Tris acetate-electrophoresis buffer 1xTBE buffer (Tris, Boric acid and Disodium EDTA) and a 100–2,000 bp DNA ladder (Roche, ,Germany) as molecular marker.

B.cereus DSM 4384 and *B. toyonensis* BCT7112T served as positive control. Also, *Staphylococcus aureus* DSM 2569 was used as a negative control. Reference strains were obtained from Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Germany.

3. RESULTS

The total bacillus count in market milk, ultra high temperature milk packs (UHT) , condensed milk , Milk powder, Damietta cheese, Kariesh cheese and Ras cheese were $5.4x10^5 \pm 1.09x10^5$, $1.3x10 \pm 9$, $7x10 \pm 4.2x10$, $4.5x10^2 \pm 1.6x10^2$, $2.3x10^5 \pm 8.5x10^4$, $1.5x10^6 \pm 2.4x10^5$ and $3.8x10^5 \pm 6.6x10^4$ cfu/ml or g, respectively (.Table 2).

Table2. Total *Bacillus* count in milk and dairy products.

Milk and dairy products	Sample	+ ve sample		Min	Max	Mean ± SE
		no	%			
Market milk	25	25	100%	$4.3x10^4$	$1.7x10^6$	$5.4x10^5 \pm 1.09x10^5$
UHT	15	2	13.3 %	$1.0x10^2$	$1.0x10^2$	$1.3x10 \pm 9$
Condensed milk	10	3	30%	$1.0x10^2$	$4.0x10^2$	$7.0x10 \pm 4.2x10$
Milk powder	25	17	68%	$1.0x10^2$	$3.5x10^3$	$4.5x10^2 \pm 1.6x10^2$
Damietta cheese	25	21	84%	$6.0x10^3$	$1.6x10^6$	$2.3x10^5 \pm 8.5x10^4$
Kariesh cheese	25	25	100%	$1.6x10^4$	$4.2x10^6$	$1.5x10^6 \pm 2.4x10^5$
Ras cheese	25	25	100%	$1.0x10^4$	$1.2x10^6$	$3.8x10^5 \pm 6.6x10^4$

(UHT): ultr high temperature.

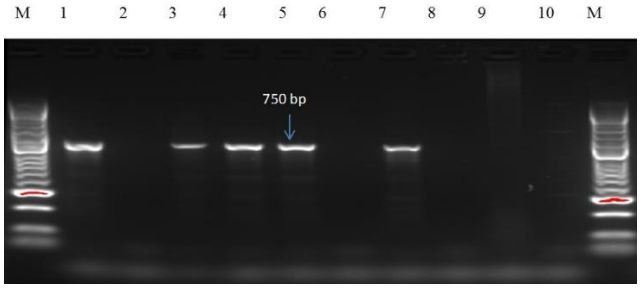

Figure 1. Typical amplification of hbl C gene, lanes 1 to 6 show positive result except number 2 and 6, 7 and 8 control positive, lane 9 control negative. M marker 100bp ladder (Promega).

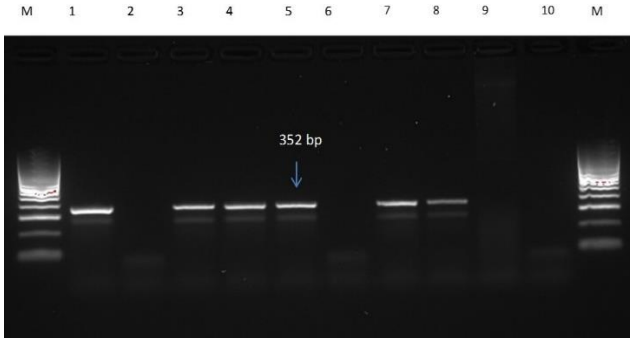

Figure 2. Typical amplification of hbl A gene, lanes 1 to 6 show positive result except number 2 and 6, 7 and 8 control positive, lane 9 control negative. M marker 100bp ladder (Promega).

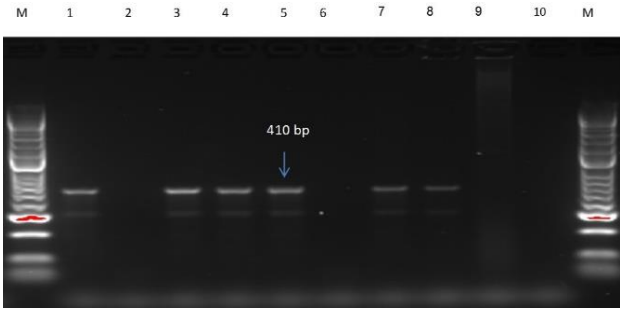

Figure 3. Typical amplification of hbl D gene, lanes 1 to 6 show positive result except number 2 and 6, 7 and 8 control positive, lane9 control negative. M marker 100bp ladder (Promega).

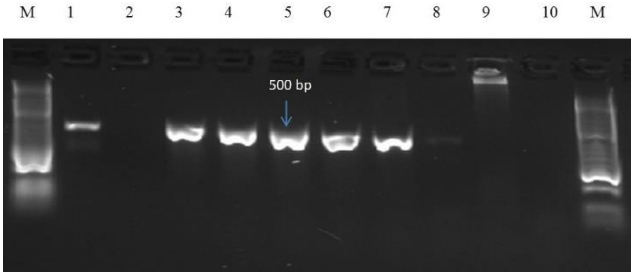

Figure 4. Typical amplification of nhe A gene Lanes from 1 to 6 show positive result except number 2, 7 and 8 control positive, Lane 9 control negative, M marker 100bp ladder (Promega).

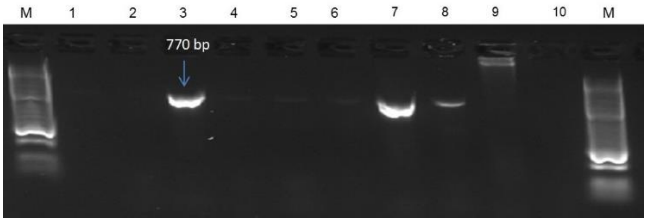

Figure 5. Typical amplification of nhe B gene, number 3 is positive, 7 and 8 control positive, Lane 9 control negative, M marker 100bp ladder (Promega).

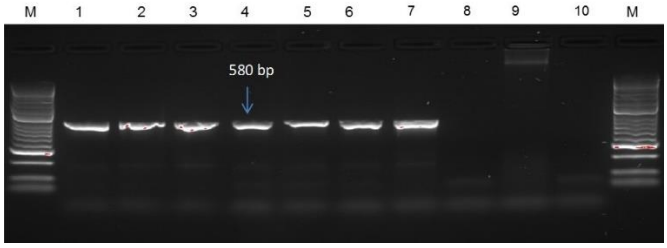

Figure 6. Typical amplification of *nhe* C gene Lanes from 1 to 6 show positive result, 7 and 8 control positive, Lane 9 control negative, M marker 100bp ladder (Promega).

Table 3 shows the prevalence of Bacillus spp. In eamined samples. The number of positive raw milk samples with *Bacillus* spp. was 100% (76% *B. subtilis*, 36% *B. pumilus*, 52% *B. cereus* and 2% *B. lichenformis)*

The number of positive *Bacillus* spp. in UHT milk samples was 20% divided in 6.6% *B. subtilis*, 13.3% *B. cereus* while both *B. pumilus* and *B. lichenformis* were not detected. In condensed milk, the positive samples for Bacillus spp. were 30% (30% *B. subtilis*, 10% *B. pumilus*, 10% *B. cereus),* while *B. lichenformis* was not detected.

In milk powder the *Bacillus* spp. was detected in 68% of samples (64% *B. subtilis*, 8% *B. pumilus* and 8% *B. cereus)*. However, *B. lichenformis* was not detected.

Regarding the Damietta cheese samples, our work revealed that the number of positive samples for *Bacillus* spp. was 84% (36% *B. subtilis*, 12% *B. pumilus*, 44% *B. cereus* and 4% *B. lichenformis*).

The number of positive *Bacillus* spp in Kariesh cheese was 96% (84% *B. subtilis* and 16% *B. pumilus), whereas* both of *B. cereus* and the *B. lichenformis* were not detected. Moreover, samples of Ras cheese were 100% positive for *Bacillus* spp. (92% *B. subtilis*, 28% *B. pumilus,* 16% *B. cereus* and 4% *B. lichenformis*).

Table 4 shows that the antimicrobial resistance, 100% of *B. cereus* isolates were resistant to colistin, ampicillin and amoxicillin followed by 83.01% resistant to Ampicillin-Sulbactum, 67.9% resistant to Streptomycin, 45.2% resistant to Spiramycin, 35.8% resistant to Lincomysin, 22.6% resistant to Tetracyclin, 5.6% resistant to Erythromycin.

Molecular examination of 46 isolates from raw milk and Damietta cheese to *hbl* A, C, D and *nhe* A, B, C showed that 27 (58.6%) isolates were positive to *hbl* A, C, D genes, 35 (76%) isolates possessed the 3 components of *nhe*., 26 (56.5%) isolates were positive to 6 genes and one isolate negative to all genes.

Additionally, the present result show that *hbl* gene was detected in (58.6%) of the tested isolates.

4. DISCUSSION

The present study revealed that total Bacillus count in market milk, ultra high temperature milk packs (UHT) , condensed milk , Milk powder, Damietta cheese, Kariesh cheese and Ras cheese were $5.4x10^5 \pm 1.09x10^5$, $1.3x10 \pm 9$, $7x10 \pm 4.2x10$, $4.5x10^2 \pm 1.6x10^2$, $2.3x10^5 \pm 8.5x10^4$, $1.5x10^6 \pm 2.4x10^5$ and $3.8x10^5 \pm 6.6x10^4$ cfu/ml org, respectively. This finding indicates that *B. cereus* is a common contaminant of milk. It can contaminate milk during production, processing and handling, it founds in soil, faeces and bedding [2, 3].

Table 3: Comparative study between direct and indirect isolation of *Bacillus spp*.in milk and dairy products.

product	Positive samples %		*B. subtilis* %		*B. pumilus* %		*B. cereus* %		*B. lichenformis* %	
	D	ID	D	ID	D	ID	D	ID	D	ID
Market milk	100%	100 %	76%	80 %	36 %	40 %	52 %	64 %	12 %	20 %
UHT milk	20%	93.3	6.6 %	46.4	-	26.6%	13.3	20 %	-	6.6 %
Condensed milk	30%	90 %	30%	70 %	10 %	20 %	10 %	20 %	-	10 %
Milk powder	68%	100 %	64%	72 %	8 %	12 %	8 %	48 %	-	40 %
Damietta cheese	84%	92 %	36%	44 %	12 %	8%	44 %	52 %	4 %	8%
Kariesh cheese	96%	100 %	84%	80 %	16 %	16 %	-	40 %	-	4%
Ras cheese	100%	100 %	92%	68 %	28 %	24 %	16 %	36 %	4 %	24 %

D: direct isolation, ID: indirect isolation

Table 4: Antimicrobial susceptibility pattern of *Bacillus cereus* (n=53)

Antimicrobial agent	S		I		R	
	No	%	No	%	No	%
Colistin (CT)	-	-	-	-	53	100%
Lincomysin (MY)	-	-	34	64.15%	19	35.8%
Pefloxacin (PEF)	24	45.28%	29	54.7%	-	
Ampicillin (AM)	-	-	-	-	53	100%
Norfloxacin (NOR)	47	88.6%	6	11.3%	-	-
Neomycin (N)	-	-	53	100%	-	-
Ampicillin-Sulbactum (SAM)	-	-	9	16.9%	44	83.01 %
Tetracyclin (TE)	3	5.6%	38	71.6%	12	22.6%
Amoxicillin (AML)	-	-			53	100%
Gentamycin (CN)	11	20.7%	42	79.24%	-	-
Cephradine (CE)	12	22.6%	41	77.3%	-	-
Spiramycin (SP)	8	15.09%	21	39.6%	24	45.2%
Vancomycin (VA)	-	-	53	100%	-	-
Erythromycin (E)	-	-	50	94.3%	3	5.6%
Clindamycin (DA)	6	11.3%	47	88.6%	-	-
Streptomycin (S)	-	-	17	32.07%	36	67.9%

S: sensitive, I: intermediate, R: resistant.

Table 5. Antimicrobial susceptibility profile of *B. cereus (n=53).*

No of isolates	Antimicrobial agents	*MAR average
3	CT,MY,AM,TE,AML,SAM,E,S	0.5
3	CT,MY,AM,AML,SP,DA,S	0.43
3	CT,MY,AM,SAM,AML,SP,S	0.43
2	CT,AM,SAM,TE,AML,SP,S	0.43
3	CT,MY,AM,SAM,AML,SP	0.37
3	CT,AM,SAM,AML,S,SP	0.37
2	CT,AM,SAM,TE,AML,S	0.37
5	CT,MY,AM,SAM,AML,S	0.37
3	CT,AM,TE,AML,SP	0.31
5	CT,AM,SAM,AML,SP	0.31
2	CT,AM,AML,SP,S	0.31
3	CT,MY,AM,SAM,AML	0.31
8	CT,AM,SAM,AML,S	0.31
6	CT,AM,SAM,AML	0.25
2	CT,AM,AML,S	0.25

***MAR:** Multiple Antimicrobial Resistance index.
colistin (CT), lincomysin (MY), pefloxacin (PEF), ampicillin (AM), norfloxacin (NOR), neomycin (N), ampicillin-sulbactum (SAM), tetracyclin (TE), amoxicillin (AML), gentamycin (CN), cephradine (CE), spiramycin (SP), vancomycin (VA), erythromycin (E), clindamycin (DA) and streptomycin (S).

Our study showed that number of positive raw milk samples with *Bacillus* spp. was 100% divided into 76% *B. subtilis*, 36% *B. pumilus*, 52% *B. cereus* and 2% *B. lichenformis*. Similarly, *B. cereus* was detected in 44% [16] , 60% [17] , 51.6% [18] and 60% [19] of the tested raw milk samples.

On contrary to our results, lower incidences of *B. cereus*; 35% [20] and 30% [21] was reported in the examined samples. Such difference may be due the high contamination of milk samples with *Bacillus* spp. *Bacillus* spp. Were found a common contaminant of milk due to their wide environmental distribution leading to milk contamination during production, handling and processing [22]. It should be noted that the Egyptian standard [23] stated that raw milk must be free from pathogenic organisms and their toxins.

In the current investigation, the number of positive *Bacillus* spp. in UHT milk samples was 20% (6.6% *B. subtilis*, 13.3% *B. cereus),* while both *B. pumilus* and *B. lichenformis* were not detected in any of samples. This finding indicates contamination of UHT milk. This may occur due to the presence of heat resistant organisms that can tolerate the process of heat treatment or by contamination with spoilage organisms after heat treatment [24]. The detection rate of *B. cereus* in UHT milk in our study was nearly similar to that obtained by other studies that reported a rate of 13.8% [18] ,18.3% [25] and 17.8% [26] . On the other hand, a study reported a higher incidence (61.3%) [27]. According to the Egyptian standard [28], UHT milk must be free from pathogenic organisms and their toxins.

The present result revealed that the number of positive Bacillus samples in condensed milk was 30%, (30% *B. subtilis*, 10% *B. B. pumilus*, 10% *B. cereus),* while *B. lichenformis* was not detected. Our detection rate of *Bacillus cereus* (10%) was lower than that of 56% obtained in another work [29], whereas another study[30] could not detect it in their samples. Moreover, Egyptian standard [31] stated that condensed milk must be free from pathogenic organisms and their toxins.

The current study revealed that the number of positive samples for *Bacillus* spp. in milk powder was 68% divided into 64% *B. subtilis*, 8% *B. pumilus* and 8% *B. cereus*. However, *B. lichenformis* was not detected.Similarly, two studies obtained nearly similar results regarding milk powder contamination by *B. cereus* which were 10.7% [32] and 8.3% [33]. On the other hand, higher percentages of 15% [19] , 27.9% [34] and 42% [35] were reported. Moreover, the current finding doesn't meet the requirement of Egyptian standard which indicates that the milk powder must be free from pathogenic organisms and toxins.

The detection of *B. cereus* in milk powder samples may be explained by the use of pasteurization and spray drying during milk powder manufacture causes induction of germination and outgrowth of *B. cereus* spores [36].

Regarding the Damietta cheese samples, our study revealed that the number of positive samples for *Bacillus* spp. was 84% (36% *B. subtilis*, 12% *B. pumilus*, 44% *B. cereus* and 4% *B. lichenformis). B. cereus* was found in 44% of the examined samples while other studies reported lower incidences of 20% [37] and 33.3% [38] . However, another study could not detect it in the examined samples [39] . Higher incidence of 50% was also recorded [40]. Interestingly, Egyptian standard [41] stated

that soft cheeses like damietta cheese must be free from pathogenic organisms and their toxins.

In case of kariesh cheese samples, the number of positive *Bacillus* spp. was 96% (84% *B. subtilis* and 16% *B. pumilus),* whereas both the *B. cereus* and the *B. lichenformis* were not detected. The extent of *B. cereus* contamination depends on the effectiveness of hygienic measures applied during processing, handling and distribution of milk products [42]. The absence of *B. cereus* in our karish cheese samples comes in agreement with other reports [39, 41] Such absence is explained by acidity kariesh cheese [43]. Contradictory, other studies reported percentages of 28% [44] and 10% [37]. Additionally, samples of ras cheese were 100% positive for *Bacillus* spp. (92% *B. subtilis*, 28% *B. pumilus,* 16% *B. cereus* and 4% *B. lichenformis)*.

Compared to our study results, both higher (48%) and lower (7%) incidences of *B. cereus* were detected in other studies [45, 46] . Generally, detection of *B. cereus* in ras cheese is inconsistent with the Egyptian standard [47] which stated that hard cheese must be free from pathogenic organisms and their toxins.

Regarding the antimicrobial resistance, 100% of *B. cereus* isolates were resistant to colistin, ampicillin and amoxicillin followed by 83.01% resistant to Ampicillin-Sulbactum, 67.9% resistant to Streptomycin, 45.2% resistant to Spiramycin, 35.8% resistant to Lincomysin, 22.6% resistant to Tetracyclin, 5.6% resistant to Erythromycin which agree withKim, Cho [48] who found that all *B. cereus* strains were resistant to β- lactam including Ampicillin, Penicillin and Amoxicillin and susceptible to Ciprofloxacin, Gentamycin, Tetracycline and Vancomycin. Therefore, the use β- lactam is ineffective for B. *cereus* infection, but use Norfloxacin and Ciprofloxacin may be of value.

Molecular examination of 46 isolates from raw milk and damietta cheese to *hbl* A, C, D and *nhe* A, B, C shows that 27 (58.6%) isolates were positive to *hbl* A, C, D genes, 35 (76%) isolates poosses the 3 components of *nhe*., 26 (56.5%) isolates were positive to 6 genes and one isolate negative to all genes. *B. cereus* secretes a group of enterotoxins which cause food poisoning symptoms (diarrheal type). These enterotoxins are hemolysin BL (*hbl*), nonhemolytic enterotoxin (*nhe*) and cytotoxin K (Cytk) [49] *hbl* is considered to be the first *B. cereus* enterotoxin to be characterized [50, 51]. Nonhemolytic enterotoxin nhe was characterized in Norway after an outbreak of food poisoning involving 152 people [51].

Nearly, all tested *B. cereus* strains produce *nhe*, the finding is in agreement with our results, which showd that 70% of isolates were *nhe* gene positive. Other studies [52, 53] found that 100% of isolates were positive to *nhe* gene, while it was also found that only less than 54.8% of isolates were positive to *nhe* gene [54]

The present result show that *hbl* gene was detected in 58.6% of the tested isolates, whereas another study [52] found *hbl* genes were also highly frequent in the tested strains (92%). In this study, all of the tested isolates contained at least one of the six genes tested indicating the high enterotoxigenicity of *B. cereus* and a potential risk to milk and dairy.

Conclusion

The results of the present study indicate that *Bacillus* spp. are established in milk and dairy products. Therefore, it is recommended use of high quality raw milk for the manufacture of milk products, proper cleaning and sanitization of equipment, employment healthy workers with health certificate in dairy industry, and effective sanitation in dairy industry in order to minimize contamination of milk and dairy products.

Conflict of interest statement

The authors declare that there is no any conflict of interest in the current research work.

Research Ethics Committee permission

The current research work was conducted according to standards of Research Ethics committee, Faculty of Veterinary Medicine, Mansoura University.

Authors´ contribution

Rowayda osama performed the experiment and drafted the MS. Marwa F. E. ahmed and Amir Abdulmawjood performed the molecular experiment in Institute of Food Quality and Food Safety, Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Germany. Maha Al-Ashmawy supervised the whole research work and revised the MS.

5. REFERENCES

[1] Waser M, Michels KB, Bieli C, Flöistrup H, Pershagen G, von Mutius E, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. Clin Exp Allergy 2007;37:661-70. https://doi.org/10.1111/j.1365-2222.2006.02640.x

[2] Velázquez-Ordoñez V, Valladares-Carranza B, Tenorio-Borroto E, Talavera-Rojas M, Antonio Varela-Guerrero J, Acosta-Dibarrat J, et al. Microbial Contamination in Milk Quality and Health Risk of the Consumers of Raw Milk and Dairy Products. Nutrition in Health and Disease - Our Challenges Now and Forthcoming Time: IntechOpen 2019. https://doi.org/10.5772/intechopen.86182

[3] Elmoslemany AM, Keefe GP, Dohoo IR, Wichtel JJ, Stryhn H, Dingwell RT. The association between bulk tank milk analysis for raw milk quality and on-farm management practices. Prev Vet Med 2010;95:32-40. https://doi.org/10.1016/j.prevetmed.2010.03.007

[4] Ledenbach LH, Marshall RT. Microbiological Spoilage of Dairy Products. Compendium of the Microbiological Spoilage of Foods and Beverages: Springer New York; 2009. p. 41-67. https://doi.org/10.1007/978-1-4419-0826-1_2

[5] Soni A, Oey I, Silcock P, Bremer P. BacillusSpores in the Food Industry: A Review on Resistance and Response to Novel Inactivation Technologies. Compr Rev Food Sci F 2016;15:1139-48. https://doi.org/10.1111/1541-4337.12231

[6] Hwang J-Y, Park J-H. Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of Bacillus cereus isolated from infant formulas and ready-to-eat foods. J Dairy Sci 2015;98:1652-60. https://doi.org/10.3168/jds.2014-9042
[7] Tewari A, Abdullah S. Bacillus cereus food poisoning: international and Indian perspective. J Food Sci Tech 2014;52:2500-11. https://doi.org/10.1007/s13197-014-1344-4
[8] Rasko DA, Altherr MR, Han CS, Ravel J. Genomics of theBacillus cereusgroup of organisms. FEMS Microbiol Rev 2005;29:303-29. https://doi.org/10.1016/j.fmrre.2004.12.005
[9] Bottone EJ. Bacillus cereus, a Volatile Human Pathogen. Clin Microbiol Rev 2010;23:382-98. https://doi.org/10.1128/CMR.00073-09
[10] Hauge S. FOOD POISONING CAUSED BY AEROBIC SPORE-FORMING BACILLI. J App Bacteriol 1955;18:591-5. https://doi.org/10.1111/j.1365-2672.1955.tb02116.x
[11] Authority EFS. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2006. EFSA J 2007;5. https://doi.org/10.2903/j.efsa.2007.130r
[12] Roberts D., Greenwood. Practical food microbiology. 3rd ed. Blackwell. 2003. https://doi.org/10.1002/9780470757512
[13] Jorgensen JH, Turnidge JD. Susceptibility Test Methods: Dilution and Disk Diffusion Methods*. Manual of Clinical Microbiology, 11th Edition: American Society of Microbiology p. 1253-73. https://doi.org/10.1128/9781555817381.ch71
[14] CLSI. Clinical and Laboratory Standard Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing. 20th Informational Supplement CLSI document M100-S20 CLSI, Wayne, PA: Clinical and Laboratory Standard Institute; Available from: https://clsiorg/. 2010.
[15] Melnick RL, Testen AL, Poleatewich AM, Backman PA, Bailey BA. Detection and expression of enterotoxin genes in endophytic strains of Bacillus cereus. Lett Appl Microbiol 2012;54:468-74. https://doi.org/10.1111/j.1472-765X.2012.03232.x
[16] ABDALLAH MIM. Aerobic sporeforming bacteria in milk and some dairy products in Damietta Governorate. MD Thesis Vet Sci Zagazig University. 1997.
[17] Martins, Albuquerque. Quality of commercial pasteurized type C milks in Fortaleza. Bacteria with multiple resistance to antibiotics. Higiene Alimentar 1999;13: 39-42. .
[18] Vidal AMC, Rossi Junior OD, Abreu ILd, Bürger KP, Cardoso MV, Gonçalves ACS, et al. Detection of Bacillus cereus isolated during ultra high temperature milk production flowchart through random amplified polymorphic DNA polymerase chain reaction. Ciência Rural 2015;46:286-92. https://doi.org/10.1590/0103-8478cr20141539
[19] Mohamed AS, Alnakip ME, Aal SF. Occurrence of Bacillus cereus in raw milk and some dairy products in Egypt. Japanese J Vet Res 2016;64:S95-S103.
[20] Te Giffel M, Beumer R. Isolation, identification and characterization of Bacillus cereus in the dairy industry. Tijdschrift voor diergeneeskunde 1998;123:628.
[21] Hassan GM, Al-Ashmawy MAM, Meshref AMS, Afify SI. STUDIES ON ENTEROTOXIGENICBACILLUS CEREUSIN RAW MILK AND SOME DAIRY PRODUCTS. J Food Safety 2010. https://doi.org/10.1111/j.1745-4565.2010.00226.x
[22] Walker SJ. Major spoilage micro-organisms in milk and dairy products. Int J Dairy Technol 1988;41:91-2. https://doi.org/10.1111/j.1471-0307.1988.tb00606.x
[23] ES. Egyptian Standards. Raw milk. Egyptian Organization for Standardization and quality Control ES: 154-2001.
[24] Hassan A, Amjad I, Mahmood S. Microbiological and physicochemical analysis of different UHT milks available in market. African J Food Sci (ACFS) 2009;3:100-6.
[25] Bahout A. Prevalence of Bacillus species in UHT milk. Assiut Vet Med J. 2000;42:47-53.
[26] Sobeih A, Al-Hawary I, Aman I. Microbiological quality of milk and ice cream sold in Kafr El-Sheikh and El-Gharbia governorates. Minufyia Vet J 2002;2:79-89.
[27] Ali ZI, Saudi AM, El-Esawy HA. Incidence and public health significance of aerobic spore forming bacteria in Ultra Heat Treated (UHT) milk.
[28] ES. UHT milk. Egyptian Standards Egyptian Organization for Standardization and quality Control ES: 1623-2005.
[29] Abdel-Hameed. Studies on Bacillus cereus and related species in heat treated milk and some milk products. Fac Vet Med, Assuit univerisity. 2004.
[30] Abdallah MIMI. Hygienic quality of concentrated and raw milks sold in Damietta governorate. 2002.
[31] ES. Condensed milk. Egyptian Standards Egyptian Organization for Standardization and quality Control ES: 1008-2000.
[32] Hammer P, Wiebe C, Walte H, Teufel P. Incidence and properties of Bacillus cereus strains from a milk powder plant-risk consideration and quality assurance. Kieler Milchwirtschaftliche Forschungsberichte 2001;53:123-46.
[33] Di Pinto A, Bonerba E, Bozzo G, Ceci E, Terio V, Tantillo G. Occurence of potentially enterotoxigenic Bacillus cereus in infant milk powder. Eur Food Res Technol 2013;237:275-9. https://doi.org/10.1007/s00217-013-1988-8
[34] AbdelKhalek. Incidence and characterization of - enterotoxigenic Bacillus cereus in some dairy products. Suez Canal VetMedJ ; 5: 1-10 2002.
[35] Rodriquez MH, Barrett EL. Changes in Microbial Population and Growth of Bacillus cereus During Storage of Reconstituted Dry Milk. J Food Prot 1986;49:680-6. https://doi.org/10.4315/0362-028X-49.9.680
[36] Arevalo-Rodriguez I, Smailagic N, Roqué I Figuls M, Ciapponi A, Sanchez-Perez E, Giannakou A, et al. Mini-Mental State Examination (MMSE) for the detection of Alzheimer's disease and other dementias in people with mild cognitive impairment (MCI). The Cochrane database of systematic reviews. 2015;2015:CD010783-CD. https://doi.org/10.1002/14651858.CD010783.pub2
[37] El Sayed M, Hosny I, El Kholy W, El Dairouty R, Mohamed HS. Microbiological evaluation of Egyptian white soft cheeses style. J American Sci 2011;7:517-26.
[38] El-Gamal M, El Dairouty R, Okda A, Salah SH, El-Shamy S. Incidence and interrelation of Cronobacter sakazakii and other foodborne bacteria in some milk products and infant formula milks in Cairo and Giza area. World Appl Sci J 2013;26:1129-41.
[39] Ibrahim GA, Sharaf OM, El-Khalek ABA. Microbiological quality of commercial raw milk, domiati cheese and kareish cheese. Middle East Journal Appl Sci 2015;5:171-6.
[40] Helmy ZA, Abd-El-Bakey A, Mohamed EI. Occurrence of Bacillus cereus in milk and milk products in Egypt. Zentralblatt für Mikrobiologie 1984;139:129-33. https://doi.org/10.1016/S0232-4393(84)80006-2
[41] ES. Egyptian Standards. Soft cheese. Egyptian Organization for Standardization and quality Control. ES: 1008-2000.
[42] FDA. Food and Drug administration (FDA), Center for Food Safety and Applied Nutrition (SFSAN). Bad book bug: Food borne Pathogenic Microorganisms and Natural Toxins Handbook. 2000.
[43] Clavel T, Carlin F, Lairon D, Nguyen-The C, Schmitt P. Survival of Bacillus cereus spores and vegetative cells in acid media simulating human stomach. J App Microbiol 2004;97:214-9. https://doi.org/10.1111/j.1365-2672.2004.02292.x
[44] Sadek ZI, Fathi FA, Salem M. Incidence, survival and biocontrol of psychrotrophic Bacillus cereus and its potential for toxin production in milk and Tallaga cheese. Polish J Food Nutr Cci 2006;15:419-25.
[45] Nawar. Toxicoinfection organisms in milk and some street- vended dairy products. Fac vet med Alexandria University,. 2007.
[46] Sadek Z, Hosny I, El-Kholy W, El-Dairouty R. Comparative investigations for detection of foodborne microorganisms in Egyptian hard cheese" Ras" using conventional and fast biochemical tests. Global Veterinaria 2009;3:189-95.
[47] ES. Egyptian Standards. Rascheese.Egyptian Organization for Standardization and quality Control ES 1183-1998.
[48] Kim C-W, Cho S-H, Kang S-H, Park Y-B, Yoon M-H, Lee J-B, et al. Prevalence, Genetic Diversity, and Antibiotic Resistance ofBacillus cereusIsolated from Korean Fermented Soybean Products. J Food Sci 2014;80:M123-M8. https://doi.org/10.1111/1750-3841.12720
[49] Sastalla I, Fattah R, Coppage N, Nandy P, Crown D, Pomerantsev AP, et al. The Bacillus cereus Hbl and Nhe Tripartite Enterotoxin Components

Assemble Sequentially on the Surface of Target Cells and Are Not Interchangeable. PLoS ONE 2013;8:e76955. https://doi.org/10.1371/journal.pone.0076955

[50] Beecher DJ, Macmillan JD. Characterization of the components of hemolysin BL from Bacillus cereus. Infect Immun 1991;59:1778-84. https://doi.org/10.1128/IAI.59.5.1778-1784.1991

[51] Beecher DJ, Wong AC. Improved purification and characterization of hemolysin BL, a hemolytic dermonecrotic vascular permeability factor from Bacillus cereus. Infect Immun 1994;62:980-6. https://doi.org/10.1128/IAI.62.3.980-986.1994

[52] Guinebretiere MH, Broussolle V, Nguyen-The C. Enterotoxigenic Profiles of Food-Poisoning and Food-Borne Bacillus cereus Strains. J Clin Microbiol 2002;40:3053-6. https://doi.org/10.1128/JCM.40.8.3053-3056.2002

[53] Andersen Borge GI, Skeie M, Sørhaug T, Langsrud T, Granum PE. Growth and toxin profiles of Bacillus cereus isolated from different food sources. International J Food Microbiol 2001;69:237-46. https://doi.org/10.1016/S0168-1605(01)00500-1

[54] Abbas BA, Khudor MH, Saeed BM. Detection of hbl, nhe and bceT Toxin Genes in Bacillus cereus Isolates by Multiplex PCR. Int J Curr Microbiol App Sci. 2014;3:1009-16.