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Original article

INCIDENCE AND BACTERIAL LOAD OF VEGETATIVE *BACILLUS* SPECIES IN DRIED MILK-BASED PRODUCTS SOLD IN UPPER EGYPT

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Summary

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Bacillus spp. are foodborne pathogens important in food contamination, especially in dried milk products. They are associated with foodborne outbreaks, spoilage of dairy products (sweet curdling and bitterness), and bovine mastitis. This study investigated the contamination rate of *Bacillus* spp. in a total of 105 samples of the dried milk products kishk, Cerelac and whole milk powder, marketed in Assiut city, Upper Egypt. The identification of the *Bacillus* spp. strains was completed using conventional biochemical methods and PCR protocols for the confirmation of isolates. *Bacillus* spp. were isolated from 57 out of 105 (54%) [95% CI: 44.6–64.0%] of the examined samples at levels of up to 7.7 $\log_{10}$ cfu/g. The prevalence of *Bacillus* spp. was significantly ($P<0.05$) higher in kishk (74%) than in the other food categories with a mean count of 4.04±0.49 $\log_{10}$ cfu/g. Regarding the species of *Bacillus* detected, 39.05% of the examined samples contained *B. cereus*, 8.57% contained *B. subtilis*, 2.86%: *B. pumilus,* another 2.86%: *B. megatrium* and 0.95%: *B. licheniformis. B. cereus* was isolated from 18 (51.43%) of the kishk samples, 9 (25.71%) of the Cerelac samples and 14 (40%) of the whole milk powder samples. Out of the 41 *B. cereus* strains previously identified by biochemical tests, 19 (46.34%) isolates were also confirmed using PCR (61.11%, 42.86% and 22.22% for kishk, whole milk powder and Cerelac, respectively). In conclusion, incorporating preventive measures to reduce bacterial contamination in the Egyptian dairy environment are warranted in order to avoid the contamination of milk products with these life-threatening pathogens.

Key words: *Bacillus cereus, Bacillus* spp., Cerelac, kishk, PCR, whole milk powder

INTRODUCTION

The genus *Bacillus* is one of the most concerning foodborne pathogens in the dairy industry due to production of enterotoxins which cause infections as well as their ability to contribute to food spoilage. These bacteria produce highly heat-resistant spores that are difficult to inactivate in food products leading to subsequent growth causing spoilage, particularly in dairy products (Beattie, 1997). *Bacillus* spp. are Gram-positive, aerobes-to-facultative anaerobes, spore-forming,

rod-shaped microorganisms. *Bacillus* is an opportunistic pathogen and is ubiquitously distributed in reheated or inadequately cooked foods such as fried rice, milk, and other dairy products (Stenfors Arnesen *et al.*, 2008; Hwang & Park, 2015). Despite the fact that *B. cereus* is the only pathogenic organism of the *Bacillus* genus, various species such as *B. subtilis*, *B. sphaericus*, *B. licheniformis*, *B. megaterium* and *B. pumilus* have been implicated in serious infections (Nieminen *et al.*, 2007; Kosikowska *et al.*, 2015). Notably, *B. cereus* and *B. licheniformis* are the most common pathogenic *Bacillus* species isolated from raw milk and at all stages of dairy processing (Scheldeman *et al.*, 2006).

Bacillus cereus is a major cause of food poisoning in the industrialised world with bacteral loads ranging from 10^2 to 10^8 cfu/g food (Machaiah & Krishnan, 2014; Hwang & Park, 2015). This pathogen is known to induce two syndromes in people: diarrhoeal and emetic. Both syndromes occur via the action of specific toxins. Heat-labile enterotoxins are produced during the growth of the pathogenic bacteria in the small intestine, causing diarrhoeal syndrome. The emetic type of food poisoning is likely due to the heat- and acid-stable cyclic dodecapeptide cereulide (Granum, 1994; Agata *et al.*, 1995). Although food poisoning caused by *B. cereus* is not usually serious, some severe cases have been reported (EFSA & ECDC, 2014) after ingestion of food contaminated with high amounts of the emetic toxin. Furthermore, three deaths due to the necrotic *B. cereus* enterotoxin have been acknowledged (Lund *et al.*, 2000). The morbidity and mortality of these infections are largely due to haemolysin BL (hbl) protein complexes and the non-haemolytic enterotoxin (nhe) genes harboured in *B. cereus,* and other enterotoxins containing a single protein which encodes for bceT (*B. cereus* enterotoxin), cytK, and one emetic toxin (ces) (Ehling-Schulz *et al.*, 2006; Hwang & Park, 2015).

Animals are more seriously affected by *B. cereus,* which is a common pathogen of bovine mastitis, severe systematic and pyrogenic infections, gangrene, septic meningitis, cellulitis, panophthalmitis, lung abscesses and endocarditis (Ghazali *et al.*, 2022). *Bacillus cereus* group is known to produce several types of extracellular or intracellular thermoresistant enzymes in contaminated dairy products including proteases, lipases and amylases. These enzymes of bacterial origin are responsible for the detrimental effects on the organoleptic quality of milk and dairy products. An example of this is the bitter flavour, clotting and gelation of milk due to the presence of the bacterial protease (Chen *et al.*, 2003).

In Egypt, fresh milk and dairy products are an important component of the daily Egyptian diet. Raw milk is mostly used as substrate for manufacturing milk-based products such as kishk, Labneh, milk powder, Cerelac, ice cream, etc. Kishk is a homemade milk-based product that is known as wheat fermented milk in Upper Egypt. Kishk is considered traditional food and is made of boiled, dried and crushed grains with Laban Zeer (Abou-Donia, 2008). Cerelac is a nutritious wheat-based cereal mixed with milk powder used for infants after 6 months of age. Milk powders are a common and widely used product in Egypt, due to their extended shelf-life and their high nutritional value including proteins, minerals, fats, and vitamins (Sadek *et al.*, 2018).

Prior studies have globally detected *Bacillus* in many types of food, including meat, eggs and dairy products due to the ubiquitous nature of these organisms (Zhu

et al., 2016). The excessive use of higher pasteurisation temperatures in the dairy industry and the extended shelf-life of dried milk and other milk-based products may lead to an increasing incidence of the *Bacillus* group in them (Meer *et al.*, 1991). Rahimi *et al*. (2013) and Cetin-Karaca & Morgan (2018) reported that the most dominant food matrices for this foodborne pathogen are milk powder products and the infant formulas.

Available data on the prevalence of *Bacillus* species in milk-based products in Upper Egypt are lacking, so, the aim of our investigation was to quantify and isolate *Bacillus* spp. in the most representative Egyptian milk-based products. Additionally, the molecular identification of *B. cereus*, the most serious foodborne pathogen, using polymerase chain reaction (PCR) was also included. The outcome of this study will help to determine the main contamination routes in order to establish improved manufacturing standards in the Egyptian dairy industry.

MATERIALS AND METHODS

Samples collection and preparation for microbiological analysis

A total of 105 samples of milk-based products which included kishk, Cerelac and whole milk powder, were collected from April to July 2022. Thirty-five samples of each product were collected on a random basis at retail outlets and at farmers' houses in the Assuit province, Egypt. According to the procedure described by Roberts & Greenwood (2003), 25 g of each of the kishk samples were transferred into a sterile mortar containing sterile white sand. These samples were mashed thoroughly in a stomacher (Seward® 400) until complete homogenisation, and then 225 mL of sterile saline (0.9% NaCl) were added to make a decimal dilution. Twenty-five grams of each of the Cerelac and whole milk powder samples, were directly diluted in sterile saline using ten-fold serial dilution (Roberts & Greenwood, 2003).

Quantitative enumeration of Bacillus spp.

After serial dilution of the prepared samples, 0.1 mL aliquots were aseptically transferred and inoculated on mannitol-egg yolk-polymyxin (MYP) agar (Oxford, UK), supplemented with polymyxin B (50,000 IU/500 mL medium) and egg yolk emulsion (25 mL/500 mL medium) in duplicate. The inoculated plates were incubated at 30 °C for 48 h and the number of presumptive colonies on each agar plate was counted. The plates were examined for the expected *B. cereus* colonies, which were large and pink (an indication that mannitol fermentation did not occur) and generally surrounded by a zone of precipitation (an indication that lecithinase was produced). Other members of the *Bacillus* spp. were 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 were used for calculation of the total cultural bacteria per g or mL of each sample.

Isolation and identification of Bacillus spp.

Isolation procedures were completed according to Roberts & Greenwood (2003) with minor modifications. Twenty-five mL or g of each prepared sample was inoculated into 100 mL of brain heart infusion (BHI) broth and incubated at 37 °C for 24 h. A sampling loop of the incubated broth from each tube was streaked on MYP agar, and incubated at 30 °C for 24 h. Suspected colonies were lifted onto

nutrient agar slants and incubated at 37 °C for 24 h before being subjected to identification procedures.

The suspected *Bacillus* spp. colonies were purified and identified using conventional biochemical tests including egg yolk lecithinase, sugar fermentation, citrate utilisation, nitrate reduction test, methyl red (MR), Voges-Proskauer (VP) tests and anaerobic growth on blood agar (Aruwa & Olatope, 2015). The smear was prepared from the isolated culture on clean, grease-free microscopic glass slide and stained according to Gram. The stained smear was evaluated by light microscopy to verify bacterial morphology. Details of morphological and biochemical tests of the tested bacilli are shown in Table 1.

Molecular identification of B. cereus using PCR

DNA was extracted from the overnight BHI cultures using the boiling method. The *B. cereus* cultures were incubated overnight in BHI broth and then centrifuged (600×g; 5 min) to obtain the pellet (10^6 to 10^7 cfu) using a labelled 1.5 mL safe-lock tube. The pellet was re-suspended into 100 µL of phosphate buffered saline. The solution was heated at 95 °C for 5 min and centrifuged at >10,000 g for 5 min to pellet the cellular debris. The supernatant (lysate) was transferred into a properly labeled 1.5 mL safe-lock tube and stored at −20 °C for further use.

The PCR technique was performed according to the methods described by Altayar & Sutherland (2006). A volume of 20 µL of reaction mixture was used consisting of 4.2 µL genomic DNA, 10 µL 2× PCR Master Mix (Green Master, Promega, USA), 1 µL of each primer (BcAPPRI [C T T (C/T) TT GGC CTT CTT CTAA] and BcFF2 [GAG ATT TAA ATG AGC TGT AA] (Applied Biosystem, USA) (Altayar & Sutherland, 2006), and nuclease free water up added to 20 µL in a PCR tube. PCR was performed under the following conditions: denaturation at 95 °C for 1 min, 40 cycles of annealing at 50 °C for 45 seconds, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products were electrophoresed in 1% agarose gel (GX 040.90, Gen Agarose, L.E., Standard DNA/RNA agarose, Molecular Biology Grade, Inno–Train Diagnostic, D–61476, Kronberg/Taunus) containing ethidium bromide as 1 µL/mL electrophoresis buffer at 100 V for 60 min using 100 bp DNA-ladder (SCiE–PLAS, HU 10, 5636, UK).

Statistical analyses

The statistical analysis includes mean comparison tests, univariate analysis of variance followed by Tukey's *post-hoc* test ($P<0.05$) to evaluate significant differences in *Bacillus* spp. incidence and counts between all of the examined samples of milk-based products. The observed data were statistically analysed using SPSS statistics 21 for Windows (IBM SPSS, Amonk, NY, USA).

RESULTS

The prevalence and concentration of *Bacillus* spp. from a total of 105 samples of milk-based products (kishk, Cerelac and whole milk powder) were shown on Fig. 1. Overall, 57 samples (54%) [95% CI: 44.6–64.0%] were contaminated with *Bacillus* spp. with a mean count of 2.65±0.28 $\log_{10}$ cfu/g.

The occurrence of *Bacillus* spp. obtained from the examined samples ranged from 34% [95% CI: 17.7–50.8%] to 74%

Table 1. Some morphological and biochemical characteristics of *Bacillus* spp. using conventional method.

Bacillus spp.	Morphological and biochemical tests								
	Gram stain	Morphology	Lecithinase production	Lactose fermentation	Citrate utilization	Nitrate reduction	Methyl red (MR)	Voges-Proskauer (VP)	Anaerobic growth on blood agar
B. cereus	+	R	+	+	+	+	+	+	+
B. subtilis	+	R	+	+	+	+	–	+	+
B. licheniformis	+	R	+	–	–	+	+	+	+
B.megatrium	+	R	+	+	+	–	–	–	+
B. pumilus	+	R	+	+	+	+	–	+	+

(+): positive reaction, (–): negative reaction, R: rods.

Table 2. Prevalence and frequency distribution of different isolates of *Bacillus* spp. in the examined samples of milk-based products.

Isolated *Bacillus* spp.	Positive samples								
	Kishk			Cerelac			Whole milk powder		
	No	%	Frequency distribution (%)	No	%	Frequency distribution (%)	No	%	Frequency distribution (%)
B. cereus	18	51.43	69.23	9	25.71	75.00	14	40	73.68
B. subtilis	2	5.71	7.69	2	5.71	16.67	5	14.29	26.32
B. licheniformis	1	2.86	3.85	–	–	–	–	–	–
B. megatrium	3	8.57	11.54	–	–	–	–	–	–
B. pumilus	2	5.71	7.69	1	2.86	8.33	–	–	–
Total	26	74.29	100	12	34.29	100	19	54.29	100

(–): non-detectable.

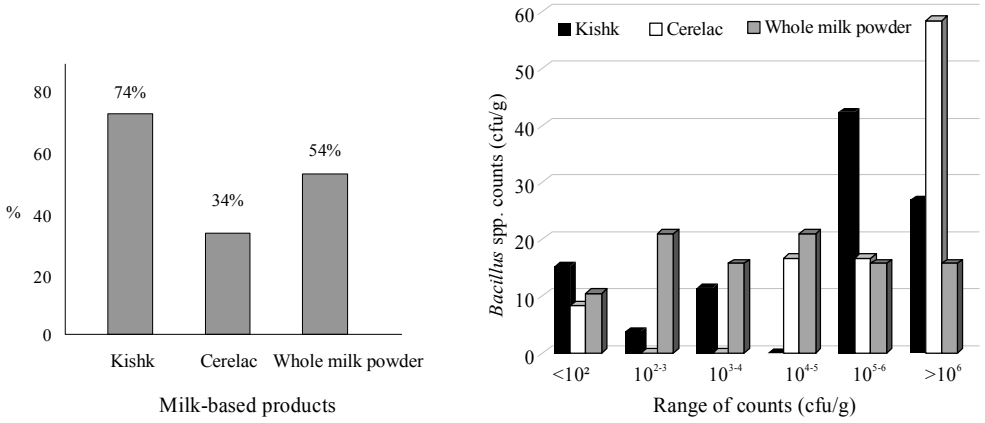

Fig. 1. Distribution (A) and concentrations (B) of *Bacillus* species in the examined samples of milk-based products (kishk, Cerelac and whole milk powder).

[95% CI: 59.1–89.5%] in Cerelac and kishk, respectively. Importantly, a significant increase ($P<0.05$) in the prevalence of *Bacillus* spp. was recorded in kishk comparing to Cerelac (Fig. 1A). Additionally, minimum to maximum levels of *Bacillus* species were < 2–7.7, < 2–6.5 and < 2–6.1 $\log_{10}$ cfu/g in 74% of kishk, 34% of Cerelac and 54% of whole milk powder, respectively. There was an increase ($P<0.05$) in the mean count of the obtained bacilli in kishk comparing to both Cerelac and whole milk powder (Fig. 1B).

The examined samples showed high level of contamination with *Bacillus* spp. The concentration of *Bacillus* spp. was more than 10^5 cfu/g in most of the tested samples (57.9%). Furthermore, the highest count (above 10^6 cfu/g) was reported in 58.33% and 26.92% of Cerelac and kishk, respectively (Fig. 1B).

In the current study, all isolates showed common morphological and biochemical characteristics consistent with the identification of *Bacillus* spp. (Table 1). Different species of the *Bacillus* group were identified in this study including *B. cereus, B. subtilis, B. licheniformis, B. megatrium* and *B. pumilus*. Strinkingly, *B. cereus* was the most prevalent isolate in the examined samples (71.93%) followed by *B. subtilis* (15.79%). *B. cereus* was identified in 51.43%, 40% and 25.71% of kishk, whole milk powder and Cerelac, respectively (Table 2), while 14.29% of whole milk powder and 5.71% of kishk as well as Cerelac were positive for *B. subtilis*. The prevalence of other identified *Bacillus* species (*B. licheniformis, B. megatrium* and *B. pumilus*) was shown in Table 2. *B. licheniformis* and *B. megatrium* were not detected in either Cerelac or whole milk powder samples. Whole milk powder samples were devoid of *B. pumilus* (Table 2).

Biochemical analysis and molecular identification of *B. cereus*, the most dangerous and frequently isolated strain in the current study, are presented in Table 3 and Fig. 2. In total, 41 *Bacillus* strains out of 57 (71.93%) isolated from the examined samples of milk-based products were identified as *B. cereus* using conventional

Table 3. Molecular identification of *B. cereus* isolates isolated from the examined samples of milk-based products (Kishk, Cerelac and Whole milk powder) using PCR.

Examined samples	No. of tested *B. cereus* isolates	No. (%) of identified isolates
Kishk	18	11 (61.11)
Cerelac	9	2 (22.22)
Whole milk powder	14	6 (42.86)
Total	41	19 (46.34)

biochemical method (sugar fermentation, citrate utilisation, nitrate reduction test, MR test, VP tests etc.). Then, the identified strains were subjected to confirmation using the PCR technique. Herein, 19 out of 41 (46.34%) conventionally identified *B. cereus* strains were confirmed. Moreover, the distribution of *B. cereus* recovered from kishk, Cerelac and whole milk powder was 61.11%, 42.86% and 22.22%, respectively (Table 3).

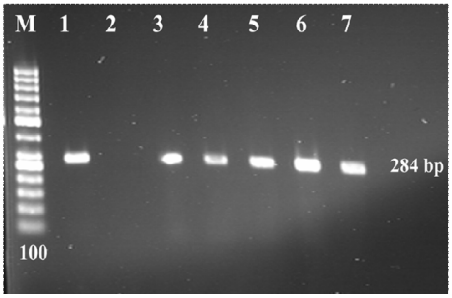

Fig. 2. Agarose gel electrophoresis of the PCR product for DNA extracted from the analysed *B. cereus* isolates. Lane (M): DNA ladder 100 bp, lane 1: positive control, lane 2: negative control and lanes 3–7: positive strains with specific bands at 284 bp.

DISCUSSION

Spore-forming *Bacillus* spp. are one of the most common contaminants of dairy products. They can contaminate milk during milking, and at each stage of manufacturing, storage and even during ripening (Tirloni *et al.*, 2022). As *B. cereus* is a dominant cause of food poisoning, and other members of the *Bacillus* genus have been implicated in bacterial spoilage of milk and milk products including *B. subtilis, B. licheniformis* and *B. pumilus*, our study was focused on detecting the prevalence and concentration of *Bacillus* spp. in certain frequently used dried milk-based products. From the present study, high incidence of *Bacillus* spp. in the examined milk-based products (kishk, Cerelac and whole milk powder) marketed in Upper Egypt was demonstrated. A variety of isolates such as *B. cereus*, *B. subtilis*, *B. licheniformis*, *B. megatrium* and *B. pumilus* was obtained. Additionally, high level of contamination was recorded in the examined dairy products. Similar results were reported in India by Bedi *et al.* (2005) who found that 53.8% of the examined samples of milk products were contaminated with *Bacillus* and the level of contamination was $>10^5$ cfu/g in 20% of the positive samples. Additionally, Heini *et al.* (2018) reported a higher prevalence (78%) of such pathogens in powdered infant formula in Switzerland. On the other side, our rate was higher than that documented by Zhao *et al.* (2020) where

the occurrence of *Bacillus* in Chinese milk products was 10.8%.

In the current study, the higher percentages of *Bacillus* spp. in examined samples may be attributed to the environmental contamination since the bacterium is ubiquitous in the nature (Mohamed *et al.*, 2016). *Bacillus* group strongly tolerates the adverse environmental conditions (Zhao *et al.*, 2020). Moreover, the excessive use of food additives such as wheat, rice, cereals and starch in milk-based products (e.g. kishk, Cerelac and whole milk powder) could be one of the main causes of the heavy contamination of such food matrices with *Bacillus* spp. (Rahimi *et al.*, 2013). Most bacilli are considered harmless if they are found at low concentration ($<10^3$ cfu/g). However, *B. cereus* could multiply within 20 min and reach more than 10^4 cfu/g at suitable temperatures (20–50 °C). Therefore, bad storage conditions after manufacturing of milk products could be a risk factor for both multiplication of the pathogen and for production of its toxic metabolites (Logan, 2012).

Importantly, different strains of *Bacillus* species such as *B. cereus*, *B. subtilis*, *B. licheniformis*, *B. megatrium* and *B. pumilus* were isolated from the examined milk-based products. Our findings showed that the majority of isolates obtained from the examined samples were categorised as *B. cereus* (71.9%). According to the Egyptian standards (ES, 2005; 2014), the cereal-based foods and milk powders must be free from pathogenic microorganisms and their toxins. Thus, the samples contaminated with *Bacillus* are not compliant with that standard and considered unacceptable. Consequently, 25.7%, 65.7% and 45.7% of kishk, Cerelac and whole milk powder, respectively, were satisfactory and safe for human consumption. In the present study, *B. subtilis* was also frequently isolated from the given samples, while *B. licheniformis* was detected in only one kishk sample. Similarly, several studies identified *B. licheniformis* and *B. subtilis* in dairy products (Crielly *et al.*, 1994; Lukášová *et al.*, 2001; Pavić *et al.*, 2005; Mohamed *et al.*, 2016). In contrast to our findings, Lukášová *et al*. (2001) revealed that *B. licheniformis* was the most common isolate detected in the tested milk samples (85%) and in the farm environment. Previous cellular assays have confirmed that *B. subtilis*, *B. licheniformis* and *B. pumilus* could produce functional heat-labile toxins (Beattie & Williams, 1999; Lindsay *et al.*, 2000). Therefore, adequate preventive measures are critical to reduce microbial contamination of milk-based products with such foodborne pathogens.

As the demand for milk products increases, the risk of *B. cereus*-associated contamination also increases. In the current study, the highest incidence of *Bacillus* spp. was detected in kishk which is the basic traditional, dried and fermented dairy product in Upper Egypt. Although kishk has a very high nutritive value, it could be a source of a variety of foodborne pathogens. There is little data on the prevalence of *Bacillus* spp. in this food matrix; however, Awad *et al*. (2015) studied the ability of *B. cereus* to grow artificially in kishk during various stages of processing and storage. The authors noticed that when the contamination occurred just before drying, *Bacillus* was able to maintain its viability in the dried product for two months when stored at ambient temperatures. Additionally, they concluded that the shaping of kishk (spherical shape) and the drying steps were the most critical points during production, particularly when kishk was

produced under uncontrolled conditions by traditional methods in farmers' houses (Awad *et al.*, 2015). Herein, all kinds of *Bacillus* species (*B. cereus*, *B. subtilis*, *B. licheniformis*, *B. megatrium* and *B. pumilus*) were isolated from kishk at high concentrations. As referred by EFSA (2016), vegetative cells or spores of *B. cereus* in a count of $>10^4$ cfu/g will produce diarrhoeal toxins in the human gut and intestine. Hence, kishk could be a source of *Bacillus* or its toxins; and the establishment of strict preventive measures and food safety management is warranted during the manufacturing and storage of such product to improve its safety.

In whole milk powder, *Bacillus* spp. was detected in 19 (54%) out of 35 samples and 40% of the obtained *Bacilli* was characterised as *B. cereus*. This high incidence in whole milk powder was in concurrence with a study conducted by Cetin-Karaca & Morgan (2018) who reported that the most common source of this bacterium was milk powder. However, our result showed higher rate than those obtained by Aman *et al*. (2016), where the occurrence of *B. cereus* was 19% of the examined infant milk powder with a load up to 9×10^2 cfu/g. Additionally, Osama *et al*. (2020) isolated *B. cereus* from only 8% of the tested milk powder in Egypt, while a higher incidence was detected by Kumari & Sarkar (2014) and Ibrahim *et al*., (2022) who found that the examined samples were contaminated with *B. cereus* at percentages of 52% and 64%, respectively. However, the mean value of *B. cereus* concentration obtained by Ibrahim *et al*. (2022) was lower ($0.57\times10^2\pm0.182\times10^2$ and $0.15\times10^2\pm0.027\times10^2$ cfu/g for whole and skim milk powder, respectively) than the mean count obtained in the current study. From the current findings and previous studies, it is obvious that milk powder may be contaminated with high level of vegetative *B. cereus* forms. Furthermore, many previous studies detected the spores of this bacterium in both milk powder and in infant formulas with prevalences ranging from 6.8% to 68% and concentrations up to 10^4 cfu/g (Zhang *et al.*, 2017; Cetin-Karaca & Morgan, 2018; Tirloni *et al.*, 2022). Presence of bacilli at a high percentage of milk powder samples may be attributed to the environmental contamination (Mohamed *et al.*, 2016). Tirloni *et al*. (2022) postulated that the milk dehydration process did not efficiently decontaminate the dried products from *B. cereus* due to the presence of thermotolerant spores which germinate rapidly in reconstituted milk powder at a temperature around 37 °C after being in its dormant state. Besides, Shaheen *et al*. (2010) announced that biofilm formed on the milk evaporator could be implicated in the recurrent contamination of milk powder with this pathogen, especially in dried infant formula (Sadek *et al.*, 2018). Of note, dried milk products may be contaminated during packaging steps as long as many of these products are imported and repackaged in the Egyptian factories.

In the current study, *B. subtilis* was detected in 14.29% of the examined samples of whole milk powder. In line with our study, Ronimus *et al.* (2003) reported that *B. subtilis* has been found to be a fairly ubiquitous microorganism in global milk powders. Importantly, *B. subtilis* has been involved in food poisoning outbreak in 2005 in a kindergarten through consumption of milk powder (Logan, 2012). However, we failed to isolate *B. licheniformis* from whole milk powder, reported as the second most common thermophilic spore-former detected in whole milk

powder in different countries with a total occurrence of 39.2% (Rückert *et al.*, 2004). Additionally, Yuan *et al.* (2012) identified *B. licheniformis* in 36.8% out of 801 isolates recovered from Chinese milk powders. Overall, the Egyptian whole milk powder could be implicated in some of food poisoning outbreaks as a result of *Bacillus* spp. contamination since there is a lack of outbreaks records and their causes in Egypt.

Cerelac is a very popular baby food worldwide. The occurrence of *Bacillus* spp. in the examined samples was low (34%) compared to other examined milk-based products. Cerelac contained a lot of food additives in addition to dried milk such as wheat, rice, cereals, sugar, etc. In the current study, *B. cereus* was isolated in abundance (25.71%) from such product. Although, little is known about the prevalence of *Bacillus* spp. in Cerelac, a previous study reported that rice containing food have been known to cause *B. cereus* food poisoning of the emetic kind (Mohamed *et al.*, 2016). Furthermore, Zhou *et al*. (2010) noticed the existence of *B. cereus* in dairy products where other food additives or vegetative materials are incorporated. Overall, the present study was focused on the most prevalent strains (*B. cereus*) obtained from the tested samps and PCR-based identification was applied for simultaneous confirmation. It was remarkable that the confirmed strains using the PCR technique were fewer than those identified by biochemical methods. This difference may be due to the similarities between the members of *Bacillus* spp. that hinder the discrimination of *B. cereus* from others via biochemical tests. Hence, it is better to use a combination of conventional biochemical method and PCR technique for the confirmation of *B. cereus* isolates.

CONCLUSIONS

Altogether, this study reported that *Bacillus* spp. widely contaminated the examined samples of milk-based products (kishk, Cerelac and whole milk powder). A variety of isolates were obtained such as *B. cereus*, *B. subtilis*, *B. licheniformis*, *B. megatrium* and *B. pumilus*. The tested products appeared to be a reservoir for particularly virulent strains of pathogenic bacteria that could pose a possible food safety risk and significant economic loss. Hygienic manufacturing practices by milk producers and traditional dairy processors are needed to reduce the level of contamination in the Egyptian dairy industry. The implementation of food safety monitoring during the processing of dried milk products will most certainly be beneficial for ensuring that safe dairy products would reach the consumers.

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REFERENCES

Abou-Donia, S. A., 2008. Origin, History and manufacturing process of egyptian dairy products: An overview. *Alexandria Journal of Food Science & Technology*, **5**, 51–62.

Agata, N., M. Ohta, M. Mori & M. Isobe, 1995. A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus*. *FEMS Microbiology Letters*, **129**, 17–20.

Altayar, M. & A. D. Sutherland, 2006. *Bacillus cereus* is common in the environment but emetic toxin producing isolates are rare. *Journal of Applied Microbiology*, **100**, 7–14.

Aman, I., M. Esraa & M. Walaa, 2016. Safety of infant milk powder sold at Kafrelsheikh governorate markets, Egypt. *International Journal of Innovative Research in Science & Engineering*, **2**, 2347–3207.

Aruwa, C. & S. Olatope, 2015. Characterization of *Bacillus* species from convenience foods with conventional and API kit method: A comparative analysis. *Journal of Applied Life Sciences International*, **3**, 42–48.

Awad, S., N. Kamal, A. Ibrahim & A. Elattar, 2015. Kishk Challenge test against *Bacillus cereus.* In: P*roceedings from the 12th Egyptian Conference of Dairy Science and Technology*.

Beattie, S. H., 1997. Incidence and importance of *Bacillus* species in raw milk and in the dairy environment, PhD Thesis, University of Glasgow (United Kingdom).

Beattie, S. H. & A. G. Williams, 1999. Detection of toxigenic strains of *Bacillus cereus* and other *Bacillus* spp. with an improved cytotoxicity assay. *Letters in Applied Microbiology*, **28**, 221–225.

Bedi, S. K., C. S. Sharma, J. P. S. Gill, R. S. Aulakh & J. K. Sharma, 2005. Incidence of enterotoxigenic *Bacillus cereus* in milk and milk products. *Journal of Food Science and Technology*, **42**, 272–275.

Cetin-Karaca, H. & M. C. Morgan, 2018. Inactivation of *Bacillus cereus* spores in infant formula by combination of high pressure and trans-cinnamaldehyde. *Food Science and Technology*, **97**, 254–260.

Chen, L., R. M. Daniel & T. Coolbear, 2003. Detection and impact of protease and lipase activities in milk and milk powders. *International Dairy Journal*, **13**, 255–275.

Crielly, E. M., N. A. Logan & A. Anderton, 1994. Studies on the *Bacillus* flora of milk and milk products. *The Journal of Applied Bacteriology*, **77**, 256–263.

EFSA, 2016. European food safety authority. Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. *EFSA Journal,* **14**, 4524.

EFSA & ECDC, 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA Journal*, **12**, 3547.

Ehling-Schulz, M., M. H. Guinebretiere, A. Monthán, O. Berge, M. Fricker & B. Svensson, 2006. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. *FEMS Microbiology Letters*, **260**, 232–240.

ES, 2005. Processed Cereal-based for foods for Infants and Children. Egyptian Organization for Standardization and Quality Control, Ministry of Industry (ES: 3284/2005).

ES, 2014. Milk powder and cream powder. . Egyptian Organization for Standardization and Quality Control, Ministry of Industry (ES: 1780/2014).

Ghazali, M. F., M. Z. Sukiman, M. H. Chai, N. M. Mohamad & S. M. Z. Ariffin, 2022. Molecular detection and antibiogram of *Bacillus cereus* isolated from dairy goat with mastitis in Malaysia. *International Journal of Infectious Diseases*, **116**, S63–S64.

Granum, P. E., 1994. *Bacillus cereus* and its toxins. Journal of *Applied Bacteriology Symposium Supplement*, **76**, 61s–66s.

Heini, N., R. Stephan, M. Ehling-Schulz & S. Johler, 2018. Characterization of *Bacillus cereus* group isolates from powdered food products. *International Journal of Food Microbiology*, **283**, 59–64.

Hwang, J. Y. & J. H. Park, 2015. Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of *Bacillus cereus* isolated from infant formulas and ready-to-eat foods. *Journal of Dairy Science*, **98**, 1652–1660.

Ibrahim, A. S., N. M. Hafiz & M. F. Saad, 2022. Prevalence of *Bacillus cereus* in dairy powders focusing on its toxigenic genes and antimicrobial resistance. *Archives of Microbiology*, **204**, 339.

Kosikowska, U., D. Stepien-Pysniak, D. Ozga, A. Wernicki & A. Malm, 2015. Identification of *Bacillus* spp. colonizing the nasal mucosa of healthy adults living in the suburban area using the matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) system. *Current Issues in Pharmacy and Medical Sciences*, **27**, 137–141.

Kumari, S. & P. K. Sarkar, 2014. Prevalence and characterization of *Bacillus cereus* group from various marketed dairy products in India. *Dairy Science & Technology*, **94**, 483–497.

Lindsay, D., F. M. Mosupye, V. S. Brözel & A. Von Holy, 2000. Cytotoxicity of alkaline-tolerant dairy-associated *Bacillus* spp. *Letters in Applied Microbiology*, **30**, 364–369.

Logan, N. A., 2012. *Bacillus* and relatives in foodborne illness. *Journal of Applied Microbiology*, **112**, 417–429.

Lukášová, J., J. Vyhnálková & Z. Pácová, 2001. *Bacillus* species in raw milk and in the farm environment. *Milchwissenschaft*, **56**, 609–611.

Lund, T., M. L. De Buyser & P. E. J. M. M. Granum, 2000. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. *Molecular Microbiology*, **38**, 254–261.

Machaiah, M. I. & M. H. Krishnan, 2014. Immunodetection of *Bacillus cereus* haemolytic enterotoxin (HBL) in food. *Analytical Methods*, **6**, 1841.

Meer, R. R., J. Baker, F. W. Bodyfelt & M. W. Griffiths, 1991. Psychrotrophic *Bacillus* spp. in fluid milk products: A review. *Journal of Food Protection*, **54**, 969–979.

Mohamed, A. S., M. E. A. Alnakip & S. F. A.-E. Aal, 2016. Occurrence of *Bacillus cereus* in raw milk and some dairy products in Egypt. *Japanese Journal of Veterinary Research*, **64**, S95–S102.

Nieminen, T., N. Rintaluoma, M. Andersson, A. M. Taimisto, T. Ali-Vehmas, A. Seppälä, O. Priha & M. Salkinoja-Salonen, 2007. Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk. *Veterinary Microbiology*, **124**, 329–339.

Osama, R., M. Ahmed, A. Abdulmawjood & M. Alashmawy, 2020. Prevalence and Antimicrobial Resistance of *Bacillus cereus* in Milk and Dairy Products. *Mansoura Veterinary Medical Journal*, **21**, 11–18.

Pavić, S., M. Brett, I. Petric, D. Laštre, M. Smoljanović, M. Atkinson, A. Kovačić, E. Cetinić & D. Ropac, 2005. An outbreak of food poisoning in a kindergarten caused by milk powder containing toxigenic *Bacillus subtilis* and *Bacillus licheniformis*. *Archiv für Lebensmittelhygiene*, **56**, 1–24.

Rahimi, E., F. Abdos, H. Momtaz, Z. T. Baghbadorani & M. Jalali, 2013. *Bacillus cereus* in infant foods: prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. *The Scientific World Journal*, **2013**, 292571.

Roberts, D. & M. Greenwood, 2003. Practical Food Microbiology, pp. 91–104; 105–129; 131–191.

Ronimus, R. S., L. E. Parker, N. Turner, S. Poudel, A. Rückert & H. W. Morgan, 2003. A RAPD-based comparison of thermophilic *bacilli* from milk powders. *International Journal of Food Microbiology*, **85**, 45–61.

Rückert, A., R. S. Ronimus & H. W. Morgan, 2004. A RAPD-based survey of thermophilic *bacilli* in milk powders from different countries. *International Journal of Food Microbiology*, **96**, 263–272.

Sadek, Z. I., M. A. Abdel-Rahman, M. S. Azab, O. M. Darwesh & M. S. Hassan, 2018. Microbiological evaluation of infant foods quality and molecular detection of *Bacillus cereus* toxins relating genes. *Toxicology Reports*, **5**, 871–877.

Scheldeman, P., L. Herman, S. Foster & M. Heyndrickx, 2006. *Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk. *Journal of Applied Microbiology*, **101**, 542–555.

Shaheen, R., B. Svensson, M. A. Andersson, A. Christiansson & M. Salkinoja-Salonen, 2010. Persistence strategies of *Bacillus cereus* spores isolated from dairy silo tanks. *Food Microbiology*, **27**, 347–355.

Stenfors Arnesen, L. P., A. Fagerlund & P. E. Granum, 2008. From soil to gut: *Bacillus* cereus and its food poisoning toxins. *FEMS Microbiology Reviews*, **32**, 579–606.

Tirloni, E., S. Stella, F. Celandroni, D. Mazzantini, C. Bernardi & E. Ghelardi, 2022. *Bacillus cereus* in dairy products and production plants. *Foods*, **11**, 2572.

Yuan, D.-D., G.-C. Liu, D.-Y. Ren, D. Zhang, L. Zhao, C.-P. Kan, Y.-Z. Yang, W. Ma, Y. Li & L.-B. Zhang, 2012. A survey on occurrence of thermophilic bacilli in commercial milk powders in China. *Food Control*, **25**, 752–757.

Zhang, Y., J. Chen, C. Feng, L. Zhan, J. Zhang, Y. Li, Y. Yang, H. Chen, Z. Zhang, Y. Zhang, L. Mei & H. Li, 2017. Quantitative prevalence, phenotypic and genotypic characteristics of *Bacillus cereus* isolated from retail infant foods in China. *Foodborne Pathogens and Disease*, **14**, 564–572.

Zhao, S., J. Chen, P. Fei, H. Feng, Y. Wang, M. A. Ali, S. Li, H. Jing & W. Yang, 2020. Prevalence, molecular characterization, and antibiotic susceptibility of *Bacillus cereus* isolated from dairy products in China. *Journal of Dairy Science*, **103**, 3994–4001.

Zhou, G., D. Zheng, L. Dou, Q. Cai & Z. Yuan, 2010. Occurrence of psychrotolerant Bacillus cereus group strains in ice creams. *International Journal of Food Microbiology*, **137**, 143–146.

Zhu, L., J. He, X. Cao, K. Huang, Y. Luo & W. Xu, 2016. Development of a double-antibody sandwich ELISA for rapid detection of *Bacillus Cereus* in food. *Scientific Reports*, **6**, 16092.

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