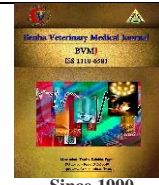




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Occurrence of aerobic spore formers including *Bacillus cereus* and its virulence genes in some vacuum packed chilled meat products

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ABSTRACT

Ninety random samples of different Vacuum Packed meat products represented by (salami, frankfurter and sausage) were gathered randomly from different supermarkets in Menofia governorates to be investigated for occurrence of aerobic spore formers, *Bacillus cereus* and its virulence genes. The results showed that the prevalence of aerobic spore formers was 11(36.67%), 17(56.67%) and 19 (63.33%) in salami, Frankfurter and Sausage, respectively. The mean counts of aerobic spore formers vacuum packed salami, Frankfurter and Sausage were $8.81 \times 10^2 \pm 1.74 \times 10^2$, $2.56 \times 10^3 \pm 0.38 \times 10^3$ and $5.03 \times 10^3 \pm 0.97 \times 10^3$, respectively. The prevalence of *Bacillus cereus* in the vacuum packaged Salami, Frankfurter and Sausage were 23.33%, 46.67% and 53.33%, respectively. Furthermore, the mean values of *Bacillus cereus* counts in Salami, Frankfurter and Sausage were $5.29 \times 10^2 \pm 1.06 \times 10^2$, $1.14 \times 10^3 \pm 0.21 \times 10^3$ and $2.75 \times 10^3 \pm 0.44 \times 10^3$ CFU/g, respectively. Based on the contamination of vacuum packaged Salami, Frankfurter and Sausage with *B. cereus* the acceptability were 76.67%, 53.33% and 46.67 accepted, while 23.33%, 46.67% and 53.33% were unaccepted, respectively. Moreover, the incidence of virulence genes of *Bacillus cereus* were 25%, 25% and 0% for *cytK*, 50%, 25% and 0 % for *hblC* and 25%, 50% and 100% for *cytK* & *hblC* isolated from the Salami, Frankfurter and Sausage meat products respectively. Sausage samples were the most contaminated products.

1. INTRODUCTION

Due to their nutritional richness, foods of animal origin play a significant role in the evolution of humans and are essential to a healthy and well-balanced diet. A great supply of protein, vitamin B-complex, calcium, iron, and phosphorus, are found in meat and its products (Pereira and Vicente 2013). Aerobic spore contamination of food at various phases of production and processing is unavoidable. The microbial contamination of the meat during the slaughtering operations is a result of a variety of factors, including air, water, soil, intestines, and lymph nodes, processing tools, human beings, and handling procedures (De maître et al. 2020). On the other side, fecal infection can contaminate meat. In addition to continuing to exist in the meat's final products, spore-forming bacteria like *Bacillus cereus*, which has caused significant health concerns, may also activate (Vandeweyer et al. 2020). In nature, the genus *Bacillus* of aerobic spore-forming bacteria is pervasive and extensively spread. According to descriptions, the genus *Bacillus* consists of gram-positive, aerobic, generally catalase-positive rods that generate endospores and are very resilient to a variety of challenging environmental factors (Saad and Ahmed 2013). Heat, desiccation, disinfectants, ionizing radiation, and ultraviolet light cannot harm *Bacillus* spores (Pirttijarvi, et al. 2000). *Bacillus* spp. is among the main spoilage organisms in food due to their versatile metabolism and heat-resistant spores (André et al. 2017). Therefore, the goal of the current study was to examine the aerobic spore-

forming bacteria in samples of vacuum-packed salami, Frankfurter and sausage. Therefore, the goal of the current investigation was to shed light on salami, frankfurter, and sausage for the identification of *B. cereus* virulence genes (*hblC*, *cytK*) using multiplex PCR as well as for the enumeration of aerobic spore formers and *B. cereus* bacteria.

2. MATERIAL AND METHODS

2.1. Collection of samples

In Menofia governorate, Egypt, 90 random samples of vacuum-packed meat products, including salami, frankfurters, and sausage (30 of each), were gathered from various supermarkets at various times. The presence of aerobic spore formers, such as *B. cereus*, and its virulence genes will be investigated

2.2. Preparation of samples

The collected samples were prepared according to APHA (2001).

2.3. Enumeration of *B. cereus* (Rhodehamel and Harmo 2001)

2.4. Identification of *B. cereus* (De Vos et al., 2009)

2.5. Detection of Virulence genes of *B. cereus* by PCR (Rather et al, 2016)

2.6. Statistical Analysis

The data were analyzed using SPSS software (Version 13.0, 2009). Statistical significance was assessed through the implementation of a one-way analysis of variance (Feldman et al., 2003). The statistical significance was established as $p \leq 0.05$.

3. RESULTS

In table (1) the results showed that the prevalence of aerobic spore formers was 11(36.67%), 17(56.67%) and 19 (63.33%) in salami, Frankfurter and Sausage samples, respectively. The table (2) revealed that the mean counts aerobic spore forming bacteria of vacuum packed salami, Frankfurter and Sausage were $8.81 \times 10^2 \pm 1.74 \times 10^2$, $2.56 \times 10^3 \pm 0.38 \times 10^3$ and $5.03 \times 10^3 \pm 0.97 \times 10^3$, respectively. Table (3) reported that the prevalence of *Bacillus cereus* in the vacuum packaged Salami, Frankfurter and Sausage were 23.33%, 46.67% and 53.33%, respectively. Table (4) also, revealed that the mean values of *Bacillus cereus* counts for Salami, Frankfurter and Sausage were $5.29 \times 10^2 \pm 1.06 \times 10^2$, $1.14 \times 10^3 \pm 0.21 \times 10^3$ and $2.75 \times 10^3 \pm 0.44 \times 10^3$ CFU/g, respectively. In table (5) the acceptability of vacuum packaged Salami, Frankfurter and Sausage meat products based on their contamination with *B. cereus* was 76.67%, 53.33% and 46.67 accepted, while 23.33%, 46.67% and 53.33% was unaccepted, respectively. Moreover, in table (5) the incidence of virulence genes of *Bacillus cereus* were 25%, 25% and 0% for *cytK*, 50%, 25% and 0 % for *hblC* and 25%, 50% and 100% for *cytK*& *hblC* isolated from the Salami, Frankfurter and Sausage, respectively. Photograph (1) proved the presence of *cytK* (565 bp) and *hblC* (695bp) virulence genes of *Bacillus cereus* that isolated from meat products by using agarose gel electrophoresis of multiplex PCR

Table (1): Prevalence of aerobic spore formers in the examined samples of vacuum packed meat products (n=30/each product)

Meat products	No of positive samples	%
Salami	11	36.67
Frankfurter	17	56.67
Sausage	19	63.33
Total	47	52.22

Table (2): Statistics of aerobic spore formers counts in the examined samples of vacuum packed meat products (n=30/each product)

Meat products	Min	Min	Mean \pm S.E ^a
Salami	1.0×10^2	3.2×10^3	$8.81 \times 10^2 \pm 1.74 \times 10^{2a}$
Frankfurter	2.0×10^2	7.9×10^3	$2.56 \times 10^3 \pm 0.38 \times 10^{3b}$
Sausage	4.0×10^2	1.1×10^4	$5.03 \times 10^3 \pm 0.97 \times 10^{3c}$

S.E^a = standard error of mean, the different superscripted small letters represent a the significance difference at level $p \leq 0.05$

Table (3): Prevalence of *B. cereus* in the examined samples of vacuum packed meat products (n=30/each product)

Meat products	No of positive samples	%
Salami	7	23.33
Frankfurter	14	46.67
Sausage	16	53.33
Total	37	41.11

Table (4): Statistics of *B. cereus* counts in the examined samples of vacuum packed meat products (n=30/each product)

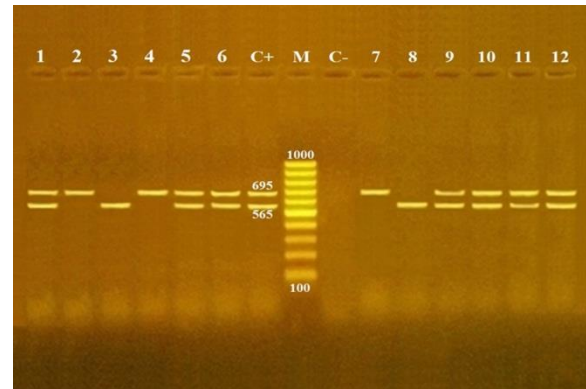
Meat products	Min	Min	Mean \pm S.E ^a
Salami	1.0×10^2	1.6×10^3	$5.29 \times 10^2 \pm 1.06 \times 10^{2a}$
Frankfurter	1.0×10^2	4.5×10^3	$1.14 \times 10^3 \pm 0.21 \times 10^{3b}$
Sausage	2.0×10^2	6.3×10^4	$2.75 \times 10^3 \pm 0.44 \times 10^{3c}$

S.E^a = standard error of mean, the different superscripted small letters represent a the significance difference at level $p \leq 0.05$

Table (5): Acceptability of vacuum packed meat products based on their contamination with *B. cereus* (n=30/each product)

Meat products	<i>B. cereus</i> count /g*	Accepted		Unaccepted	
		No	%	No	%
Salami	Free	23	76.7	7	23.3
Frankfurter	Free	16	53.3	14	46.7
Sausage	Free	14	46.7	16	53.3
Total (90)		53	58.9	37	41.1

*Egyptian Organization for Standardization "EOS" (2005)



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *cytK* (565 bp) and *hblC* (695bp) virulence genes of *Bacillus cereus* isolated from meat products. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *B. cereus* for *hblC* and *cytK* genes. Lane C-: Control negative. Lanes 3and 8: Positive *B. cereus* strains for *cytK* gene. Lanes 2, 4and 7: Positive *B. cereus* strains for *hblC* gene. Lanes 1, 5, 6, 9, 10, 11 and 12: Positive *B. cereus* strains for *hblC* and *cytK* genes

4. DISCUSSION

Foodborne pathogens are a growing issue that can result in a wide range of illnesses brought on by bacteria, viruses, parasites, and fungi (Lee et al. 2014). Consuming ready-to-eat (RTE) meals leads to an increase in the incidence of foodborne disease (Ijabdeniyi et al. 2019). The data in table (1) showed that sausage samples were the most contaminated with aerobic spore forming bacteria followed by frankfurter then salami. While Heikal et al. (2006) recovered aerobic spore forming bacteria from 70% of sausage samples, Ghanyem- Hanan (2015) isolating aerobic spore forming bacteria in lower results from sausage (40%) Moreover Schlegelova et al. (2003), recorded that salami samples were free of aerobic spore formation. It is believed that during the handling process, cutting, slicing, and repackaging in small bits, chunks, or slices has a key role in promoting the development and survival of these diseases (Ferrentino et al. 2015). Additionally, sausage had a higher incidence of *B. cereus* than frankfurters and salami. Nortjé et al. (1999) examined 51 samples and the results showed that the *B. cereus* numbers ranged from 1.00 to 2.10 CFU/g. Lower results was recorded by YÖRÜK and GÜNER (2017) who isolated *B. cereus* from sausage and salami by 16.66%. Salami and frankfurter had a substantially lower *B. cereus* count than sausage, which was a significant difference. As *B. cereus* is frequently present in food ingredients like spices and could survive on the processing equipment, it could contribute to the contamination of meat (YRÜK and GÜNER, 2017). The average number of *B. cereus* found in the samples was 1.00-8.18 CFU/g in the sausage samples and 1.00-2.00 CFU/g in the salami samples. Due to heat treatment during manufacturing, quick cooling procedures, or incorrect sausage storage (extended durations of storage at insufficient temperatures), the prevalence of *B. cereus* in salami was lower than that in sausages (Fernández et al. 2011). The acceptability of the examined sample revealed that the most refused sample was sausage followed by frankfurter then salami as the Egyptian Organization for Standardization "EOS", (2005) stated that the samples must be free from *B. cereus*. In other research, such as Aragon et al. (2008), Chon et al. (2012), Forghani et al. (2014), Anita Tewari et al. (2015), Hblc (55.2%) and CytK (41.4%), and Rather et al. (2016), Hblc and CytK (67.78%), it was noted that hblc (86%) and CytK (77%). El-Wahaab et al. 2018 extracted cytK (50%) from banger, as well as hblc (20%) and hblc and cytK (30%). *B. cereus* cytK (565 bp) and hblC (695 bp) virulence genes were identified from meat products, and multiplex PCR is a rapid method for their identification.

5. CONCLUSIONS

From the obtained results it could be concluded that sausage contains spices, which may be the most significant source of *B. cereus*, the results showed that sausage had the greatest amount of *B. cereus* and its virulence genes.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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