

## CONTENTS

<b><i>Title</i></b>	<b><i>Page</i></b>
<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. REVIEW OF LITERATURE</b>	<b>5</b>
<b>2.1. Sources of contamination of meat products with <i>Bacillus cereus</i></b>	<b>5</b>
<b>2.2. incidence of <i>Bacillus cereus</i> in meat products</b>	<b>10</b>
<b>2.3. PCR for charactrisation of <i>Bacillus cereus</i> and its virulence factor</b>	<b>19</b>
<b>2.4. Control of <i>Bacillus cereus</i> using some essential oils</b>	<b>36</b>
<b>2.5. Public health hazards of <i>Bacillus cereus</i></b>	<b>42</b>
<b>3. MATERIAL AND METHODS</b>	<b>54</b>
<b>3.1. Collection of Samples</b>	<b>54</b>
<b>3.2. Bacteriological examination</b>	<b>54</b>
<b>3.3. Polymerase Chain Reaction (PCR)</b>	<b>57</b>
<b>3.4. <i>Experimental part</i></b>	<b>60</b>
<b>3.5. Statistical analysis</b>	<b>62</b>
<b>4. RESULTS</b>	<b>63</b>

<b>5. DISCUSSION</b>	<b>76</b>
<b>6. CONCLUSION AND RECOMMENDATIONS</b>	<b>84</b>
<b>7. SUMMARY</b>	<b>86</b>
<b>8. REFERENCES</b>	<b>89</b>
<b>9. ARABIC SUMMARY</b>	<b>-</b>

## LIST OF TABLES

Table	Title	Page
1	Statistical analytical results of <i>Bacillus cereus</i> count in the examined samples meat products (n=25)	63
2	Incidence of <i>Bacillus cereus</i> in examined meat products samples (n=25)	65
3	Analysis of variance (ANOVA) of <i>Bacillus cereus</i> counts in the examined samples of meat products	67
4	Occurrence of virulence genes ( <i>cytK</i> and <i>hblC</i> ) in <i>Bacillus cereus</i> strains isolated from examined meat products samples	68
5	Effect of different essential oils (1%) on viability of <i>B. cereus</i> inoculated into minced meat by intensity of $3 \times 10^6$ /g (n=5).	74

## List of Figures

Figure No.	Title	Page
1	Mean value of <i>Bacillus cereus</i> count in the examined samples of meat products ( $\times 10^3$ )	64
2	Number and percentage of <i>Bacillus cereus</i> in the examined samples	66
3	Mean value of virulence genes in <i>Bacillus cereus</i> strains isolated from examined meat products samples	69
4	Agarose gel electrophoresis of multiplex PCR of <i>cytK</i> (565 bp) and <i>hblC</i> (695bp) virulence genes for characterization of <i>Bacillus cereus</i> isolated from Rice kofta.	70
5	Agarose gel electrophoresis of multiplex PCR of <i>cytK</i> (565 bp) and <i>hblC</i> (695bp) virulence genes for characterization of <i>Bacillus cereus</i> isolated from Kobeba	71
6	Agarose gel electrophoresis of multiplex PCR of <i>cytK</i> (565 bp) and <i>hblC</i> (695bp) virulence genes for characterization of <i>Bacillus cereus</i> isolated from sausage	72
7	Agarose gel electrophoresis of multiplex PCR of <i>cytK</i> (565 bp) and <i>hblC</i> (695bp) virulence genes for characterization of <i>Bacillus cereus</i> isolated from beef burger	73
8	Effect of different essential oils (1%) on viability of <i>B. cereus</i> strains	75

# List of Abbreviations

<b>B.Cereus</b>	<b>Bacillus cereus</b>
<b>CDC</b>	<b>Centers for Disease Control and Prevention</b>
<b>CytK</b>	<b>Cytotoxin K</b>
<b>FDA</b>	<b>Food and Drug Administration</b>
<b>GMP</b>	<b>Good manufacturing practice</b>
<b>HACCP</b>	<b>Hazard Analysis and Critical Control Point</b>
<b>Hbl</b>	<b>Heamolysine BL</b>
<b>Nhe</b>	<b>Non heamolytic enterotoxin</b>
<b>PCR</b>	<b>Poly merase chain reaction</b>
<b>WHO</b>	<b>World Health Organization</b>

14. Using Marjoram oil the best added essential oil as it was the most effective on *B. cereus* strains followed by clove and cinnamon oils.

## 7. Summary

*Bacillus cereus* considered as one of the most important pathogens and the presence of them in meat products constitutes serious problems for consumers. So, the present study was conducted to throw light over the prevalence of *B. cereus* in common meat products (rice kofta; kobeba; sausage and beef burger) sold in different supermarkets at Kaliobia Governorate, beside the phenotypic characterization of the isolated strains ; detection some virulence genes(*hblC*, *cytK*) and trials to control them by using some essential oils.

The results revealed that, 47 isolates of *B. cereus* were isolated from 100 examined meat products samples (rice kofta; kobeba; sausage and beef burger) represented as 15 (60%) from rice kofta samples; 13 (52%) from kobeba samples; 10 (40%) from sausage samples and 9 (36%) from beef burger samples.

In addition, the minimum and maximum *B. cereus* count in the examined meat products samples (rice kofta; kobeba; sausage and beef burger) were ranged from  $6.0 \times 10^2$  to  $4.9 \times 10^4$ ;  $3.0 \times 10^2$  to  $2.1 \times 10^4$ ;  $1.0 \times 10^2$  to  $1.3 \times 10^4$  and  $1.0 \times 10^2$  to  $8.5 \times 10^3$  respectively, with a mean value of  $1.57 \times 10^4 \pm 0.39 \times 10^4$ ;  $9.14 \times 10^3 \pm 2.06 \times 10^3$ ;  $7.82 \times 10^3 \pm 1.65 \times 10^3$  and  $2.35 \times 10^3 \pm 0.72 \times 10^3$ , respectively. So, the highest bacterial

percentage was in rice kofta followed by kobeba then sausage and finally beef burger.

The PCR results showed that, *cytK* virulence gene was detected in 15 out of 47 studied strains (3 rice kofta; 3 kobeba; 5 sausages and 4 beef burger) giving product of 565 bp. Meanwhile, *hblC* virulence gene was detected in 7 out of 47 studied strains (1 rice kofta; 2 kobeba; 2 sausages and 2 beef burger) giving product of 695 bp. Moreover, both *cytK* and *hblC* virulence genes were detected in 24 out of 47 studied strains(11 rice kofta; 8 kobeba; 3 sausage and 2 beef burger) giving product of 565 bp. and 695 bp.

Regarding to studying the effect of different essential oils (1%) on viability of *B. cereus* strains, the results cleared that, the *B. cereus* counts in examined minced meat samples after addition of 1% essentials oils (cinnamon, clove and marjoram) were as follow:

At zero time, the mean value of *B. cereus* counts for control and treated samples was  $3.0 \times 10^6 \pm 0.5 \times 10^6$ .

In control samples, *B. cereus* counts were decreased by 3.3%; 10.0% and 13.3% with mean values of  $2.9 \times 10^6 \pm 0.3 \times 10^6$ ;  $2.7 \times 10^6 \pm 0.3 \times 10^6$  and  $2.6 \times 10^6 \pm 0.2 \times 10^6$  after chilling for 2<sup>nd</sup>; 4<sup>th</sup> and 6<sup>th</sup> days, respectively; meanwhile, the mean value of *B. cereus* counts at zero time was  $3.0 \times 10^6 \pm 0.5 \times 10^6$ .

Concerning to addition of cinnamon oil 1%, *B. cereus* counts were decreased by 94.0%; 97.4% and 98.9 % with mean values of  $1.8 \times 10^5 \pm 0.2 \times 10^5$ ;  $7.9 \times 10^4 \pm 1.4 \times 10^4$  and  $3.3 \times 10^4 \pm 0.6 \times 10^4$  after 2<sup>nd</sup>; 4<sup>th</sup> and 6<sup>th</sup> days, respectively; meanwhile, the mean value of *B. cereus* counts at

zero time showed no changes since reduction effect of cinnamon oil was not initiated yet.

Meanwhile, the addition of clove oil 1% revealed the decrease of *B. cereus* counts by 97.2% and 99.8% with mean values of  $8.5 \times 10^4 \pm 2.1 \times 10^4$  and  $5.2 \times 10^3 \pm 1.0 \times 10^3$  after 2<sup>nd</sup> and 4<sup>th</sup>. But, at the 6<sup>th</sup> day of storage, it had complete inhibited effect on *B. cereus* strains that cannot be detected in treated samples.

Moreover, the addition of marjoram oil 1% revealed the decrease of *B. cereus* counts by 99.7 % with mean value of  $6.7 \times 10^3 \pm 1.5 \times 10^3$  after 2<sup>nd</sup> day of storage and after that, it had complete inhibited effect on *B. cereus* strains which cannot be detected in treated samples.

Finally, the results proved that, meat products (rice kofta; kobeba; sausage and beef burger) are considered public health hazard and the presence of *B. cereus* may be due to lack of sanitary measures during processing, handling and storage or using contaminated flesh, rice, spices and vegetables in manufacture of such product. Moreover, Marjoram oil was considered the best added essential oil as it was the most effective on *B. cereus* strains which cannot be detected in treated samples at 4<sup>th</sup> day of storage followed by clove and cinnamon oils.

Therefore, it was concluded that, *B. cereus* is meat borne pathogen of public health important and the hygienic measures are suggested for obtaining meat products with controlled bacterial pathogens to be fit for human consumption.