Occurrence of *Bacillus Cereus* and its Virulence genes in Some Meat Products by Multiplex PCR

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**ABSTRACT**

One Hundered random samples of different meat products represented by (rice kofta, kobaba, sausage and beef burger) were collected randomly from different supermarkets in Kalyobia governorates to be examined for occurrence of *B. Cereus* & its virulence genes. The incidence of *Bacillus Cereus* was 60%, 52%, 40% & 36% for rice kofta, kobaba, sausage and beef burger, respectively. Moreover, the results of PCR out of 47 of isolated *B. Cereus* from the examined samples of meat products were 15(31.91%) for cytk, 7(14.89%) for hblc and cytk & hblc 24(51.6%), respectively. Public Health significance of *B. Cereus* and its virulence genes and possible sources of meat product contamination as well as some recommendations to improve the quality of meat products were discussed.

**Key words:** rice kofta, kobeba, sausage, beef burger, PCR, *Bacillus cereus*.

**1. INTRODUCTION:**

*Bacillus Cereus* causes problems to the foodstuff industry both deteriorating the products (Eneroth et al., 2001) and by endangering people’s health upon consuming contaminated foods (Ghelardi et al., 2002). Certain strains of *B. cereus* are capable of producing heat labile diarrheal enterotoxin and/or heat stable emetic enterotoxin, as well as, other toxins leading to human gastroenteritis after ingestion of food containing preformed enterotoxins rather than a result of colonization or infection of host (Granum, 1994) PCR-based techniques are used increasingly in food-microbiology research as they are well developed and when applied as culture confirmation tests, they are reliable, fast and sensitive. PCR methods offer a sensitive and specific detection of pathogens and can discriminate virulent bacteria from a virulent member of the same species (Olsen, 2000). So, the present study was designed to throw light on rice kofta, kobeba, beef burger and sausage for the following:

1- Enumerating of *Bacillus cereus*. 
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2- Isolation and identification of *B. Cereus*.

3- Detection of *B. Cereus* virulence genes (hblC, cytK) by using multiplex PCR.

2. MATERIAL AND METHODS:

2.1. Collection of samples

One Hundred random samples of meat products represented by rice kofta, kobeba, sausage and beef burger (25 of each) were collected from different supermarkets at different times in Kalyobia governorate, Egypt. To be examined for occurrence of *B. cereus* & its virulence genes.

Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay.

2.1. Preparation of samples

The samples were prepared according to the technique recommended by ICMSF (1996). Twenty-five grams of each sample were aseptically taken using sterile forceps and scissors, and then mixed with 225 ml of sterile peptone water 0.1%, the content was homogenized for one minute by using a stomacher to provide a dilution of 10^{-1}. The homogenate was allowed to stand for 15 minutes at room temperature, then 1 ml from the original dilution was transferred aseptically with a sterile pipette into test tube containing 9 ml of sterile peptone water 0.1% to produce a dilution of 10^{-2}, and then further tenfold decimal serial dilutions were carried out. The prepared samples were subjected to the following examinations

2.2. Enumeration and isolation of *Bacillus cereus* (Harrigan and McCane, 1976)

From each previously prepared dilution, 0.1 ml was seeded evenly onto each of duplicated plates of Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue agar (PEMBA). The inoculum was spread over the entire surface of the agar with a sterile bent glass rod and using of back and forth motion, while turning the plate until the inoculum was completely dried. The plates were incubated at 37°C for 48 hours. Typical colonies of *B. cereus* characterized by blue turquoise color and surrounded by a halo zone of white precipitation.

2.3. Identification of Bacillus cereus:

The suspected bacterial isolates were identified morphologically and biochemically according to Koneman *et al.* (1992).

2.4. Polymerase Chain Reaction (PCR)

2.4.1. Amplification reaction of *B. cereus* (Nagawongsati *et al.*, 2008)

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The multiplex PCR amplification was performed in a final volume of 20 ul containing 5 μl of DNA templates with final concentration 1X PCR buffer (10mM Tris-HCl pH 8.3 and 50 mM KCl), 1.5mM MgCl2, 200 μM of each dNTP, 5U Taq DNA polymerase and 0.4μM hlbC primer and 0.2 μM cytK primer.

3. Results:

Results recorded in table (1) revealed that 60%, 52% ,40% and 36% of examined rice kofta, kobeba, sausage and beef burger samples were contaminated with *Bacillus cereus*, respectively.

It is evident from the results achieved in table (2) that the total *bacillus cereus* count in the examined samples ranged from $6.0 \times 10^2$ to $4.9 \times 10^4$ with a mean value of $1.57 \times 10^4 \pm 0.39 \times 10^4$ cfu\g for rice kofta, $3.0 \times 10^2$ to $2.1 \times 10^4$ with a mean value of $9.14 \times 10^3 \pm 2.6 \times 10^3$ cfu\g for kobeba, $1.0 \times 10^2$ to $1.3 \times$
10^4 with a mean value of 7.82 x 10^3 ± 1.65 x 10^3 cfu/g for sausage and 1.0 x 10^2 to 8.5 x 10^3 with a mean value of 2.35 x 10^3 ± 0.72 x 10^3 cfu/g for beef burger samples.

The occurrence of virulence genes cytκ of Bacillus Cereus isolated from the examined samples of meat products are shown in Table (3) and Photograph (1,2,3&4) the result of multiplex PCR indicated that in rice kofta 3(20%), 3(23.07%) for kobaba, 5(50%) for sausage and 4 (44.44) for beef burger. The presence of hblc is 1(6.67%) for rice kofta, 2(15.38%) for kobaba, 2(20%) for sausage and 2(22.22%) for beef burger. The presence of cytκ & hblc is 11(73%) for rice kofta, 8(61.54%) for kobaba, 3(30%) for sausage and 2(22.22) for beef burger.

Totally out of 47 of isolated B.cereus from the examined samples of meat products is 15(31.91%) for cytκ, 7(14.89%) for hblc and cytκ&hblc 24(51.6%).

Table (1): Incidence of Bacillus Cereus in the examined samples of meat products (n=25).

<table>
<thead>
<tr>
<th>Meat products</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice kofta</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Kobaba</td>
<td>13</td>
<td>52</td>
</tr>
<tr>
<td>Sausage</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Beef burger</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Total (100)</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>

Table (2): Statistical analytical results of Bacillus cereus count in the examined samples of meat products (n=25).

<table>
<thead>
<tr>
<th>Meat products</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± S.E^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice kofta</td>
<td>6.0x10^2</td>
<td>4.9x10^4</td>
<td>1.57x10^4 ± 0.39x10^4</td>
</tr>
<tr>
<td>Kobaba</td>
<td>3.0x10^2</td>
<td>2.1x10^4</td>
<td>9.14x10^3 ± 2.06x10^3</td>
</tr>
<tr>
<td>Sausage</td>
<td>1.0x10^2</td>
<td>1.3x10^4</td>
<td>7.82x10^3 ± 1.65x10^3</td>
</tr>
<tr>
<td>Beef burger</td>
<td>1.0x10^2</td>
<td>8.5x10^3</td>
<td>2.35x10^3 ± 0.72x10^3</td>
</tr>
</tbody>
</table>

S.E^* = standard error of mean
Occurrence of *Bacillus Cereus* and its Virulence genes in Some Meat Products by Multiplex PCR.

Photograph (1): Agarose gel electrophoresis of multiplex PCR of *cytK* (565 bp) and *hblC* (695bp) virulence genes for characterization of *Bacillus cereus* isolated from Rice kofta.
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.
Lane 6: Positive *B. cereus* strain for *hblC* gene.
Lanes 1, 2, 4, 5, 7, 8, 11, 12, 13, 14 & 15: Positive *B. cereus* strains for both *hblC* and *cytK* genes.

Photograph (2): Agarose gel electrophoresis of multiplex PCR of *cytK* (565 bp) and *hblC* (695bp) virulence genes for characterization of *Bacillus cereus* isolated from Kobeba.
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 1, 9 & 12: Positive *B. cereus* strains for *cytK* gene.
Lanes 5 & 7: Positive *B. cereus* strains for *hblC* gene.
Lanes 2, 3, 4, 6, 8, 10, 11 & 13: Positive *B. cereus* strains for both *hblC* and *cytK* genes.
Abdel-Wahaab et al., (BVMJ-34(3): 158-166, 2018)

Photograph (3): Agarose gel electrophoresis of multiplex PCR of cytK (565 bp) and hblC (695bp) virulence genes for characterization of Bacillus cereus isolated from sausage.
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 1, 6 & 8: Positive B. cereus strains for both hblC and cytK genes.

Photograph (4): Agarose gel electrophoresis of multiplex PCR of cytK (565 bp) and hblC (695bp) virulence genes for characterization of Bacillus cereus isolated from beef burger.
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 2, 5, 6 & 8: Positive B. cereus strains for cytK gene.
Lanes 1 & 4: Positive B. cereus strains for both hblC and cytK genes.
Lane 7: Negative B. cereus strain for both hblC and cytK genes.
Occurrence of Bacillus Cereus and its Virulence genes in Some Meat Products by Multiplex PCR.

Table (3): Occurrence of virulence genes (cytK and hblC) of Bacillus cereus isolated from the examined samples of meat products.

<table>
<thead>
<tr>
<th>B. cereus</th>
<th>No. of ex. Isolates</th>
<th>cytK</th>
<th>hblC</th>
<th>cytK &amp; hblC</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>%</td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td>Rice kofta</td>
<td>15</td>
<td>3</td>
<td>20</td>
<td>1</td>
<td>6.67</td>
</tr>
<tr>
<td>Kobeba</td>
<td>13</td>
<td>3</td>
<td>23.07</td>
<td>2</td>
<td>15.38</td>
</tr>
<tr>
<td>Sausage</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Beef burger</td>
<td>9</td>
<td>4</td>
<td>44.44</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>15</td>
<td>31.91</td>
<td>7</td>
<td>14.89</td>
</tr>
</tbody>
</table>

cytK: cytotoxic K gene
hblC: hemolysin BL gene

4. DISCUSSION:

The over-all prevalence of B. Cereus in rice kofta samples were 60%, which agreed with Abdallah (2005) (60%). However, these results were higher than Torky (1995) (40%).

The current results of the examined samples of kobaba were similar to those obtained by Abdallah (2005) (60%). While the current results of the examined samples of sausage were agreed with those reported by Amin (1995) (40%), Torky (1995) (51.4) & El-Mossalami (2003) (40%). However, this incidence was higher when compared with those recorded with other studies as Hefnawy et al. (1984) (18%), Torky (1995) (40%) & El-said (2005) (30%). This result considered low when compared with that reported by other studies as Ali (1987), El-Daly et al. (1988) (74.29%), Lotifi et al., (1988) (72%), Saleh et al. (1993) (74.3%), Ashmawy (1994) (98%), Abu-elnaga (2003) (88%) & Ghanaym (2014) (72%).

The incidence of B. cereus was 36% in the examined beef burger samples. This result was nearly similar to that obtained by Ghanaym (2014) (35%). However this incidence was higher when compared with those recorded with other studies as Abu-elnaga (2003) (28%), Hassanien (2004) (28%) & El-said (2005) (24%). This result considered low when compared with that reported by other studies as Ahmad (1991) (48%), El-Shewehy (1994) (48%), Torky (1995) (40%), El-Ghamry (2004) (65%), Abosrea (2005) (56%), Heikal et al. (2006) (65%) and El-Mossalami et al. (2008) (92%).

The current result in table 3, were nearly similar to those obtained by...
Guinebretiere et al. (2002) (33%) for cytk toxin. However this result considered low when compared with that recorded with other studies as Aragon et al. (2008) hblc (67.8%), Rahmati and Labbe (2008) hblc (50%), Chon et al. (2012) hblc (86%) & cytk (77%), Forghani et al. (2014) cytk (47.58) & hblc (59.47), Anita Tewari et al. (2015) hblc (55.2%) & cytk (41.4%) &Rather et al. (2016) hblc &cytk(67.78%).

From the obtained results its showed that rice kofta recorded the highest level of Bacillus cereus and its virulence genes due to the fact that it contains rice which rich in starch which act as suitable media for growth of Bacillus cereus.

So, the following recomendations should be considered when
1. Animal should be slaughtered and dressed under strict hygienic measures.
2. Periodical sanitation of animal slaughter halls, chilling rooms and freezing cold stores with suitable antifungal agents.
3. Proper sanitary precautions of the utensils, equipments and tools.
4. Education programmes and proper personal hygiene to all processors and workers sharing in production and handling of meat products.
5. Vacuum packaging or other perfect methods of storage to exclude oxygen should be adopted as a modem technique.
6. Food must be rabidly and efficiently being cooled after cooking to less than 7c⁰ and when reheated the temperature must be at least 70 c⁰. Cooked food must be separated from raw one to prevent cross contamination.

5. REFERENCES:


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