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

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A B S T R A C T

One Hundered randam 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 occurance of *B. Cereus* & its virulence genes. The incidence of *Bacillus Cereus* was 60%, 52%, 40% & 36% for rice kofta, kobeba, sausage and beef burger, respectively. Moreover, the results of PCR out of 47 of isolated *B. Cereus* from the examined samples of meat prouducts 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.*

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1. INTRODUCTION:

Bacillus Cerus 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. cerus* 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 cerus.*

2- Isolation and identification of *B. Cerus.*

3- Detection of *B. Cerus* virulence genes (*hbl*C, *cyt*K) 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 occurance of 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 (Nagamwongsatit 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 x 10^2 to 4.9 x 10^4 with a mean value of 1.57 x $10^4 \pm 0.39 \times 10^4$ cfu\g for rice kofta, 3.0 x 10^2 to 2.1 x 10^4 with a mean value of 9.14 x $10^3 \pm$ 2.6 x 10^3 cfu\g for kobeba, 1.0 x 10^2 to 1.3 x

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 amean value of 2.35 x 10^3 ± 0.72 x 10^3 cfu\g for beef burger samples.

The occurrence of virulence genes *cytk* of *Bacillus Cereus* isolated from the examined samples of meat products are showen 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(2.22%) for beef burger. The presence of cytk & 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 prouducts is 15(31.91%) for *cytk*, 7(14.89%) for *hblc* and *cytk&hblc* 24(51.6%).

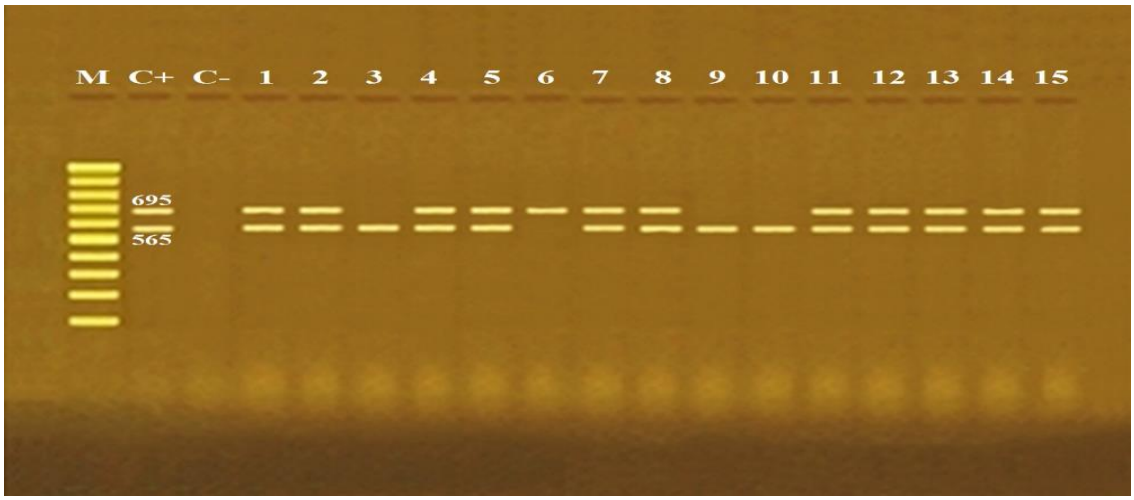
Table (1): Incidence of *Bacillus Cereus* in the examined samples of meat products (n=25).

Meat products	No.	%
Rice kofta	15	60
Kobaba	13	52
Sausage	10	40
Beef burger	9	36
Total (100)	47	47

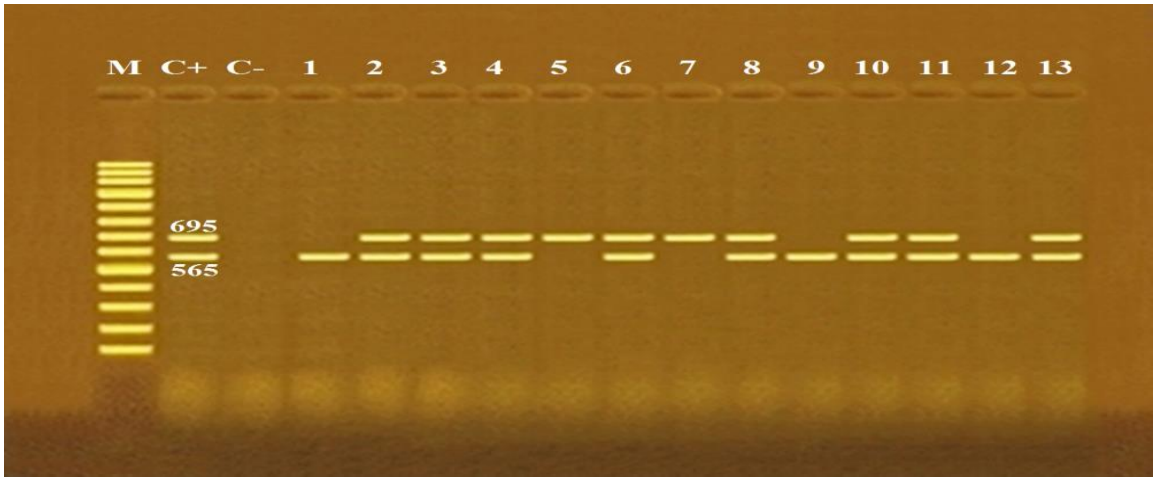
Table (2): Statistical analytical results of *Bacillus cereus* count in the examined samples of meat products (n=25).

Meat products	Min	Max	Mean ± S.E*
Rice kofta	6.0×10^2	4.9×10^4	$1.57\times10^4\pm 0.39\times10^4$
Kobeba	3.0×10^2	2.1×10^4	$9.14\times10^3\pm 2.06\times10^3$
Sausage	1.0×10^2	1.3×10^4	$7.82\times10^3\pm 1.65\times10^3$
Beef burger	1.0×10^2	8.5×10^3	$2.35\times10^3\pm 0.72\times10^3$

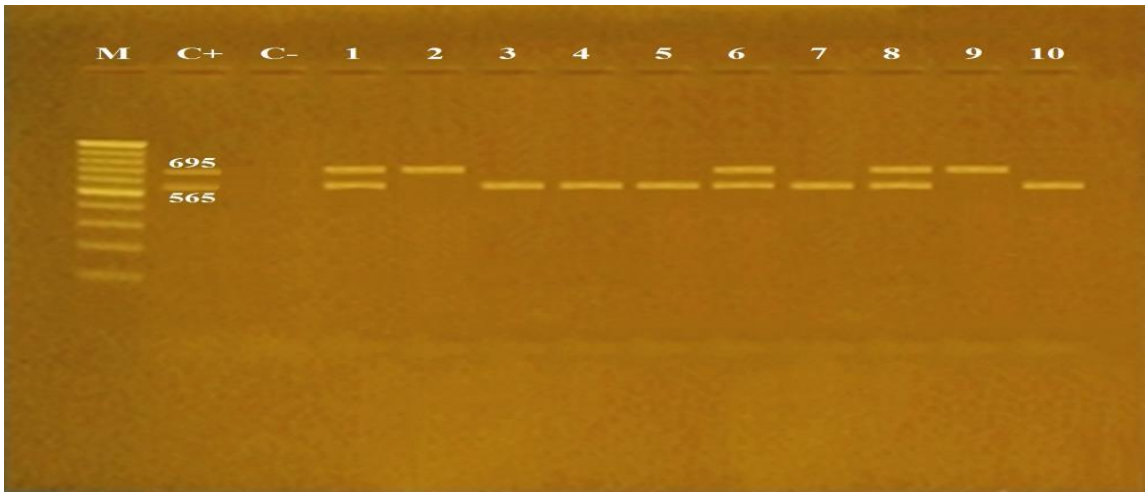
S.E* = standard error of mean



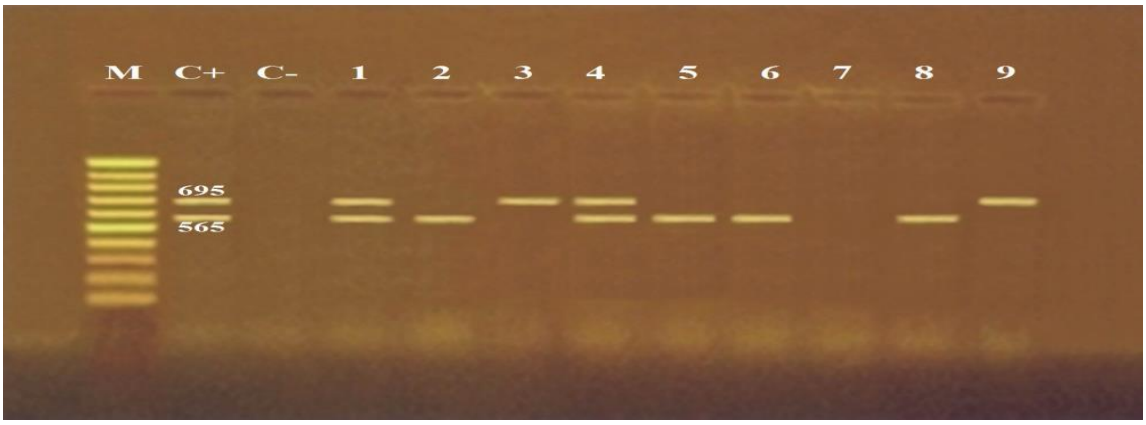
Photograph (1): Agarose gel electrophoresis of multiplex PCR of *cytK* (565 bp) and *hblC* (695bp) virulence genes for characterization of *Bacillus cereus* isolated from Rice kofta.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *B. cereus* for *hblC* and *cytK* genes.
Lane C-: Control negative.
Lanes 3, 9 & 10: Positive *B. cereus* strains for cytK gene.
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.



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 3, 4, 5, 7 & 10: Positive *B. cereus* strains for cytK gene.
Lanes 2 & 9: Positive *B. cereus* strains for *hblC* gene.
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 3 & 9: Positive *B. cereus* strains for *hblC* 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.

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

B. cereus	No. of ex. Isolates	*cytK*		*hblC*		*cytK* & *hblC*		None	
		NO	%	NO	%	NO	%	NO	%
Rice kofta	15	3	20	1	6.67	11	73.33	0	0
Kobeba	13	3	23.07	2	15.38	8	61.54	0	0
Sausage	10	5	50	2	20	3	30	0	0
Beef burger	9	4	44.44	2	22.22	2	22.22	1	11.11
Total	47	15	31.91	7	14.89	24	51.06	1	2.13

cytK: cytotoxic K gene *hblC*: heamolysin 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) (28%), El-sayed *et al.* (1999) (28%), Abu-Elnaga (2003) (26%), Hassanien (2004) 16%, El-said (2005) (30%) & Eid *et al.* (2008) (30%). This result considered low when compared with that reported by other studies as El-Daly *et al.* (1988) (60%), Ashmawy (1994) (92%), El–Ghamry (2004) (80%), Abosrea (2005) (84%), Hamouda (2005) (84%) & Heikal *et al.* (2006) (70%). (70%).

However, this incidence of sausage was 40% in the examined sausage samples was nearly similar when compared with that 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%). 4However 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 table3, 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 $7c^0$ and when reheated the temperature must be at least 70 c^0. Cooked food must be separated from raw one to prevent cross contamination.

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