Prevalence, virulence factor genes and antibiotic resistance of *Bacillus cereus* isolated from ready to eat sandwiches

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Abstract

A total of 105 random samples of ready – to – eat meat sandwiches were collected from different localities with different sanitation levels at Zagazig City, Egypt. The collected samples were steak, kofta, burger,shawarma, hawawshi, liver and sausage (15 of each). The incidence of *B. cereus* was 40%, 100%, 86.66%, 46.66%, 33.33%, 66.66 % and 80% in examined steak, kofta, burger, shawarma, hawawshi, liver and sausage sandwiches, respectively. The *B. cereus* counts were 2.73 ± 0.17, 4.16 ± 0.28, 3.98 ± 0.25, 3.17 ± 0.21, 2.84 ± 0.24, 3.34 ± 0.23 and 3.57 ± 0.31 $\log_{10}$CFU/g in examined steak, kofta, burger, shawarma, hawawshi, liver and sausage sandwiches, respectively. The resistance of *B.cereus* was 100%, 90%, 90%, 80%, 75%, 75%, 70%, 70% and 65% for colistin, metronidazole, doxycycline, cephradine, streptomycin, thiamphenicol, cephaclor and erythromycin and ciprofloxacin, respectively. Meanwhile, the sensitivity obsereved for apramycin and kanamycin was 55% and 45%, respectively. The multi antibiotic resistance (MAR) was ranged from 0.25 to 1. Six strain (30%) resist all examined antibiotics (12) moreover, 18 (90%) of *B.cereus* isolates are considered as multi antibiotic resistant (resist three or more antibiotic from different classes). The Cereulide *(ces)* gene was not detected, The Non-hemorrhagic entero-toxin *(nhe)* gene was detected in 7/10 (70%) and cytotoxin (*cyt*K) gene was detected in 5/10 (50%) in identified B. cereus isolated from the examined ready to eat meat sandwiches.

Keywords: Bacillus cereus, Sausage, Burger, cereulide, Ready to eat, Sandwiches, Meat product.

1. Introduction:

Ready to eat foods are excellent concentrated nutrient sources which contain protein with a high digestibility score, essential amino acids, fatty acids, vitamins and minerals which are considered essential to optimal human growth and development. They also provide a source of readily available and nutritious meals for the consumers and are well appreciated by consumers because of their taste, low cost, nutrient value and ready availability for an immediate consumption. Various ready-to-eat (RTE) foods are becoming increasingly popular between high and low income people in developing countries where higher income people obtained RTE foods from restaurants and on the other hand low income people obtained RTE foods from street

vendors on the street. The vendors congregate mainly in the central business district at major points of transit where large numbers of minibus taxi that are headed for different destinations. Foods are often held for several hours after cooking until sold, this included in some foods overnight at ambient temperature, although reheating could alleviate some hazards, but this action has not always been done effectively at vending site. Foodborne disease outbreaks linked with RTE foods have been associated with various foodborne pathogens **(Gilbreth et al., 2005)**. The initial microbiological load on RTE food ingredients is important, however, factors such as handling, processing, storage and display may influence the microbiological load of RTE foods at the point of sale **(Angelidis et al., 2006)**.

Ready to eat meat products may be contaminated with microorganisms from meat handlers carrying of pathogenic bacteria during the processes of manufacturing, packaging and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness. Foodborne pathogens are the leading causes of illness ranging from an upset stomach to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramps and dehydration and death depending on the etiological agents **(Van et al., 2007)**.

Foodborne illness is generally classified as an infection (ingestion of a harmful microorganism within a food. e.g. *Salmonella* and *E.coli* , and intoxication (ingestion of a harmful toxin produced within a food. e.g. *S. aureus* or toxicoinfection (ingestion of a harmful microorganism within a food that produces a toxin in the human body, e.g. *Bacillus cereus* **(Bean and Griffin, 1990).** Antimicrobial resistance is a significant public health concern all over the world. The virulent and antibiotic-resistant bacteria cloud be transmitted to humans by the consumption of RTE meat sandwiches and the presence of these bacteria such as *B. cereus*, indicating poor hygienic measures which may produce a health risk for consumers **(Gundogan et al., 2013)**.

The study was planned to determine prevalence, count, virulence genes antibiotic resistant pattern of *B.cereus* isolated from ready to eat meat sandwiches.

2. Materials and Methods:

2.1. Collection of samples:

A total of 105 random samples of ready – to – eat meat sandwiches were collected from different localities with different sanitation levels at Zagazig City, Egypt. The collected samples were steak, kofta, burger, shawarma, hawawshi, liver and sausage sandwiches (15 of each). All samples were directly transferred to the laboratory of Food Control Department in an ice box under hygienic conditions without undue delay then the core content were bacteriologically examined.

2.2. Bacillus cereus detection and identification:

The samples were prepared according to **ISO 6887-2:(2003)**. Twenty five grams of each sandwiches core were homogenized aseptically for 1 min with 225 ml of 0.1 % peptone water in a stomacher (Colworth, 400) then serially diluted to 10-fold in the same diluent.

The technique of *Bacillus cereus* count was performed according to **ISO 7932:(2004).** One hundred microlite from each prepared serial dilution of the samples under investigation was evenly spread over a dry surface of the *B. cereus* selective agar base with egg yolk and polymyxin supplement media. The plates were inverted and incubated at 37 °C for 24 hours then examined for typical colonies of *B. cereus* which are turquoise blue 5 mm diameter with hallow zone of the same colour. The plates were re-incubated again for 24 hours before being counted again for further growth. The number of such colonies were recorded as "presumptive" *B. cereus* count.Isolated organisms were identified morphologically and biochemically according to **Cowan and steel (1974).**

2.3. Antibiogram for antibiotic sensitivity of isolated *B.cereus*:

All Bacillus cereus isolates were tested for their sensitivity to antibiotics by means of a disc diffusion method **(Bauer *et al.*, 1966).** Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated strains **(Oxoid Limited, Basingstoke, Hampshire, UK)**.

Agar plate method was applied by using of nutrient agar as a substrate for growth of the tested bacterium for its antibiotic sensitivity. The bacterial culture was uniformly spread on the surface of nutrient agar. Then the antibiotic discs were placed over the surface of inoculated plate. Moreover, the plate was then incubated at suitable temperature (25 °C) for 2-7 days and checked for the growth of the bacterium around the antibiotic discs. The maximal inhibition zone for the growth of microbe is said to that antibiotic had a maximum effect on the microbe growth.

Therefore, the antimicrobial susceptibility testing was applied according to the guidelines stipulated by **National Committee for Clinical Laboratory Standards "NCCLS" (2001).** Accordingly, the antimicrobial discs and their concentrations as well as the diameters of the zones of inhibition for the tested strains are demonstrated in table (1):

The tested strains were evaluated as susceptible, intermediate and resistant.

Multiple Antibiotic Resistances (MAR) index for each strain was determined according to the formula stipulated by **Singh *et al.* (2010)** as follow:

MAR index= No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics.

2.4.

2.4. Detection of emetic and diarrheic gene by using PCR:

The DNA of 10 isolated *B.cereus* strain extracted by **QIAamp DNA Mini Kit,** Catalogue no.51304 and the PCR Master Mix used for cPCR was Emerald Amp GT PCR mastermix (2x premix). The Oligonucleotide primers used in cPCR **Metabion (Germany)** as shown in Table (2).

Table (1) Antimicrobial discs, concentration and interpretation of their action on the isolated bacteria.

Antimicrobial agent	Sensitivity disc content (ug)	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Thiamphenicol (TP)	30	18 or more	13-17	12 or less
Ampicillin (AM)	10	18 or more	14-17	13 or less
Doxycycline (DO)	30	18 or more	13-17	12 or less
Ciprofloxacin (CP)	5	20 or more	15-19	15 or less
Erythromycin (E)	15	23 or more	14-22	13 or less
Apramycin (APR)	30	18 or more	15-17	14 or less
Norfloxacin (NOR)	10	16 or more	13-15	12 or less
Sterptomycin (S)	10	15 or more	12-14	11 or less
Kanamycin (K)	30	18 or more	14-17	13 or less
Cephradine(CE)	30	25 or more	17-24	16 or less
Cephaclor (CEC)	30	17 or more	15-17	14 or less
Sulphamethoxazol (SXT)	25	16 or more	11-15	10 or less

Table (2): Oligonucleotide primers sequences

Primer	**Sequence**	**Amplified product**	**Reference**
nhe	AAG CIG CTC TTC GIA TTC	766 bp	**Ehling-Schulz et al .(2006)**
	ITI GTT GAA ATA AGC TGT GG		
cytK	ACA GAT ATC GGI CAA AAT GC	421 bp	
	CAA GTI ACT TGA CCI GTT GC		
ces	GGTGACACATTATCATATAAGGTG	1271 bp	
	GTAAGCGAACCTGTCTGTAACAACA		

Table (3): Cycling conditions of the different primers during cPCR

95˚C	94˚C	49˚C	72˚C	35 ˚C	72˚C
5 min.	30 sec.	1 min.	1 min.	1 min.	3 min.

2.3. Statistical analyses:

The experimental data were evaluated using mixed model's procedure, *post hoc* comparisons were applied, whenever appropriate, using Duncan's test. All statistical procedures were performed using PASW statistics 18 (SPSS Inc., USA). Statistical significance was considered at ($P \leq 0.05$).

3. Results and discussion:

Farinaceous foods are the most common vehicles of the emetic type whereas the diarrheal type is associated with meat and soups **(Kramer and Gilbert 1989)**.

The achieved results in table (4) declared that the incidence of *B. cereus* was 40%, 100%, 86.66%,46.66%,33.33% , 66.66 % and 80% in examined steak, kofta, burger, shawarma, hawawshi, liver and sausage sandwiches, respectively.

A lower incidence was detected (37%) in the examined ready to eat foods collected from street vendors in Egypt by **El-Sherbeeny et al. (1985)** ,in Turkey 22.4% and 23.3% **Gueven *et al.* (2006)** and **Büyükyörük *et al.* (2014)**, respectively. **Schlegelova *et al.* (2003)** found 28 % of meat samples positive for *B. cereus*. **Willayat *et al.* (2007)** and **Das *et al.* (2009)** found 23.5 % and 36.7 % contamination level of *B. cereus*, respectively. (20%) of *B.cereus* detected in RTE liver sandwiches samples by **Abd-El-Malek (2014**) This variation is due to hygienic practices followed in different localities in meat shops and restaurants.

Nearly similar incidence of *B.cereus* in examined marketed sandwiches (68%) was detected by **Enan et *al.* (2012)**, (80%) in the examined chicken and meat products detected by **Kamat *et al.* (1989)**. The results in table (4) showed that *B. cereus* counts were 2.73 ± 0.17, 4.16 ± 0.28, 3.98 ± 0.25, 3.17 ± 0.21, 2.84 ± 0.24, 3.34 ± 0.23 and 3.57 ± 0.31 $\log_{10}$CFU/g in examined steak, kofta, burger, shawarma, hawawshi, liver and sausage sandwiches, respectively. The obtained counts were in the range of the previous studies by **Konuma *et al.* (1988)** who detected the majority of the examined spices which ranged from to 2 to 4 $\log_{10}$CFU/g, **Rusul and Yaacob (1995)** from 2.7-3.9 $\log_{10}$CFU/g. **Te Giffel *et al.* (1996)** 2 to 6 $\log_{10}$CFU/g in different food samples. **Aksu *et al.* (2000)** from 3 to4 $\log_{10}$CFU/g. and **Morshdy et al.,(2014)** who found *B.cereus* counts were 2.5 x 10^4 ± 6.3x10^3, 3.9x 10^4 ± 1x10^4 and 3.3 x 10^4 ± 7.6 x10^3 CFU/g in examined kofta, liver and shawarma sandwiches, respectively.

There was a significant difference ($p< 0.05$) between examined samples which was attributed to the sandwich composition (plan with sauce or with sauce dressing and green vegetables), the additives introduced in processing of meat products, environmental conditions, and potential differences in handling by personnel. According to **Food Standards Australia New Zealand (2001) ,** data in table (4) revealed that 37 samples (35.3%) of the total examined samples were categorized as good < 10^2, while 14 (13.3%) of the total examined samples fell in the acceptable category (10^2 to <10^3).

Table 6. Prevalence and count log_{10} CFU/g of *B.cereus* in comparison to standard of ready to eat meat (n= 105)

Sandwiches	prevalence	Mean ± SE (Min – Max)	Categories according Food Standards Australia New Zealand (2001).			
			Good $>10^2$	Acceptable 10^2 to $<10^3$	Unsatisfactory 10^3 to $<10^4$	Potentially hazardous $\geq 10^4$
Steak	6(40%)	2.73 ± 0.17^{a} 2 - 3.54	9	4	2	-
Kofta	15(100%)	4.16 ± 0.28^{bc} 3.24 - 5.03	-	-	6	9
Burger	13(86.66%)	3.98 ± 0.25^{ab} 3.1 - 4.54	2	-	7	6
Shawarma	7(46.66%)	3.17 ± 0.21^{bc} 2.56 - 3.87	8	2	5	-
Hawawshi	5(33.33%)	2.84 ± 0.24^{c} 2 - 3.36	10	3	2	-
Liver	10(66.66%)	3.34 ± 0.23^{a} 2.95 - 4.24	5	2	4	4
Sausage	12(80%)	3.57 ± 0.31^{b} 2.85 - 4.24	3	3	5	4
Total			37 (35. 3%)	14 (13.3%)	31(29.5%)	23(21.9%)

(a,b and c) Means within the same column bearing different small superscript letters are significantly different ($p < 0.05$).

Min= Minimum Max= Maximum SE= Standard Error

The unsatisfactory samples were 31 (29.5%) carry a significant risk if applied under certain conditions as excessive handling, bad hygienic conditions, and time and temperature abuse) that help *B.cereus* to proliferate and make it to fall in the category unsatisfactory. The presence of potentially hazardous level of *B.cereus* (>10^4) in 23(21.9%) of total examined RTE sandwiches as recommended by **Food Standards Australia New Zealand (2001)**.

The presence of large numbers of *B. cereus* (more than 10^4 CFU/g) in food is an indicative of active growth and proliferation of the organism and consistent with a potential hazard to health. It causes both diarrheal and emetic syndromes, each associated with distinct enterotoxin **(Slabyj et al., 2003)**.

Toxin producing *B. cereus* plays an important role as the causative agent of two types of food poisoning: diarrhea and emesis. The emetic syndrome is mainly characterized by vomiting a few hours after ingestion of the contaminated food. In the diarrheal syndrome, symptoms appear 8–16h after ingestion, and include abdominal pain and diarrhea. In general, both types of food borne illness are relatively mild and self-limiting. Nevertheless, more severe cases have occasionally been reported involving hospitalization or even deaths **(Lund *et al.*, 2000; Dierick *et al.*, 2005)**.

In the absence of control measures, virulent and antibiotic-resistant organisms might be transmitted to humans by the consumption of meat and their product and the presence of these organisms such as *B.cereus* in examined chicken meat products, indicating poor sanitary conditions which may create a health risk for consumers (**Gundogan *et al.*, 2006**). Especially, multidrug resistant strains which are more dangerous and of great food safety concern **(Van *et al.*, 2012).** As, the emergence of infectious disease caused by drug-resistant bacteria requires alternatives to conventional antibiotics and the search for new drugs is becoming critical because of the growing concern over the failing antibiotic drug discovery pipeline **(Sulakvelidze *et al.*, 2001).**

The data in figure (1) showed that the resistance of *B.cereus* was 100%, 90%, 90%, 80%, 75%, 75%, 70%, 70% and 65% for colistin, metronidazole,doxycycline, cephradine, streptomycin ,thiamphenicol, cephaclor and erythromycin and ciprofloxacin,respectively. Meanwhile, the sensitivity obsereved for apramycin and kanamycin 55% and 45%, respectively. Antibiotic resistance of *Bacillus cereus* previously detected by **Rusul and Jaacob (1995), Rahmati and Labbé (2008) and Ankolekar *et al.* (2009)** for different types of antibiotics such as tetracycline, streptomycin and ceftriaxone. The data in table (5) showed that multi antibiotic resistance (MAR) ranged from 0.25 to 1. Six strain (30%) resist all examined antibiotics (12) moresover, 18 (90%) of *B.cereus* isolates are considered as multi antibiotic resistant (resist three of more antibiotic from different classes). The multidrug resistant *Bacillus cereus* is increased. It may be attributed to the use of antibiotics in animals raised for food for different purposes such as prophylaxis, and growth promotion, or therapeutics and these resistant bacteria can be transmitted to human through foods (**Nygard *et al.*, 2008**) *Bacillus cereus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier **(Gundogan *et al.*, 2006),** and carry a wide variety of multi –drug resistant genes on plasmids, which can be exchanged and spread among different species of bacillus **(Neihart *et al.*, 1988).**

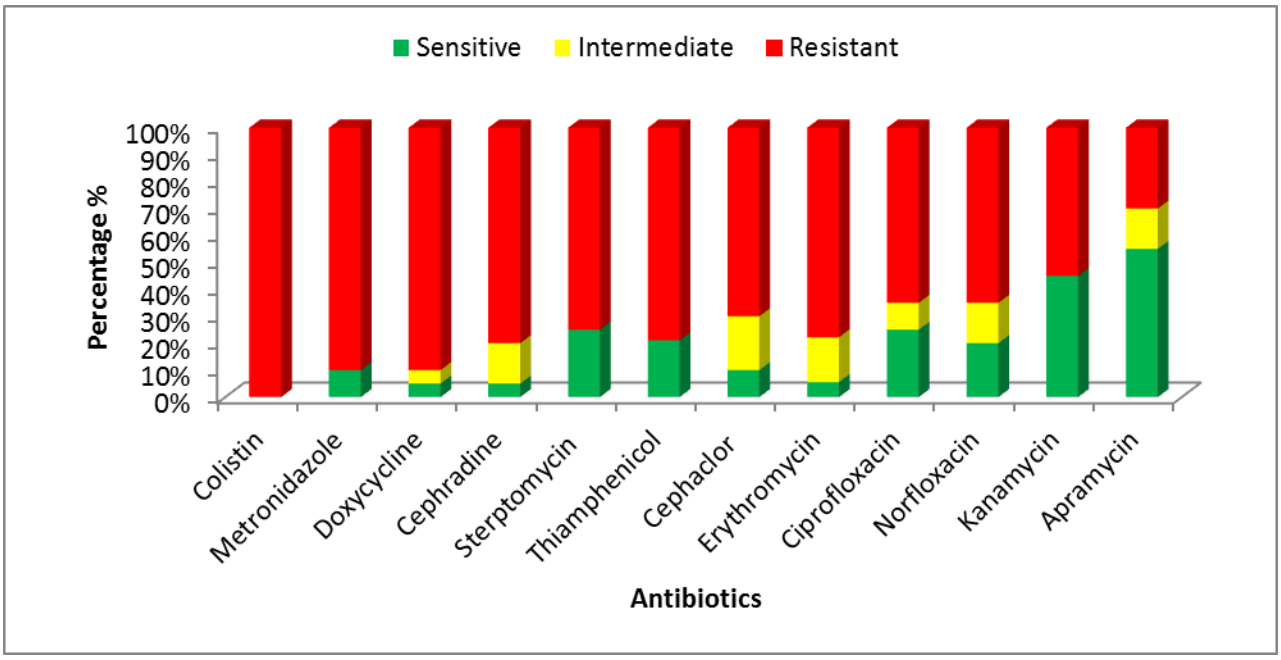


Figure (1): Antimicrobial susceptibility of *B. cereus* isolated from examined ready to eat sandwiches.

Table (5) Antimicrobial resistance profile of *B. cereus* isolated from examined ready to eat sandwiches (N=20).

	Antimicrobial resistance profile	No of Isolates	No of A.R	MAR index
I.	**CT, MTZ, DO, CE, S, TP, CEC, E, CIP, NOR, K, APR**	6	12	**1**
II.	**CT, MTZ, DO, CE, S, TP, CEC, E, CIP, NOR, K**	5	11	**0.916**
III.	**CT, MTZ, DO, CE, S, TP, CEC, E, CIP, NOR**	2	10	**0.833**
IV.	**CT, MTZ, DO, CE, S, TP, CEC, E**	1	8	**0.666**
V.	**CT, MTZ, DO, CE, S, TP**	1	6	**0.5**
VI.	**CT, MTZ, DO, CE**	1	4	**0.333**
VII.	**CT, MTZ, DO**	2	3	**0.25**
VIII.	CT	2	1	**0.083**

Colistin (CT) Metronidazole (MTZ) Doxycycline (DO)
Cephradine(CE) Sterptomycin (S) Thiamphenicol (TP)
Cephaclor (CEC) Erythromycin (E) Ciprofloxacin (CIP)
Norfloxacin (NOR) Kanamycin (K) Apramycin (APR) No:number
A.R: antibiotics resisted by S.aureus
MAR: multi antibiotic resistant (No antibiotics resisted by *B.cereus* / No examined antibiotics).

To compare the antibiotic resistance of the isolated *B. cereus* from foods in other studies the resistance of 50 randomly selected enterotoxin-positive isolates was determined using a panel of antibiotics previously employed in a study of *B. cereus* from fish **(Rahmati and Labbé, 2008)**.Moreover, half or more of 50 isolates were resistant to three of the 10 antibiotics tested: ceftriaxone, streptomycin and tetracycline **Ankolekar *et al.* (2009)**. Resistance to tetracycline by foodborne *B. cereus* was observed previously **(Rusul and Jaacob, 1995)**.

Thus, the antimicrobial susceptibility would have differed between each study. Thus, the rates of resistance to antibiotics of bacteria were high in developing countries, possibly as the result of the inappropriate or uncontrolled use of antibiotics with or without prescription **(Chang *et al*., 2003)**.

Prevalence of virulent genes among isolated *B.cereus* strains isolated from the examined chicken meat products samples:

The spores of *B.cereus* survive normal cooking temperatures **(Dierick *et al.*, 2005)** and proliferate when the additives stored at room temperatures for long times; such temperature-abused can cause foodborne illness either by intoxication (emetic type) or infection (diarrheal type). Toxin producing *Bacillus cereus* plays an important role as the causative agent of two types of food poisoning: diarrhea and emesis. The emetic syndrome is mainly characterized by vomiting a few hours after ingestion of the contaminated food. In the diarrheal syndrome, symptoms appear 8–16h after ingestion, and include abdominal pain and diarrhea. In general, both types of food borne illness are relatively mild and self-limiting. Nevertheless, more severe cases have occasionally been reported involving hospitalization or even deaths **(Lund *et al.*, 2000 and Dierick *et al.*, 2005).**

- **Cereulide *(ces)* gene:**

The achieved results in Photograph (1) revealed that the *(ces)* gene not detected in identified *B. cereus* isolated from the examined ready to eat meat sandwiches.

Similar results revealed that the *(ces)* gene not detected during examination of *B.cereus* isolates by **Chang *et al.* (2011)** and **Hariram and Labbé (2015**) who reported that none one of the 88 isolates obtained in their study possessed the emetic toxin ***(ces)*** gene.

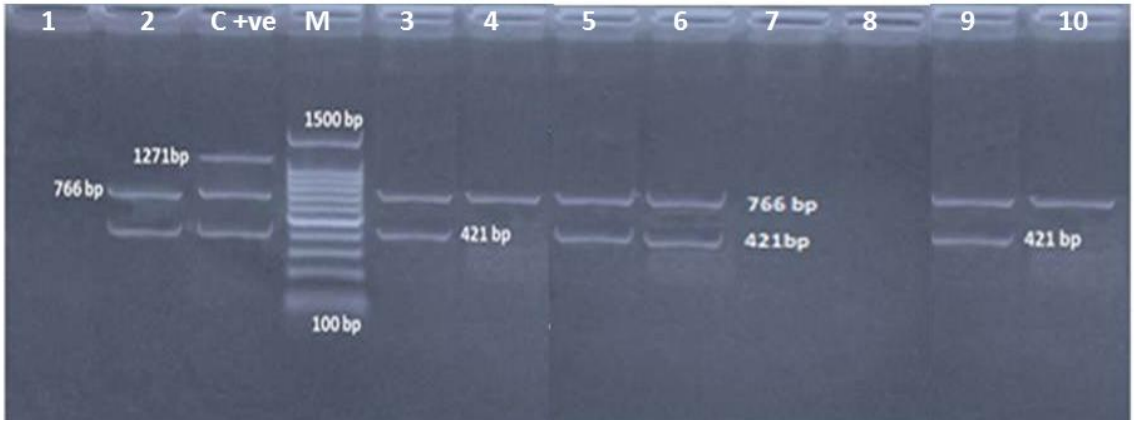
Lower incidence of ***(ces)*** gene detected by **Kim *et al.* (2011*)*** who found that only nine from 68 sample of red pepper carrying the ***(ces)*** gene in comparison with the current study this result is considered to be low. **Kim *et al.* (2015)** they detected only one emetogenic ***(ces)*** gene.

Cereulide ***(ces)*** gene causes vomiting, potentially by binding to the 5-HT3 receptors in the stomach/small intestine to stimulate the vagus nerve and brain **(Agata *et al.*, 1995)**. However, in recent studies, isolates harbouring emetic toxin genes were reported for *B. cereus* isolates from rice, spices, cooked rice and *Sunsik* and also farinaceous foods are known to be mainly associated with the emetic syndrome of *B. cereus* intoxications **(Schoeni and Wong 2005).**

- **Non-hemorrhagic entero-toxin *(nhe)* gene:**

The achieved results Photograph (1) revealed that the ***(nhe)*** gene detected in 7/10 (70%) in identified *B. cereus* was isolated from the examined ready to eat meat sandwiches.

The study coincides with the result obtained by **Te Giffel *et al.* (1997).** They reported that (*nhe)* seem to be commonly distributed among *B. cereus*, in more than 50% of foodborne *B. cereus* isolates. **Anderson *et al.* (2001)** found that (*nhe)* genes were detected among most *B. cereus* strains tested **Kim *et al.* (2011)** found *(nhe) genes* and were highly frequent among *B. cereus* strains tested in this study in a comparison with the other genes., which is in a good agreement with previous reports that most isolates from different food samples contained the *(nhe)* genes.



Photograph (1.) Agarose gel electrophoresis of multiplex PCR products amplified from genomoic DNA of *Bacillus cereus*.

<u>cytotoxin K *(cytK)* gene:</u>

The achieved results Photograph (1) revealed that the (*cyt*K) gene detected in 5/10 (50%) in identified B. cereus was isolated from the examined ready to eat meat sandwiches.

The recently discovered cytotoxin K ***(****cytK)* was the cause of the symptoms in a severe outbreak of *B. cereus* food poisoning, which included production of bloody diarrhea in several people and three fatalities, in France in 1998 **(Lund *et al*. 2000).** The result is in line with the previous work reported by **Lund *et al.* (2000)** who assumed that the occurrence of strains that possess only *(cytK)* is quite limited. **Ehling-Schulz *et al.* (2006)** found that *(cytK)* gene was mainly found in a combination with the two other enterotoxin genes, none of the tested isolates carried only *(cytK).* Higher prevalence was obtained by **Ngamwongsatit *et al.* (2008).** They found *(cytK)* gene was frequently detected with a higher incidence in the *B. cereus* with a percentage of (88.81%). **Chitov *et al.* (2008)** found *(cytK)* gene occurred in (70.4%) of the foodborne isolates tested. Generally, it was found that meat sandwiches from street vendors harbor *B.cereus* that has the ability to resist many antibiotics and contain harmful genes. Therefore, the executive authorities must intervene to protect consumers.

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المخلص العربى

مدى تةاجد الجينات الضارية ومقاومة المضادات الحيوية لعصيات سيرس المعزولة من سندوتشات اللحوم

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يزداد الاقبال علي منتجات اللحوم الجاهزة للأكل لدى الباعة الجائلين من قبل المواطنين لسد جوعهم خاصة فئة العمال البسطاء، الذين يجدون أنفسهم مجبرون علي تناولها لتوافقها مع دخلهم اليومي،غير مبالين اذا كانت صحية ام لا. تباع هذه المنتجات دائما على عربات مصنوعة من الحديد في أغلب الأحيان أو من الخشب، لها فاترينة زجاج حجمها دائما ما يكون صغيرا أو متوسطا ليقدر البائع منهم على جرها، بجوارها أسطوانة غاز لتحضير الطعام عليها. تعتبر هذه المنتجات مصدر من مصادر التسمم الغذائي وتشكل خطرا علي صحة المستهلك وذلك نتيجه لتعرضها للتلوث بميكروبات التسمم الغذائى. تتواجد بكتريا عصيات سيرس بنسب بلغت ٤٠٪ و ١٠٠٪ و ٨٦.٦٦٪ و ٤٦.٦٦٪ و ٣٣.٣٣٪ و ٦٦.٦٦٪ و ٨٠٪. وكانت متوسطات قيم أعدادها ٢.٧٣ ± ٠.١٧ و ٤.١٦ ± ٠.٢٨ و ٣.٩٨ ± ٠.٢٥ و ٣.١٧ ± ٠.٢١ و ٢.٨٤ ± ٠.٢٤ و ٣.٣٤ ± ٠.٢٣ و ٣.٥٧ ± ٠.٣١ مستعمرة بكتيرية لوغاريتم ١٠/ جرام من سندوتشات اللحم ؛ الكفتة ، البرجر الشاورما؛ الحواوشي؛ الكبده والسجق. تم تصنيف ٣٧ عينة (٣٥.٣٪) من إجمالي العينات التي تم فحصها على أنها جيدة تحتوى على أقل من^{2}١٠ ، في حين أن ١٤ (١٣.٣٪) من إجمالي العينات التي تم فحصها كانت ضمن الفئة المقبولة (^{2}١٠ إلى <^{3}١٠). وكانت العينات غير المرضية ٣١ (٢٩.٥٪) ووجد أن نسبة العينات التى تحتوى علىأكثر من^{4}١٠هى ٢٣ (٢١.٩٪) من إجمالي السندوتشات التى تم فحصها طقبا لأعداد عصيات سيرس. وكانت نسب مقاومة المضادات الحيوية لعصيات سيرس١٠٠% ، ٩٠ ٪ ، ٩٠٪ ، ٨٠ ٪ ، ٧٥٪ ، ٧٥ ٪ ، ٧٠ ٪ ، ٧٠ ٪ و ٦٥ ٪ للكوليستين ، الميترونيدازول ، الدوكسيسيكلين ، السيفرادين ، الستربتومايسين ، الثيامفينيكول ، السيفاكلور والاريثروميسين و سيبروفلوكساسين ، على التوالي. ووجد أن العزلات كانت حساسة لكلا من أبراميسين وكاناميسين بنسب ٥٥ ٪ و ٤٥ ٪ ، على التوالي. تراوح مؤشر المقاومة للمضادات الحيوية المتعددة لعصيات سيرس المعزولة من ٠.٢٥ إلى ١ . علاوة على ذلك ، كانت ٦ سلالات (٣٠ ٪) متعددة المقاومة لجميع المضادات الحيوية التي تم اختبارها و ١٨ (٩٠ ٪) من سلالات عصيات سيرس تعتبر مقاومة للعديد للمضادات الحيوية. وباستخدام تكنولوجيا تفاعل البلمرة المتسلسل لعشرة عزلات من عصيات سيرس لم يتم تحديد جين السيريوليد بينما تم تحديد الجين المعوى غير النزفى فى ٧ (٧٠%) والسيتوتوكسن جين فى ٥(٥٠%) من عصيات سيرس المعزولة من سندوتشات اللحوم من الباعة الجائلين.