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Prevalence of bacillus cereus and their antibiotic resistance of meat products

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ABSTRACT

A total of 100 random samples of meat products were collected from different localities super markets with different sanitation levels in Menofia governrorate. The collected Samples were sausage,kofta,beef burger and luncheon (25 of each).The prevalence of Bacillus cereus was 44%, 36%, 32% and 24% in examined ,sausage,kofta,beef burger and luncheon, respectively. B. cereus counts were 6.75×103 ± 0.81×104 , 4.88×103 ± 0.37×103 , 3.14×103 ± 1.65×103 and 1.67×103 ± 0.13×103 log10CFU/g in examined sausage,kofta,beef burger and luncheon, respectively. The resistance of B.cereus was 100%,91.2% and 85.3% for Kanamycin, Clindamycin and Penicillin , respectively. Meanwhile, the sensitivity obsereved for Meropenem and Gentamicin 32% and 27%,respectively. The multi antibiotic resistance (MAR) was ranged from 0.062 to 1. 34 strain were resistance to kanamycin,30 strain were resistance to both kanamycin and clindamycin and only 1 strain was resistance to all 16 antibiotic discs..

1. INTRODUCTION

Meat products are considered to be a good source of protein, vitamins, especially and other essential minerals for human growth and health. In addition, it is becoming more and more popular because it is quick and easy to prepare, it is cheap and has good taste, but it takes a long chain of handling, processing, distribution and storage. Meat tends to harbor a wide variety of microorganisms that transmit diseases through it [1] Food-borne pathogens are a major cause of illness and death in developing countries [1] Contaminated raw meat is the main cause of food poisoning [2] *B. cereus is an aerobic spore-forming Gram-positive bacterium ,This bacteria is normally disseminated in the environment. It is usually isolated from the soil, plant materials, raw meat and processed meat products .The pathogenesis of B. cereus induced food poisoning is mostly still indistinct. The microorganism conveys an expansive number of potentially toxic components, including hemolysins, phospholipases, and proteases [3].) Most of the pathophysiology of food poisoning caused by B. cereus is still unclear. [4].B. cereus* food poisoning were two types , emetic and diarrheal syndromes[5]. Diarrheal type Symptoms are abdominal pain and diarrhoea while emetic type are emesis by stimulating the vagus afferent nerve through binding to the 5-HT3 receptor [6]. In order to ensure that the food reaches its destination in good conditions, special requirements are needed, mainly to prevent contamination and spoilage during processing, preparation, packing, packaging, transport or holding of such food [7] [8]. Meat preservation became necessary for transporting meat and meat products for long distances without spoiling or changes in texture, color and nutritional value [9]. The level of contamination of meat products with *B. cereus* cause serious problems for consumers, So, the aim of this study is to

evaluate the prevelence of *B. cereus* in meat products (kofta; Lanchun; sausage and beef burger) at Menoufia governorate and their resistance to different types of antibiotics.

S0 the aim of the study is to :

- Isolation and identification of *B. cereus*.
- Antibiotic senstivity test for *B.cereus*.

2. Material and Methods

2.1. Samples collection:

A total of one hundred randomly selected samples of meat items, consisting of 25 pieces of each of beef sausage, kofta, beef burger and luncheon, were acquired from several supermarkets located in Shibin Elkom city, Menoufia governorate, Egypt. The samples that were gathered were stored individually in sterile plastic bags and kept cold in an ice box. Without unnecessary delay, all samples were brought into the laboratory and evaluated as soon as possible under stringent aseptic condition

The gathered samples were examined bacteriologically to determine whether they were contaminated with *B. cereus* and, if so, whether they were fit for human consumption.

Bacteriological examination:

2.2.1 Samples preparation :

Sample preparation step three: Weighing 25 gm of the sample, it was then put into a sterile homogeniser flask with 225 millilitres of sterile peptone water (0.1%) within, all while maintaining perfect aseptic conditions. After homogenising the contents of the flask for three minutes at 14,000 rpm, they were left to stand at room temperature for five minutes.

2.2.2 Enumeration and isolation of *Bacillus cereus* [11]:

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 were picked up and spread over the surface of slope nutrient agar slant then incubated at 37°C for 24 hours then kept in the refrigerator at 4°C for further identification of such bacteria.

2.2.3 Proteolytic activity (Lecithinase activity):

Detection of *B.cereus* lecithinase activity was carried out according to the method recommended by [10].Mannitol Egg Yolk Polymyxin Agar (MYP) is designed to detect the proteolytic activity of certain bacteria especially *B. cereus*. Mannitol fermentation was indicated by production of yellow color for mannitol fermenting colonies. Further, the medium contains egg yolk emulsion for differentiation of Lecithinase - Producing Colonies which are surrounded by a zone of white sediment .

Therefore, the diagnostic properties of the medium depend on the non-utilization of mannitol by *B.cereus* and the ability of most strains to produce phospholipase C. The medium is made selective by the addition of polymyxin B which will inhibits gram-negative bacteria. MYP agar has been shown to be very effective for detecting *Bacillus cereus* even for challenging ratios between one cell of *B. cereus* to 106 cells of other organisms.

2.2.4. Lipolytic activity[11]:

A type of nutrient based on tributyrin (glycerol tributyrate); it is used to detect the lipolytic activity of organisms such as *B. cereus*. Production of lipase enzyme splits tributyrin resulting in lipolytic colonsurrounded by a clear zone in an opaque medium.

2.2.5 Antibiotic resistance of isolated *Bacillus cereus* (Antibiogramme):

The susceptibility of antimicrobial agents was determined using the single diffusion method, as described in [11], for bacillus cereus strains. Different sensitivity discs with varying concentrations were employed to assess the vulnerability of the isolated strains (oxoid limited, basingstoke, hampshire, uk) .

The agar plate method was utilized by incorporating nutrient agar as a growth medium for the tested bacterium, allowing for the assessment of its antibiotic sensitivity. The bacterial culture was evenly distributed on the

surface of nutrient agar. After that, the antibiotic discs were positioned on top of the inoculated plate. Additionally, the plate was then incubated at a suitable temperature (25°C) for 2-7 days and examined for the presence of bacterial growth around the antibiotic discs. The maximal inhibition zone, which represents the area where the growth of microbe is significantly reduced, is determined by the antibiotic that has the greatest impact on the microbe's growth.

4. RESULTS

When looking at different meat products like sausage, kofta, beef burger, and luncheon, 34 out of 100 samples tested positive for *B. cereus*. The highest incidence was found in sausage at 44%, followed by kofta at 36%, beef burger at 32%, and luncheon at 24%.

Meat products	No. of examined samples	No.	%
Sausage	25	11	44
Kofta	25	9	36
Beef burger	25	8	32
Luncheon	25	6	24
Total	100	34	34

The results shown in table (2) indicate that the average number of *B. cereus* in the tested meat products samples (sausage, kofta, beef burger, and luncheon) was calculated. $6.75\times10^3\pm 0.81\times10^4$, $4.88\times10^3\pm 0.37\times10^3$, $3.14\times10^3\pm 1.65\times10^3$ and $1.67\times10^3\pm 0.13\times10^3$, respectively. Moreover, the statistical results revealed that,Mean values with different superscript litters in the same column are significantly different ($P<0.05$)

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Table (2): Enumeration of *Bacillus cereus* in the examined meat samples products (n=25).

Meat products	Min	Max	Mean ± S.E*
Sausage	5×10^2	3×10^4	$6.75\times10^3\pm 0.81\times10^4$ **A**
Kofta	2×10^2	1×10^4	$4.88\times10^3\pm 0.37\times10^3$ **B**
Beef burger	2×10^2	8×10^3	$3.14\times10^3\pm 1.65\times10^3$ **C**
Luncheon	1×10^2	5×10^3	$1.67\times10^3\pm 0.13\times10^3$ **D**

S.E* = standard error of mean

**Mean values with different superscript litters in the same column are significantly different ($P<0.05$).

.. **Table (3): Acceptability of the examined samples of meat products depending on their contamination with *Bacillus cereus* (n=25).**

Meat products	*B. cereus* /25 g*	Fit samples		Unfit samples	
		No.	%	No.	%
Sausage	Free	14	56	11	44
Kofta	Free	16	64	9	36
Beef burger	Free	17	68	8	32
Luncheon	Free	19	76	6	24
Total	92.2	66	66	34	34

***Center for Food Safety (2014)**

Table (4): Proteolytic and lipolytic activity of *Bacillus cereus* isolated from the examined samples of meat products (n=25).

Meat products	Proteolytic activity		Lipolytic activity	
	No.	%	No.	%
Sausage	17	68	12	48
Kofta	15	60	8	32
Beef burger	11	44	7	28
Luncheon	10	70	4	16
Total (100)	53	53	31	31

***Center for Food Safety (2014)**

Table (5): Percentages of antimicrobial susceptibility of *Bacillus cereus* isolated from the examined meat products samples (n=25).

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Kanamycin (K)	-	-	-	-	34	100
Clindamycin (CL)	-	-	3	8.8	31	91.2
Penicillin G (P)	4	11.8	1	2.9	29	85.3
Colistin (CO)	7	20.6	2	5.9	25	73.5
Sulphamethoxazol (SXT)	8	23.5	2	5.9	24	70.6
Nalidixic acid (NA)	9	26.5	3	8.8	22	64.7
Ampicillin (AM)	16	47.1	-	-	18	52.9
Tetracycline (T)	16	47.1	1	2.9	17	50
Cefotaxime (CF)	19	55.9	2	5.9	13	38.2
Azithromycin (AZ)	21	61.7	2	5.9	11	32.4
Erythromycin (E)	23	67.7	1	2.9	10	29.4
Ciprofloxacin (CP)	26	76.5	-	-	8	23.5
Gentamicin (G)	27	79.4	2	5.9	5	14.7
Daptomycin (DA)	27	79.4	3	8.8	4	11.8
Linezolid (LZ)	31	91.2	1	2.9	2	5.8
Meropenem (M)	32	94.1	1	2.9	1	2.9

Table (6): Antimicrobial resistance profile of *Bacillus cereus* isolated from the examined meat products samples (n=25).

NO	Antimicrobial resistance profile	MAR index
1	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP, G, DA, LZ, M**	1
2	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP, G, DA, LZ**	0.938
3	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP, G, DA**	0.875
4	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP, G, DA**	0.875
5	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP, G**	0.812
6	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP**	0.750
7	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP**	0.750
8	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E, CP**	0.750
9	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E**	0.687
10	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ, E**	0.687
11	**K, CL, P, CO, SXT, NA, AM, T, CF, AZ**	0.625
12	**K, CL, P, CO, SXT, NA, AM, T, CF**	0.563
13	**K, CL, P, CO, SXT, NA, AM, T, CF**	0.563
14	**K, CL, P, CO, SXT, NA, AM, T**	0.500
15	**K, CL, P, CO, SXT, NA, AM, T**	0.500
16	**K, CL, P, CO, SXT, NA, AM, T**	0.500
17	**K, CL, P, CO, SXT, NA, AM, T**	0.500
18	**K, CL, P, CO, SXT, NA, AM**	0.437

19	**K, CL, P, CO, SXT, NA**	0.375
20	**K, CL, P, CO, SXT, NA**	0.375
21	**K, CL, P, CO, SXT, NA**	0.375
22	**K, CL, P, CO, SXT, NA**	0.375
23	**K, CL, P, CO, SXT**	0.312
24	**K, CL, P, CO, SXT**	0.312
25	**K, CL, P, CO**	0.250
26	**K, CL, P**	0.188
27	**K, CL, P**	0.188
28	**K, CL, P**	0.188
29	**K, CL, P**	0.188
30	**K, CL**	0.125
31	**K, CL**	0.125
32	**K**	0.062
33	**K**	0.062
34	**K**	0.062

K: Kanamycin CL: Clindamycin P: Penicillin-G CO: Colistin SXT: Sulphamethoxazol NA: Nalidixic acid AM: Ampicillin T: Tetracycline CF: Cefotaxime DA: Daptomycin AZ: Azithromycin E: Erythromycin CP: Ciprofloxacin G: Gentamycin LZ: Linezolid M: Meropenem Linezolid M: Meropenem

5. Disscussion

B.cereus has emerged as a major foodborne pathogen during the last few decades which produce different enterotoxins that cause two types of illness,emetic type and diarrial type. [12]and :[13]

The results obtained in table (1) revealed that 34isolates of *B.Cereus* were isolated from 100 examined meat products samples (sausage,kofta, beef burger and luncheon) represented by 11 (44%) from sausage samples, 9 (36%) from kofta samples, 8 (32%) from beef burger samples and 6 (24%) from luncheon samples .

Regarding to sausage samples, the obtained results were nearly similar to that recorded by [14], but disagree with those obtained by [15] who recorded lower incidence (25%),(30% .(%15)

Meanwhile, for kofta samples, the obtained results were nearly similar to that obtained by [16][17]) who recorded that. The percentage of *B.Cereus* in kofta was 37.5%,.but disagree with those obtained by [18] who recorded that the incidence of *B. cereus* in kofta was 37.5% but disagree with those obtained by [19] who recorded lower incidence.(%25)and [19]who recorded higher incidence, 11 (% 55)

Regarding to beef burger samples, the obtained results were similar to that recorded by[20] ,[21]), [22]and . [23] who recorded similar incidences 35% ,36.67%,36% and 31.25%, respectively, but disagree with those obtained by [24] [25]and [26] who recorded lower incidences, 28%, 28% and 24%, respectively, and with those of [27]), El-Shewehy (1994), [28],[29],[30], [31]and [32]who recorded higher incidence 48%, 48%, 40%, 65%, 56%,65% and 92%,respectively.

Moreover, for luncheon samples, the obtained results were similar to that recorded [33] who recorded similar incidence 26.7% and 26.7% respectively, while disagree with those obtained by [34] , [35] who recorded lower incidence, (13.3%) and (15%)respectively. and with those of [36]and [37]) who recorded higher incidence (55%)and(44%),respectively.So, the obtained results reflected that, the highest bacterial percentage was in sausage followed by kofta then beef burger and finally luncheon and this may be attributed to spices added as a major source of contamination [38]and[20]. Also, when proper hygiene practices are not followed during the processing, handling, and storage of food, it allows the spores of *B. cereus* to grow and multiply into harmful cells. These spores also have the ability to stick to surfaces and create biofilms, which can lead to food poisoning[39]and[40] . The results from Table (2) showed that the amounts of *B. cereus* bacteria in the meat samples (sausage, kofta,

beef burger, and luncheon) ranged from $5\times10^2$to 3×10^4; $2\times10^2$to 1×10^4; $2\times10^2$to $8\times10^3$and $1\times10^2$to 5×10^3, respectively, with a mean value of $6.75\times10^3\pm 0.81\times10^4$; $4.88\times10^3\pm 0.37\times10^3$; $3.14\times10^3\pm 1.65\times10^3$ and $1.67\times10^3\pm 0.13\times10^3$, respectively. Moreover, the statistical results table(2) revealed that, the mean value within examined samples of meat products showed high significant differences ($P<0.05$).

Regarding for sausage samples, these results came in agreement with [20][41][42] and [43] but disagree with those of [44] and [45] who found that the count was 9 x $10^5$and 2 x 10^5,respectively .

On the other hand, the results from the kofta samples were close to the findings of [46] but disagree with those obtained by [47] who found that the mean values of *B. cereus* count (cfu/g) in the examined samples of kofta was $6.08x10^5$/g..

In addition, for luncheon samples, these results came in agreement with [48]and [49]who found that, the count was3.37 x 10^3 and $3.73\times10^3\pm 0.51\times10^3$,but dissagree with [50] who found that the average values of *B.cereus* count(cfu/g) in the examined samples of luncheon was $8.58\times10^4\pm1.62\times10^4$.

Moreover, for beef burger samples, the obtained results were nearly similar to that recorded by [51]and [52]

The reason why there are a lot of *B. cereus* bacteria in frozen rice kofta is likely because of the wet rice and spices used. Similarly, the high amount of *B. cereus* found in sausage could be because of the curing salts, spices [53]and [54] and temperature changes during cooking[55]. The bacterial count in beef burger samples may be due to cross-contamination during processing. Poor hygiene practices when handling, storing, transporting, and selling food can greatly contribute to the spread of bacteria.the obtained results revealed that, the meat products contained high *B. cereus* count and this may be attributed to contamination of flesh used for manufacture, mincing machine, grinders, equipment and knives that considered as a source of contamination of meat during processing [20] and[56]. The presence of high concentrations of *B. cereus* (more than10^5 cfu/g) in food is stimulates the growth and reproduction of the organism and is associated with health problems. It causes both diarrheal and emetic syndrome, each associated with a specific enterotoxin[57] and [58]

The obtained results in table (4) revealed that the acceptance rate for the examined meat products samples (sausage ,kofta ,beef burger and luncheon) depending on their contamination with *B. cereus,* were 56%, 64%, 68% and 76%, respectively were approved for human consumption, while 44, 36, 32 and 24%, respectively were unfit for human consumption ,according to [59]

Proteolytic and Libolytic activity of *B.Cereus*:

In the data chart, it was found that *B. cereus* bacteria in the meat samples had 53% proteolytic activity and 31% lipolytic activity. The proteolytic activity for sausage, kofta, beef burger, and luncheon was 68%, 60%, 44%, and 70% respectively. Lipolytic activity was 48%, 32%, 28%, and 16% in sausage, kofta, beef burger, and luncheon respectively

6.Antimicrobial susceptibility of *B. Cereus*

Food contamination by antibiotic-resistant bacteria poses a serious risk to public health. The resistance genes can spread to other harmful bacteria, making it harder to treat severe infections. [60] The results in Table (5) show that *B.cereus* isolates were highly resistant to Kanamycin (K) at 100%, followed by Clindamycin (CL) at 91.2%, Penicillin G (P) at 85.3%, and Colistin (CO) at 73.5%. Additionally, they showed resistance to Sulphamethoxazol (SXT) at 70.6%, Nalidixic acid (NA) at 64.7%, Ampicillin (AM) at 52.9%, and Tetracycline (T) at 50%. Lower resistance percentages were seen in Cefotaxime (CF), Ciprofloxacin (CP), Azithromycin (AZ), Erythromycin (E), and Ciprofloxacin (CP) at 32.4%, 29.4%, and 23.5%, respectively. Isolated bacteria showed resistance to Gentamicin (G) (14.7%), Daptomycin (DA) (11.8%), Linezolid (LZ) (5.8%), and Meropenem (M) (2.9%). The study suggests that Linezolid and Meropenem are the best choices for treating most cases of *B.cereus* infection since the bacteria were mostly susceptible to these drugs. A table with six rows is displayed, indicating how often *B.cereus* isolates showed resistance to various antimicrobials. It was found that 34 strains

were resistant to kanamycin, 30 strains were resistant to both kanamycin and clindamycin, and only one strain was resistant to all 16 antibiotic discs. Contaminated food with bacteria that are resistant to antibiotics can be a big problem for public health. This is because the resistance traits can be passed on to other harmful bacteria, making it harder to treat serious bacterial infections. The data in Table 5 shows that *B.cereus*. bacteria were highly resistant to Kanamycin (K), Clindamycin (CL), Penicillin G (P), and Colistin (CO). They also showed resistance to Sulphamethoxazol (SXT), Nalidixic acid (NA), Ampicillin (AM), Tetracycline (T), Cefotaxime (CF), Ciprofloxacin (CP), Azithromycin (AZ), and Erythromycin (E), but to a lesser extent. The findings showed that some *B.cereus* isolates were not affected by Gentamicin (G) (14.7%), Daptomycin (DA) (11.8%), Linezolid (LZ) (5.8%), and Meropenem (M) (2.9%). Based on this, Linezolid and Meropenem seem to be the best options for treating *B.cereus* since most of the isolates responded well to these drugs. A table was made to show how often *B. cereus* bacteria were resistant to different antibiotics. The table showed that 34 strains were resistant to kanamycin, 30 strains were resistant to both kanamycin and clindamycin, and only 1 strain was resistant to all 16 types of antibiotics.

6. CONCLUSION

By looking at the data we have, we can see that when beef products get contaminated with *B. cereus*, it raises the risk of people getting sick from the food. To lower this risk, it's important to keep equipment, machines, and knives clean, and only use additives from reliable sources. These steps can help reduce the chances of getting infected with Bacillus spores. Scheduling antibiotic testing for *B. cereus* found in beef products can help in selecting the right antibiotic. The information also confirms the importance of detecting *B. cereus*. Cereus is used in programs to control diseases and prevent them, as well as in labs for clinical and food quality control in Egypt.

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