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SOME AEROBIC AND ANAEROBIC SPORE FORMERS IN SOME READY-TO-EAT CHICKEN AND CHICKEN PRODUCTS

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ABSTRACT

Fifty samples of fried chicken and chicken burger sandwiches and nuggets were collected from different fast food restaurants in Alexandria province and examined bacteriologically to isolate and enumerate pathogenic spore forming bacteria. Bacillus cereus (aerobic spore formers) could be isolated with a percentage of 68% and with an average count of 1.2 x 10^4 cfu/g while Clostridium perfringens (anaerobic spore formers) could be isolated with a percentage of 12% and with an average count of 1.7 x 10 cfu/g. The isolated bacteria were identified microscopically and biochemically, and the public health hazard of the isolated microorganisms were discussed.

Key words: chicken products, *Bacillus cereus, Clostridium perfringens.*

INTRODUCTION

Bacterial food poisoning is the most common cause of food borne illness all over the world. The temperature range in which most bacterial grow is between 5°c - 60°c so cooked foods should not be kept in this danger zone for longer time than those absolutely necessary. A large number of food poisoning bacteria

may be present and cause illness, therefore illness can be prevented by (1) controlling the initial number of bacteria present. (2) preventing the small number from growing, (3) destroying the bacteria by proper cooking and (4) avoiding re-contamination.

Recently, a wide spread of fast food restaurants chains are taking place in Egypt which encouraged a large number of people from different ages leading to incidence of many food poisoning cases.

Aerobic spore forming bacteria are widely spread in nature and gains entrance into foods either through contaminated utensils or from the surrounding environments ***(Wong et al., 1988)***.

Bacillus cereus is a Gram- positive, spore forming, motile, aerobic rod and commonly found in soil, water and foods. It is the most important cause of food poisoning from the genus Bacillus ***(Toril Lindback et al., 2004)***. The heat resistance of *B.cereus* spores and its non fastidious nature facilitates its survival and / or growth in a wide variety of foods ***(Shinagawa, 1990)***. Chicken and its products were the most frequently implicated food sources for Bacillus food poisoning in Ireland ***(Wilson et al., 1993)***.

Presence of anaerobic spore formers in food could be attributed to bad quality of raw materials and additives, insufficient heat treatment, unsatisfactory sanitation during handling, processing and storage ***(ICMSF, 1996)***. Processing temperature can not kill all anaerobic spore formers, but it may stimulate some of these spores to germinate and kills the competing organisms, on the other words, the oxygen derived off during cooking rendering the

conditions suitable for anaerobic spores to survive and they can germinate when the temperature is favorable ***(Davis and Board, 1998)***.

Clostridium perfringens is a Gram + positive, non motile spore forming anaerobic rod, it is widely distributed in soil, sewage, stool samples and intestine of animals and human ***(Steele and Wright, 2001)***. Anyone can get C. perfringens food poisoning by eating food stored in a large quantities at room temperature at schools, camps, bankets and buffets ***(Martel et al., 2004)***.

C. perfringens type A is usually involved in food poisoning outbreaks ***(Gilbert, 1983)***, it is considered as the third leading cause of food poisoning in the USA ***(Bos et al., 2005)*** and one of the four most important bacterial agents causing food poisoning due to its ubiquitous nature, ability to contaminate carcass intravitally, thermal resistance of spores, relatively short generation time at high incubation temperature, survival in chilled and frozen meat and ability to overcome stomach acid barrier ***(Naravan, 1982)***.

Recently, many famous fast food restaurants chains including chicken and its products are taking place in Egypt and attract many people from different ages. Preparing large quantities of foods through the day which can be contaminated by food handlers or stored in an incorrect temperature and reheated insufficiently when demand, which give the chance for spore forming bacteria to survive leading to food poisoning cases.

So, the present study was carried out to investigate some spore forming food poisoning bacteria in some ready-to-eat chicken and its products produced by such restaurants to detect the hygienic status of such products and concluded the hygienic measures to be followed to protect the consumers. The present study aimed to: (1) isolate and enumerate *B. cereus* as an aerobic spore formers, (2) isolate and enumerate *C. perfringens* as an anaerobic spore formers in some ready-to-eat chicken and its products.

MATERIAL AND METHODS

1- Collection of samples:

A total of 50 random samples of fried chicken and chicken burger sandwiches and nuggets were collected from different fast food restaurants in Alexandria province and transported immediately to Animal Health Research Institute, Alex. Lab, department of bacteriology in its original containers with a minimum of delay for recovery of *Bacillus cereus* and *Clostridium perfringens* contaminating such products.

2- Preparation of the samples *(APHA.1992)*:

Twenty five grams of each sample were homogenized in 225 ml sterile buffered peptone water 0.1% (**Oxoid**) using sterile blender (homogenizer type MPW-302 Poland) to provide a dilution of 10^{-1}, then further serial dilution were prepared up to 10^{-6}.

3- Isolation and enumeration of *B. cereus (Health protection Agency, 2004)*:

0.1 ml from each dilution was spread over previously dried surface of Peptone Egg Yolk Polymyxin Bromothymol Blue Agar (PEMBA) (**Biolife**) plates using a sterile bented glass rod for each plate then left to dry and incubated at 30° c for 24-48 hours.

The presumptive *B. cereus* colonies that having the following characteristics were counted: crenate, about 5 mm in diameter, turquoise blue in colour surrounded by a distinct opaque zone of egg yolk precipitation of the same colour as the colonies then subculture and confirmed microscopically as large Gram- positive bacilli in short-to-long chains, spores are ellipsoidal, central to sub terminal, and don't swell the sporangium, and biochemically by glucose fermentation, Voges Proskauer reaction, nitrate reduction, Haemolysis on blood agar and Motility test.

4- Isolation and enumeration of Clostridium perfringens *(ISO 7937-2004)*:

1 ml from each dilution was inoculated into a sterile petridish, then 10-15 ml of Treptose Sulphite Cycloserine Agar (TSC) (**Biolife**) at 44-47°c poured and mixed with the inoculum and left to solidify. 10 ml of the same medium (2nd layer) was added, incubated under an anaerobic conditions for 24 hours at 37° c.

The characteristic colonies (black colonies) were counted in each plate then subcultured to be confirmed microscopically as a Gram-positive short rod, arranged in pairs or short chains with rounded or

sometimes pointed or square end posses a single endospore ***(Health Protection Agency, 2008)***, and biochemically ***(Willis, 1977)*** by nitrate reduction, gelatin liquefaction and motility test.

Statistical analysis of the results was performed using T test and analysis of variance (ANOVA test) ***(Snedecor and Cochran, 1980)***.

RESULTS

Results were tabulated in the following 2 tables:

Table (1): *Bacillus cereus* isolated from ready-to-eat chicken and chicken products:

Type of the examined samples	No.	+ ve	%	Min	Max	Average
- Fried chicken	30	24	80	1x10	$3x10^4$	$1.4x10^4$
- Chicken burger	12	2	16.7	$1x10^2$	$1x10^2$	$1x10^2$
- Nuggets	8	8	100	$1x10^2$	$2x10^4$	$7.8x10^3$
Total	**50**	**34**	**68**	**$1x10^2$**	**$3x10^4$**	**$1.2x10^4$**

Table (2): *Clostridium perfringens* isolated from ready-to-eat chicken and chicken products:

Type of the examined samples	No.	+ ve	%	Min	Max	Average
- Fried chicken	30	2	6.7	2x10	2x10	2x10
- Chicken burger	12	4	33.3	1x10	2x10	1.5x10
- Nuggets	8	-	-	-	-	-
Total	**50**	**6**	**12**	**1x10**	**2x10**	**1.7x10**

DISCUSSION

Ready-to-eat (RTE) foods poultry meat and have been documented to serve as vehicle for several bacterial pathogens and food borne outbreaks have been associated with the consumption of contaminated RTE foods ***(Guerra et al., 2001; Borch and Arinder, 2002; Gudbjornsdottir et al., 2004).***

Bacillus cereus is one of pathogenic aerobic spore forming bacteria causing food poisoning. It can give rise to two distinct forms of food borne illness, the diarrhoeal type is caused by three different heat-labile enterotoxin produced during its vegetative growth in the small intestine after ingestion and the emetic type is due to production of a heat-stable emetic toxin (cereulide) during growth in foods ***(Cecilie et al., 2005)***, the diarrhoeal type has been associated mostly with meat and poultry ***(Doyle, 1988)***.

Table (1) showed that *B. cereus* could be isolated from the examined samples with a percentage of 68 % and with counts ranged from 1×10^2 to 3×10^4 cfu/g with an average of 1.2×10^4 cfu/g. Nearly similar results were reported by ***Kamat et al. (1989)*** with a percentage of 80 % from chicken and meat products with counts ranged from 2×10^2 to 5×10^5 /g; ***Vazgecer et al. (2004)*** with a percentage of 48 % and had a count less than 10^2 /g for cooked chicken dôner kebab, and ***Mira and Abuzied (2006)*** who could isolate it from all ready-to-eat chicken products samples.

The symptoms of *Clostridium perfringens* food poisoning which include watery diarrhea, intense abdominal pain, gas gangrene and inflammation have been attributed to a protein enterotoxin produced by the organism in the intestine ***(Teo and Tan, 2005)***. The neurotoxin produced by the organism causes also necrotic enteritis in animals and human ***(Wise and Siragusa, 2005)***.

Table (2) showed that *C. perfringes* could be isolated from the examined samples with a percentage of 12 % and with counts ranged from 1 x 10 to 2 x 10 cfu/g with an average of 1.7 x 10 cfu/g; the microorganism could be isolated by ***Vazgecer et al. (2004)*** with a percentage of 7 % from chicken dôner kebab.

The obtained results indicated that there is a public health hazard from consuming ready-to-eat chicken and chicken products produced by such fast food restaurants which contaminated with these serious pathogenic bacteria so there are some recommendations must be followed to prevent such hazard that include:

(1) Proper cooking of the raw materials to a temperature sufficient to kill bacterial spores.

(2) Avoid re-contamination of cooked products by handlers by following the hygienic measures.

(3) Serving of cooked products immediately or keeping in a temperature below 5°c or above 60°c and reheated to 75°c prior to serving.

In addition, authorities must pay attention to such establishments in order to protect the consumers.

REFERENCES

- ***American Public Health Association (APHA) (1992):*** Compendium of methods for microbiological examination of foods. 4th Ed., Academic press, Washington, D.C.

- ***Borch, E. and Arinder, P. (2002):*** Bacteriological safety issues in red meat and ready-to-eat products, as well as control measures. Meat sci. 62, 281-390.

- ***Bos, J; Smithee, L.; McClane, B.; Distefano, R.F.; Uzal, F.; Songer, J.G.; Mallonee, S. and Crutcher, J.M. (2005):*** Fatal necrotizing colitis following a food borne outbreak of enterotoxigenic *Clostridium perfringens type A* infection. Clin. Infect. Dis., 40: 78-83.

- ***Cecilie From; Rudiger Pukall; Peter Schumann; Victor Hormazabal and Per Einar Granum (2005):*** Toxin-producing ability among Bacillus spp. Outside the Bacillus group. App. Environ. Microbiol., 71 (3) 1178-1183.

- ***Davis, A. and Board, R. (1998):*** The microbiology of meat and poultry. A text book, 1st Ed., Edmunds bury press, Ltd, Edmunds, London.

- ***Doyle, M.P. (1988):*** *Bacillus cereus*. Food technol., 42, 4, 199.

- ***Gilbert, R.J. (1983):*** Food borne infection and intoxications. Recent trends and prospects for the future. In: Food Microbiology: Advances and prospects. Roberts, T.A. and Skinner, F.A. (Eds.). Academic press, London, pp. 47-66.

- ***Gudbjornsdottir, B.; Suihko, M.L.; Gustavsson, O.; and Brdholt, S. (2004):*** The incidence of L. monocytogenes in meat, poultry and sea food plants in Nordic countries. Food Microbiol. 21, 217-225.

- ***Guerra, M.M; Mclauchlin, J. and Bernardo, F.A. (2001):*** *Listeria* in ready-to-eat and unprocessed foods produced in Portugal. Food Microbiol. 18, 423-429.
- ***Health Protection Agency (NHS) (2004):*** Enumeration of *Bacillus cereus* and other B. species. National standard method F 15 issue 1.
- ***Health Protection Agency (NHS) (2008):*** Identification of Clostridium species. National standard method B sop I D 8 Issue 3. http:// www. hpa- standard methods.org.uk/pdf sops.asp.
- ***ICMSF (1996):*** Microorganisms in food III. Microbial specification of food pathogens. Vol. 2 Chapman and Hall, Landon, New York.
- ***ISO-7937 (2004):*** "Horizontal method for the enumeration of *Clostridium perfringens*- colony – count technique".
- ***Kamat, A.S.; Nerkar, D.P. and Nair, P.M. (1989):*** *Bacillus cereus* in some Indian foods, incidence, antibiotics, heat and radiation resistance. J. Food Safety, vol. 10 issue 1 pages 31-41.
- ***Martel, A.; L.A. Devrise; K. Cauwverts; K. DeGussem; A. Decostere and F. Haesebrouck (2004):*** Susceptibility of *Cl. Perfringens* strain from broiler chickens to antibiotics anticoccidials. Avian pathol. 1: 307.
- ***Mira, EKL and Abuzied, SMA (2006):*** Prevalence of *B. cereus* and its enterotoxin in some cooked and half cooked products. Assiut vet. Med. J., vol. 52 (no. 109).
- ***Naravan, K.G. (1982):*** Food borne infection with Clostridium perfringens type A. Int. j. zoonoses, 9 (1) 12-32.
- ***Shinagawa, K. (1990):*** Analytical methods for *B. cereus* and other bacillus. species. Int. j. Food Microbiol. Mar; 10 (2): 125-41.

- ***Snedecor GW and Cochran W (1980):*** Statistical Methods. The Iowa State University Press, Ames, IA.

- ***Steele, F.M. and K.H. Wright (2001):*** Cooling rate effect on outgrowth of *Clostridium perfringens* in cooked ready-to-eat turkey breast roasts. Poultry sci., 6: 813-816.

- ***Teo, A.Y. and H.M. Tan (2005):*** Inhibition of *Clostridium perfringens* by a novel strain of B. subtilis isolated from the gastrointestinal tracts of healthy chickens. Appl. Environ. Microbiol. 71: 4185-4190.

- ***Toril Lindback; Annette Fagerlund; Marianne Skeie Rodland and Per Einar Granum (2004):*** Characterization of the *Bacillus cereus* Nhe enterotoxin. Microbiology 150, 3959-3967; DOI 10. 1099/ MICO. 27359-0.

- ***Vazgecer, B.; Ulu, H. and Oztan, A. (2004):*** Microbiological and chemical qualities of chicken dôner kebab retailed on the Turkish restaurants. Food control, vol. 15, issue 4, June 2004, pages 261-264.

- ***Willis, A.T. (1977):*** Anaerobic bacteriology, clinical and laboratory practice. 3rd Ed. Butter Worth, London.

- ***Wilson, I.G.; Wilson, T.S. and Kramer, J.M. (1993):*** Increase in Bacillus food poisoning in Northern Ireland. Lancet, 342: 928.

- ***Wise, M.G. and G.R. Siragusa (2005):*** Quantitative detection of Clostridium perfringens in the broiler foil gastrointestinal tract by real time PCR. Appl. Environ. Microbiol., 71: 3911-3961.

- ***Wong, H.G.; Chang, M.H. and Fan, J.Y. (1988):*** Incidence and characterization of *Bacillus cereus* isolated from contaminating dairy products. Appl. Environ. Microbiol. 54 (3): 699-702.

بعض الميكروبات المتحوصلة الهوائية و اللاهوائية فى الدجاج و منتجاته المعدة للأكل

سوسن خميس محمود عبيد ، سهام فؤاد جورجى ، محمد محمد موسى

يعد التسمم الغذائى البكتيري سببا رئيسيا من أسباب التسمم الغذائي على مستوى العالم. و بما أن معظم البكتيريا تنمو في درجات حرارة تتراوح ما بين 5°م –60°م لذلك يجب عدم حفظ الأطعمة المعدة للأكل في مثل هذه الدرجات ما لم يكن هناك ضرورة . و نظرآ لأن التسمم الغذائي البكتيري يتطلب وجود عدد كبير من البكتيريا لذلك يمكن تجنبه عن طريق: منع تلوث الأغذية بالبكتيريا قبل الطهي، منع الأعداد الصغيرة للبكتيريا من النمو، القضاء على البكتيريا بالطهي السليم و تجنب التلوث بعد الطهي. و قد أدى انتشار مطاعم الوجبات السريعة في مصر حديثا إلى جذب الكثير من الناس من مختلف الأعمار و حدوث الكثير من حالات التسمم الغذائي لذلك أجريت هذه الدراسة لفحص بعض أنواع البكتيريا المتحوصلة الهوائية و اللاهوائية في بعض وجبات الدجاج و منتجاته المباعة في هذه المطاعم لتحديد الحالة الصحية لها و استنتاج الطرق الصحية الواجب إتباعها لحماية المستهلكين. و قد تم تجميع 50 عينة من سندوتشات الدجاج و البرجر و كذلك الناجتس من مختلف مطاعم الوجبات السريعة و المشهورة في مدينة الإسكندرية و تم فحصها بكتريولوجيا لعزل و عد المتحوصلات الهوائية الممرضة و التي تتمثل في *الباسيلس سيرس* و التي تم عزلها بنسبة 68% و متوسط عددى 1.2× 10^4 ميكروب/جرام و كذلك المتحوصلات اللاهوائية الممرضة المتمثلة في *الكلوستردیم بیرفرینجنز* و التي تم عزلها بنسبة 12% و متوسط عددي 1.7× 10 ميكروب/جرام و قد تمت مناقشة النتائج و الأهمية الصحية للبكتيريا المعزولة و الاحتياطات الواجب إتباعها لتجنب الإصابة بها.