Bacteriological quality of beef burger in some local markets with special reference to *C. perfringens*

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Abstract

Fifty random samples of frozen beef burger were collected from different markets in Cairo and Giza Governorates and subjected to bacteriological examination. The results revealed that the mean values of aerobic and anaerobic bacterial counts were $4.6 \times 10^5 \pm 1.69 \times 10^5$ and $1.61 \times 10^3 \pm 0.58 \times 10^3$ CFU/ gm, respectively.

Isolation of *C. perfringens* was also demonstrated in 12% of the samples. Three isolates were type A, one isolate type D and non-toxigenic type 2 isolates (4%). The rejected samples were 34% from the total examined samples according to **EOSQC (2005)**. The results and the recommendation for production of high quality beef burger were discussed.

Key words: frozen beef burger*, C. perfringens, Cairo, Giza* .

Introduction

Meat and meat products are considered as an excellent source of high quality animal protein. They are considered as an ideal culture medium for growth of many organisms because they are high in moisture, rich in nitrogenous compounds of various degrees of complexity, plentifully supply with minerals and accessory growth factors, have some fermentable carbohydrates (glycogen) and favorable pH for most microorganisms **(Jure *et al.,* 2006)**.

Microorganisms may contaminate meat products during a long chain of processing from the time of preparation, handling, processing, distribution and storage as well as marketing. Such contamination may render the product of inferior quality or even unfit for human consumption and at times may constitute a public health hazard **(Essa *et al.,* 2009)**. *Clostridium perfringens* is a leading cause of bacterial food borne illness in countries where consumption of meat is high **(Lin and Labbe, 2003)**. *C. perfringens* is an anaerobic spore forming and ubiquitous pathogen bacterium widely distributed in the environment and frequently occurs in soil, water and the intestinal tract of certain animals and humans **(Omer *et al*., 2006)**. Most food-poisoning cases involving *C. perfringens* are reported from restaurants, hospitals and homes for elderly people. Through proper cleaning and disinfection, it should be relatively easy to control food borne diseases caused by *C. perfringens*, but unfortunately, large out breaks, sometimes with fatal outcome due to *C. perfringens* food poisoning, are still frequently reported **(Labbe, 2000).**

C. perfringens food poisoning, is caused by type A isolates carrying a chromosomal enterotoxin gene (C. cpe), *C. perfringens* spore are thought to be the important infectious cell morph type and after inoculation into a suitable host, these spores must germinate and return to active growth to cause gastrointestinal disease **(Daniel *et al*., 2008)**.

The purpose of the present study was to determine aerobic plate count, Anaerobic count, the occurrence of *C. perfringens* and its enterotoxin in beef burger sold in Cairo and Giza markets.

Material and Methods

Sampling:

A total of 50 random samples of frozen beef burger were collected from different supermarkets at Cairo and Giza Governorates. The collected samples were aseptically transferred to

the laboratory and subjected to the following bacteriological exanimation according to **APHA (2001)**.

1. Bacterial counts:

1.1. Aerobic plate count (APC):

The total bacterial counts were done by using the plate count agar in duplicate plates and incubated at 30ºC for 48 hours. The total aerobic count (CFU/g) of samples were calculated.

1.2. Anaerobic plate count (ANPC):

The plate media (reinforced clostridium media RCM) were streaked with 0.1 ml of the first and second dilution then, incubated anaerobically at 37ºC for 48 hours in anaerobic jar containing gas pack. The total anaerobic count as cfu / gm of samples were calculated.

2. Isolation of *Clostridium perfringens*:

Using cooked meat medium, aneroboically incubated at 37ºC for 24 hours followed by sub culturing on to neomycin blood agar which incubated at 37ºC for 24 hours. Suspected colonies of *C. perfringens* were tested for lecithinase activity using Egg yolk Agar plates. However, further identification of *C. perfringens* was carried out according to **Koneman *et al.* (1992).**

On the other hand, the isolated strains of lecithinase positive *C. perfringens* were typed through dermonecrotic reaction by intra dermal inoculation test into Guinea pigs according to **Sterne and Batty (1975).**

3. Biological Assay for Detection of Enterotoxigenic *C. perfringens* according to Giannella, (1976) and Nasr *et al.* (2007):

Cooked meat broth culture of *C. perfringens* isolates were inoculated in Duncan and Strong (DS) sporulation medium **(Duncan and Strong, 1968)** and incubated anaerobically at 37ºC for 8 hrs. Centrifugation of the culture at 12.000 rpm was done at cooling centrifuge, then filtration of the culture using syringe filter. Infant albino mice (1-2 gm, 1-4 days old) were subjected to intra – gastric inoculation with 0.1 ml of crude filtrate. After 4 hours autopsy were performed and the small intestine weights and distention were recorded. The gut weight to the remaining body weight was calculated A ratio of less than 0.083 was considered negative.

Table (1): Statistical analytical results of bacterial counts (cfu/g) of examined beef burger samples (n=50).

Count / Value	Aerobic bacteria	Anaerobic bacteria
Minimum	1.5×10^4	$< 10^2$
Maximum	6×10^7	2×10^4
Mean	4.6×10^5	1.61×10^3
± SE	1.69×10^5	0.58×10^3

Table (2): Incidence and typing of isolated *C. perfringens* in tested samples.

No. of tested samples	No. of samples contaminated with *C. perfringens*		Toxigenic				Non-toxigenic	
			Type A		Type D			
	No.	%	No.	%	No.	%	No.	%
50	6	12	3	75	1	25	2	4

Table (3): Character of *C. perfringens* type A and its enterotoxin.

No. of type A	Lecithinase activity	Alfa and Beta haemolysis on blood agar	Virulence of enterotoxin to baby mice
3	+ve	+ve	+ve

N.B. all strains more than 0.083 (slandered ratio for +ve control in baby mice assay).

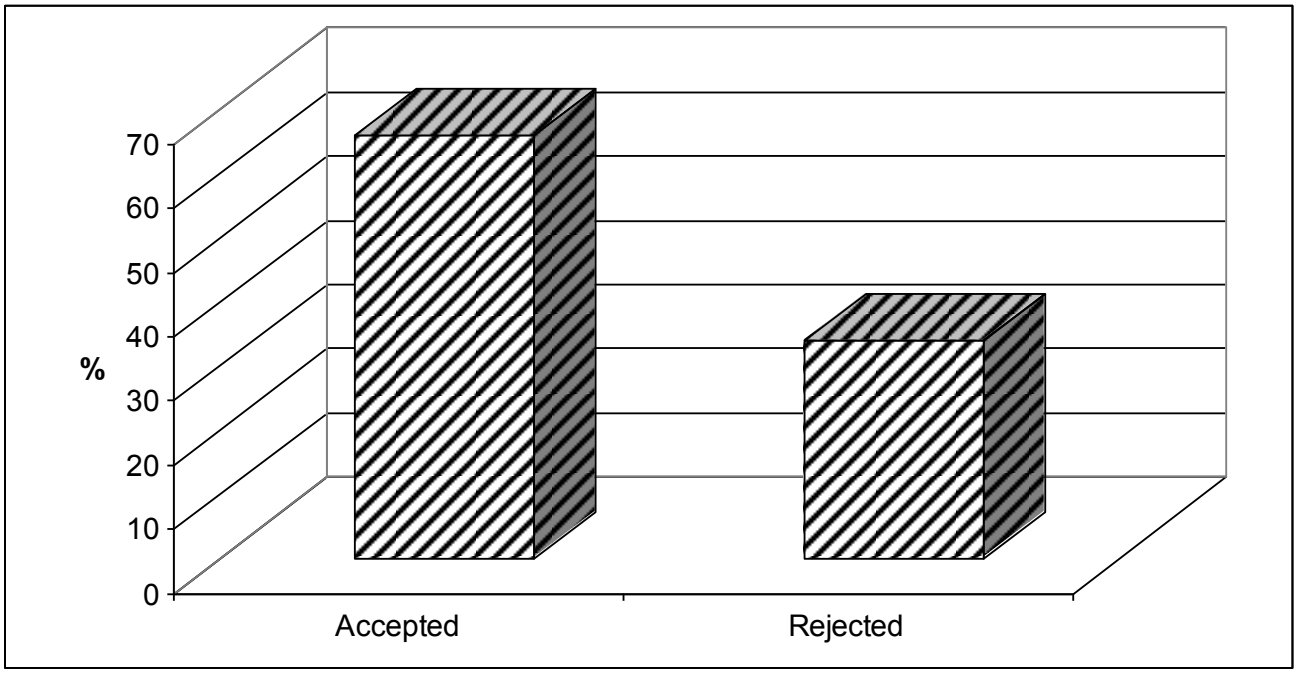

Fig. (1): Percentage of accepted and rejected beef burger samples according to EOSQC (2005).

Discussion

Meat products such as beef burger have a popularity because they represent quick easy prepared meat meals and solve the problem of the shortage in fresh meat of high price. *C. perfringens* remains as one of the major food borne health hazards, and meat plays an important role, as a reservoir, in disseminating *C. perfringens*.

In the present study, a total of fifty random samples of frozen beef burger were examined for APC, ANPC and detection of toxigenic strains of *C. perfringens* contaminating such food stuff. From the results obtained in **Table (1)**, the mean values of aerobic bacterial count were $4.6 \ 10^5 \times 1.69 \pm 10^5 \times$CFU/ g which exceeded the permissible limit (10^5) recommended by EOSQC 1688 – 2005. These results agreed with those reported by **El. Mosalami, *et al*. (1982); El. Sherif *et al*. (1991); Nassif, *et al*. (2002); Abd. El. Aziz (2002); Hassan and Antown (2005);** Lower bacterial counts were reported by **Galab *et al*. (2011),** the aerobic microbial load reflects the sanitary status of the product as well as the measures applied during processing and handling of such product.

The mean value of anaerobic bacterial count was $1.61 \times 10^3 \times 0.58 \pm 10^3$ CFU/ gm. These results exceeded the permissible limit (10^2 CFU/gm) recommended by **EOSQC (2005)**. Nearly similar results were recorded by **Mohamed (2002); El- Mossalami (2003) and Hassan and Antown (2005)**. According to **EOSQC (2005)** anaerobic count/ gm in beef burger should not exceed 10^2; in this study 34% of beef burger samples had a higher percentage of anaerobic count exceeding the permissible limit. This might be due to the addition of a plenty of additives to the meat products. The high anaerobic count may play an important role in the spoilage of meat or its products affecting their keeping quality resulting in economic loses **(Smith and Holdman, 1968)**.

The isolation of *C. perfringens* was achieved in 12% of the examined beef burger samples (Table, 2) Higher incidence of *C. perfringens* was obtained by **El. Mahrouk (2007); Skariyachan *et al*. (2010); Kouassi *et al*. (2011); Mohamed and Ebraheem (2011).** Presence of pathogens like *C. perfringens* indicates substandard hygiene during processing, storage and retailing causing risk to consumer. Meat products are generally implicated in most outbreaks, with beef products responsible for about 40% of *C. perfringens* food borne outbreaks **(Bhunia, 2008).**

Clostridium perfringens isolates are commonly classified into five types (A to E) based on the production of four typing toxins (alpha. beta, epsilon and iota toxins). Type A isolates, the most abundant toxinotype, produce alpha toxin **(Immerseel *et al*., 2004).**

Regarding the typing of the isolated *C. perfringens* strains, it was shown that 3 isolates were type A and only one isolate was type D. These results were nearly similar to that obtained by **Abd El. Aziz *et al*. (2011),** while slightly higher incidence were recorded by **Bassiony and Brr (2009)**.

It is of great importance to mention that *C. perfringens* type A has great concern for human being, where the organisms can survive cooking and have ability to produce heat resistant spores constituting a public health hazard **(Brynestad and Granum, 2002)**.

Regarding the virulence to baby mice, the results illustrated in **table (3)** proved that the isolated *C. perfringens* strains type A were positive for virulence to baby mice. Food poisoning due to *C. perfringens* usually occurs 8-24 hr after ingestion of food containing large numbers of vegetative cells. Diarrhea and severe abdominal pain usually occur **(Levin, 2010)**.

The final judgment of the examined beef burger samples showed that the accepted samples were 66% and the rejected samples were only 34% as recorded in Fig (1).

Finally, to obtain high quality beef burger, the following recommendation should be taken:

1. The meat used should be accepted in accordance to **EOSQC (2005)** for beef burger.
2. Strict control of personal hygiene and equipment cleaning.

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التقييم الميكروبيولوجى للبيف برجر فى بعض الاسواق المحلية مع أعطاء الأهمية لميكروب الكلوستريديم بيرفرنجيس

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الملخص العربى

أجريت هذه الدراسة على عدد 50 عينة عشوائية من البيف بيرجر المجمد تم تجميعها من الأسواق المختلفة فى محافظتى القاهرة والجيزة لإستبيان الحالة الصحية لهذا المنتج وتأثيره على صحة المستهلك وقد خضعت العينات للفحوص البكتريولوجية وأظهرت النتائج مايلى:

كان متوسط العد البكتيرى الكلى للميكروبات الهوائية واللاهوائية هو $4.6 \times 10^5 \pm 1.69 \times 10^5$ و $1.61 \times 10^3 \pm 0.58 \times 10^3$ خلية/جم من العينة على التوالى وكانت أعلى من الحدود المسموح بها طبقاً للمواصفات القياسية المصرية 1688 لسنة 2005.

تم عزل ميكروب الكلوستريديم بيرفرنجيس من 12% من العينات المفحوصة و كان 8% من العترات المعزولة لها القدرة على أفراز السموم (أ ، د). و2 عترة (4%) ليس لها القدرة على إفراز السموم.

كانت العينات المرفوضة 34% من أجمالى العينات المفحوصة.