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INCIDENCE OF SOME ENTEROTOXIGENIC FOOD POISONING MICROORGANISMS IN CHICKEN MEAT PRODUCTS

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SUMMARY

Forty - five packaged random samples of cooked and half cooked chicken meat products represented by chicken shawarma, fillet and wings (15 for each) were collected from different retail markets in Cairo and Giza. The incidence of *S. aureus, E. coli* and *C.perfringens* in fillet was 26.66%, 20%, 26.66%, respectively, while equals 0.0%, 13.33%, 20%, in shawarma, respectively. The incidence in wings reaches to 13.33%, 20%, 6.66%, respectively. Six number of *S.aureus* strains were isolated from fillet and wings examined for enterotoxin production and revealed that only one strain isolated from wings had the ability to produce enterotoxin types A, B, C and D (multiproducer). While *S.aureus* failed to be isolated from shawarma. Concerning *C. perfringens* 5 toxigenic strains classified as 3 *C.perfringens* type A and 2 *C. perfringens* type D, while 3 strains were non- toxigenic. For *E. coli* the serological typing revealed 8 untypable strains which were not enterotoxigenic.

INTRODUCTION

Chickens occupy the major role in production and consumption among poultry in Egypt. Chicken meat becomes the second most popular type of meat eaten after red meat. Chicken meat is characterized by ease during preparation consistent quality and the availability of the wide range of pre-packed, branded, raw, ready to eat and serve products (Shedeed, 1999).

Bacterial agents are incriminated in foodborne infection and intoxication outbreaks in industrial and developing countries, which increase day by day (Stevenson and Bernard, 1995).

Study of the different causative bacterial agents of food poisoning is one of the pioneering efforts

to improve poultry industry with the resultant protein gap covering. Determination of types of some microorganisms in most carcasses is important from the stand point of public health for judging effectiveness of sanitary handling during processing (Butler el al., 1979).

The food poisoning organisms of most concern with meat such as enteropathogenic *E. coli* and *C. perfringens* are associated with enteric contamination (ICMSF, 1980), moreover, *C. perfringens* is a very common cause of human foodborne diseases and in most outbreak cases the food involved are cooked meat or poultry products containing high levels of viable cells (Labbe, 1989; Labbe and Harman, 1992).

Beside *E. coli* and *C. perfringens, S. aureus* continues to be a major cause of foodborne intoxication and its presence in food constitutes an important hygienic problem for food processors, food service, workers and consumers (Bergdoll, 1989).

The possible sources of *E. coli* and other pathogens contaminated the ready to eat meat products included inadequate sanitary practices, inadequate heat treatment and the presence of pathogens on different surfaces continuously or occasionally contaminating the finished products (Gibbons et al., 2006).

Thus, this study was conducted to insure in what range we need to apply and control the hygienic measures on chicken meat products along its processing and retailing.

MATERIAL AND METHODS

Samples: A total of 45 random samples of chicken meat products of cooked shawarma and half-cooked fillet and wings packaged units (15 for each) purchased from different retail markets in Giza and Cairo. The packaged of samples transfered in an ice box without delay and under hygienic conditions to the laboratory for the bacteriological examination.

*** Preparation of the samples (APHA, 1992):**
Twenty five grams of food sample were homogenized with 225 ml of sterile buffered peptone water (0.1%) to give a dilution of 1/10. One ml of the clear homogenate was mixed with 9 ml of buffered peptone water (0.1%), then decimal dilutions were prepared.

*** Estimation of *Escherichia coli* count (MPN):**
The most probable number was conducted as recommended by (FAO, 1992), isolation and biochemical identification according to Feng et al. (1998) and serologically according to Cruickshank et al. (1975).

*** *Staphylococcus aureus* count:**
The procedure of FAO (1992) was followed.

*** Enumeration of Clostridia organisms:**
Enumeration of Clostridia organisms was con-

ducted according to ICMSF (1978) and Bergeyís Manual (1986).

*** Isolation of C. perfringens :**

The method was applied according to ICMSF (1978) and identification according to Buchanan and Gibbons (1975).

* Demonstration of *C. perfringens* toxin by dermonecrotic test (Sterne and Batty, 1975).

*** Detection of S. aureus enterotoxins:**

It was done according to Donelly et al. (1967); Oda et al. (1979) and Shingaki et al. (1981) using the SET-RPLA kit for the detection of staphylococcal enterotoxins A, B, C and D.

*** Detection of Enterotoxins of *E. coli* strains:**

Preparation of *E. coli* strains for recovery of enterotoxin according to Evans et al. (1973) and Alderate and Robertson (1977).

*** Detection of heat labile enterotoxin (LT.) among *E. coli* isolates:**

Serologically using Oxoid VET-RPLA kit for detection of *E. coli* heat labile enterotoxin.

*** Detection of heat stable enterotoxin (ST) among *E. coli*** isolates according method by Giannella (1976).

RESULTS

Table (1): Statistical analytical results of isolated microorganisms.

samples / Microorganisms	Fillet*				Shawarma**				Wings*			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
S. aureus	$1x10^2$	$1.1x10^3$	$6x10^2$	2.1x10	-	-	-	-	$2x10^2$	$5x10^2$	-	-
E. coli	2.8x10	$1.5x10^2$	7.4x10	1.3x10	7	2.3x10	-	-	4	75	28.5	4.6
Clostridia	$8x10^2$	$7.6x10^4$	$17.5x10^2$	$1.6x10^2$	$4x10^2$	$3x10^3$	$8.66x10^2$	8.1x10	$1.5x10^2$	$3.9x10^3$	$17.3x10^2$	3.9x10

* Half-cooked ** Cooked

Data represented as mean ± SD

Table (2): Incidence of isolated microorganisms from chicken meat products (n = 15).

samples / Microorganisms	Fillet		Shawarma		Wings	
	No.	%	No.	%	No.	%
S. aureus	4	26.66	0	0	2	13.33
E. coli	3	20.0	2	13.33	3	20.0
C. perfringens	4	26.66	3	20.0	1	6.66

Table (3): Enterotoxin types of isolated *S. aureus*.

Chicken samples	No. of isolated strains	Enterotoxigenic strains	Types of enterotoxins
Fillet*	4	-	-
Shawarma* *	-	-	-
Wings*	2	1	A,B,C,D

Table (4): Typing of *C. perfringens* strains (n =15).

Chicken samples	No. of isolated strains	Toxigenic strains				Non-toxigenic strains	
		A		D			
		No.	%	No.	%	No.	%
Fillet*	4	1	25	1	25	2	50
Shawarma**	3	2	66.6	-	-	1	33.3
Wings*	1	-	-	1	100	-	-

* Half-cooked ** Cooked

DISCUSSION

The revealed results gave a profile about the hygienic and microbiological status of some cooked (shawarma) and half-cooked (fillet and wings) chicken products and showed that these products could harbour the food poisoning microorganisms easily and the achieved results must give more attention to follow up the hygienic rules in the processing, handling and storage of such products.

Table (1) showed that the mean count levels of S. aureus was $6 \times 10^2 \pm 2.1 \times 10$ in fillet, while not detected in shawarma and wings. *E. coli* recorded mean count value/g using (MPN) equals $7.4 \times 10 \pm 1.3 \times 10$ and 28.5 ± 4.6 in each of fillet and wings, respectively. While, the mean count level of clostridia organisms reached to $17.5 \times 10^2 \pm 1.6 \times 10^2$, $8.66 \times 10^2 \pm 8.1 \times 10$ and $17.3 \times 10^2 \pm 3.9 \times 10$ in fillet, shawarma and wings, respectively.

In this regard, Hafez et al. (1987) detected *S. aureus* in chicken meat product (burger) in mean level count of 4.6×10^3 CFU/g, Hefnawy and Moustafa (1990) detected *S.aureus* 1×10^2 in ready to eat chicken products, while Hashim (2003) detected 5×10^2 CFU/g. Shabana and Ouf (2003) found *S. aureus* in chicken shawarma in range mean count of 2.72 - 3.36 log count. Ahmed (2004), Gad (2004), Essa et al. (2004) detected *S. aureus* mean count reached 6.77×10^3, 3.8×10^3, 3.8×10^3 and 1.37×10^4 CFU/g from chicken nuggets, wings, fillet and luncheon, respectively.

For *E.coli* mean count level, Soriano et al. (2000) found *E. coli* from 3 - 2.400 MPN/g, Shabana and Ouf (2003) detected *E. coli* from chicken shawarma in range of 1.94-2.11 log count and Hashim (2003) found coliform 2.9×10^2 in chicken meat product. On the other hand, Warburton et al. (1988) failed to detected *E.coli* from processed poultry products.

Concerning clostridia organisms Osman (2005) detected clostridia in a mean count ranged from 2.3×10^2 - 3.3×10^3 CFU/g. while, *C. perfringens* in count mean range 3×10^2 - 9×10^3 CFU. On the other hand, Wen and McClane (2004) failed to detect *C. perfringens* in chicken product samples.

As shown in Table (2) the incidence of *S. aureus, E. coli* and *C. perfringens* were 26.66%, 20.0% and 26.66% in fillet samples, while in shawarma was 0.0%, 13.33% and 20.0%, respectively. In wings samples the incidence represented in 13.33%, 20% and 6.66% for *S. aureus*, *E. coli* and *C.perfringens*. In this respect, many researchers studied and isolated food poisoning microorganisms in different chicken meat products as; Bryan (1988) found *S. aureus* (8%), and *C. perfringens* (5.2%); Hefnawy and Mosutafa (1990) isolated *S. aureus* and *E. coli* at rate of

20% and 10%, respectively. While, Soriano et al. (2000) found the incidence of *S.aureus* (7.6%) and *E. coli* (8.8%). High incidence (73.6%) recorded by Chang et al. (2001) for *S.aureus*. On the other hand, Girgis (2002) recorded S. aureus (7.5%) and *E. coli* (6.3%). Shaltout (2002) isolated S. aureus and *E. coli* in rate of 18% and 10%, respectively from hot wings. Shabana and Ouf (2003) isolated *S. aureus* from shawarma in 21.4% and *E. coli* (7.1-35.7%). Ahmed (2004) isolated *E. coli* (20%) and (12%) from fillet and wings, respectively. While, Sharma et al. (2005) found *E. coli* (14%) from meat and chicken products. Regarding, *C. perfringens* incidence Miwa et al. (1998) found *C. perfringens* in an average of 37% from meat and poultry samples, Osman (2005) isolated *C. perfringens* in range 10-50% from different chicken meat products, while, Nasr et al. (2007) isolated *C. perfringens* ranging between 8.3 to 14%. In this respect, the flesh of healthy live poultry is sterile but bacteria on the skin surface such as *S. aureus* may contaminate the flesh (Avens and Miller, 1970). On the other hand, Murugkar et al. (1993) studied poultry and meat product samples and he detected faecal coliforms, *S. aureus* and *C. perfringens* in all meat products and added that the levels of coliforms and *S. aureus* were particularly high in all products with conclusion that the presence of such pathogens indicated substandard hygiene practices during processing, storage and retailing.

The results in table (3) illustrated that 2 strains of *S.aureus* isolated from wings samples were examined against enterotoxins production and typing, as a result one strain only (50%) considered enterotoxigenic and could produce enterotoxin A, B, C and D types (multiproducer), while we can't detect any toxigenic *S.aureus* strains from fillet. As previously recorded in the results that shawarma is negative for presence of *S. aureus* and that may be due to exposure of these samples to efficient heat treatment (cooked) and avoid of recontamination after processing and packaging.

In this respect Adesiyun (1984) found 36% of the isolated *S. aureus* strains from chicken were enterotoxigenic, while, Zaki (1998) found 54.16% of *S. aureus* strains were enterotoxigenic isolated from chicken meat. Chang et al. (2001) isolated enterotoxigenic *S. aureus* at rate of 29.64% from chicken meat and the same author found that the enterotoxin type C was predominated. *Staphylococcal* food poisoning is one of the most common types of foodborne illness and results from the ingestion of enterotoxin produced during growth of enterotoxigenic strains of *S. aureus* in food (Anon, 1986). In the absence of competitors *S. aureus* grow better but toxin production appeared to be influenced more by growth temperature than by bacterial competition (Herten et al., 1989).

The direct extraction of examined chicken meat samples (fillet, wings and shawarma) and typing

for presence of *S.aureus* enterotoxins showed negative results (not detected). Sally Rose et al. (1989) reported that the detection of preformed enterotoxins (SETs) is therefore important in epidemiological studies outbreaks food poisoning and in routine quality appraisal monitoring of foods during manufacture. On the other hand, Wieneke et al. (1993) provided that enterotoxins were detected in foods in the absence of viable *S. aureus* and most contamination took place at home followed by restaurant and shops.

S. aureus on chicken cuts insured that there is potential incidence reached to 43.3% (Sliva et al., 2002). Colombari et al. (2007) stated that strains of S. aureus isolated from food and food handlers implicated as the etiologic agent of an outbreak of staphylococcal food poisoning involving 180 people occurred in Brazil, in April, 1998.

Regarding table (4) the results showed the toxins typing of *C. perfringens* strains by I/D inoculation in Guinea pigs as follows in fillet 4 strains isolated were classified as 2 strains toxigenic, one type A (25%) and the second (25%) type D and the other two strains (50%) were non toxogenic. Three strains isolated from shawarma and classified as 2 (66.6%) type A and one (33.3%) was non-toxigenic. Only one strain of *C. perfringens* type D was isolated from wings.

In this respect, Osman (1999) reported that out of 22 strains of *C. perfringens* 17 of them provided enterotoxigenic type A, which is usually involved in food poisoning outbreaks and noticed that all *C. perfringens* recovered from cooked products proved toxigenic and the non-toxigenic were only allocated to the frozen raw products.

By examination of chicken meat products for enterotoxigenicity of *C. perfringens.* Osman (2005) found 23% of the strains provided to be toxigenic while, Nasr et al. (2007) detected 83.9% toxigenic *C. perfringens* strains and typing as 70.8% type A and 12.9% were type D. In this regard, Lin and Labbe (2003) stated that *C. perfringens* is a leading cause of bacterial foodborne illness in countries where consumption of meat and poultry is high, and not all strains of these microorganisms possess the enterotoxigenicity. The toxigenic strains of *C. perfringens* causes pronounced diarrhea and abdominal cramps generally appeared 8-12 hrs after consumption of contaminated food (Abigail and Dixie, 1994), and the epidemiological reports incriminated *C.perfringens* as a source of outbreaks of human food poisoning transmitted through poultry (Mulder, 1997) and (Dalton et al., 2004).

To prevent *C. perfringens* infections cooked beef and poultry meat should be refrigerated promptly and reheated thoroughly (internal temperature 75°C) before serving (Enan, 2006).

Examination of *E.coli* isolated strains (8 strains) for serological identification, the results revealed

untypable strains, in addition the enterotoxigenicity of isolated *E. coli* strains for detection of heat labile (LT) and heat stable (ST) yielding negative results (non-toxigenic). It is benefit to mention that the properity of enterotoxigenicity of *E. coli* is clearly not restricted to enterotoxigenic serotypes (Klipstein et al., 1978), and cooked sample must be free from *E.coli* according to E.S. (2000). On the other hand, Sosa et al. (1988) tested 45 strains of *E. coli* isolated from food for enterotoxigenicity, and found none produced thermolabile toxin (LT), while 4 only produced heat stable toxin (ST). From the public health point the serotyped *E. coli* causes human cases of gastroenteritis, acute infantile diarrhea, sporadic diarrhea in children, food poisoning outbreaks (Foster, 1987).

It could be concluded that the potentially of some enterotoxigenic microorganisms (*S. aureus*, *E. coli* and *C. perfringens*) that may be present or harbour the chicken meat products, so that barriers could be put at the processing lines to minimize the likelihood of such organisms surviving or multiplying to levels which may limit these products acceptability.

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مدى تواجد بعض الميكروبات المفرزة للسموم المعوية فى منتجات لحوم الدجاج

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تم تجميع عدد ٤٥ عينة عشوائية من منتجات لحوم الدجاج المطهية والنصف مطهية متمثلة فى ١٥ عينة من كل من (شاورمة – فيليه – اجنحة). واسفرت النتائج عن تواجد الميكروب المكور العنقودى الذهبى ، الميكروب القولونى ، ميكروب الكلوستردیم بيرفرنجنيس فى عينات الفيليه بنسبة ٢٦,٦٦% ، ٢٠% ، ٢٦,٦٦% على التوالى ؛ بينما عينات الشاورمة سجلت ٠,٠ (صفر%) ، ١٣,٣٣% ، ٢٠% لذات الميكروبات مع ملاحظة أن عينات الشاورمة سالبة لسموم الميكروب المكور العنقودى الذهبى. أما الأجنحة فقد أشارت النتائج أن نسبة التواجد للميكروبات المذكورة بعالية كانت ١٣,٣٧% ، ٢٠% ، ٦,٦٦% على التوالى.

وبتصنيف ٦ عترات عزلت من هذه المنتجات من الميكروب المكور العنقودى الذهبى لتواجد السموم المعوية كانت النتيجة عترة واحدة عزلت من الأجنحة موجبة لإفراز السموم المعوية أنواع (A, B, C and D) وبإختبار ٨ عترات من ميكروب الكلوستردیم بيرفرنجينس لإنتاج السموم كانت النتيجة ٥ عترات موجبة وكانت أنواعها كالآتى : ٣ عترات مفرزة للسم نوع A بينما ٢ عترة للسم نوع D.

وبأجراء الإختبارات السيرولوجية لعترات الميكروب القولونى المعزولة من المنتجات وعددها ٨ عترات تبين أنها (untypable) وبإختبارها لتواجد السموم المعوية LT , ST أعطت نتائج سلبية.

وقد تم مناقشة أهم أسباب تلوث منتجات لحوم الدواجن بهذه الميكروبات المسببة للتسمم الغذائى وأهمية التأكد من تفعيل الشروط الصحية الواجب توافرها لحماية صحة المستهلك وسلامة المنتج.