Bacteriological evaluation of meat and chicken meals served in one of Menufia Governorate hospitals.

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Abstract

Forty random samples (20 cooked chicken meals and 20 cooked meat meals) were collected from one of Menufia governorate hospitals for bacteriological evaluation. The mean value ± SE of Aerobic plate count of cooked chicken meals and cooked meat meals were $1.97x10^4 \pm 1.48x10^4$ and $1.39x10^4 \pm 3.12x10^4$ CFU/gm respectively; the Anaerobic plate count were $3.1x10^3 \pm 2.8x10^3$ and $3.2x10^3 \pm 6.5x10^2$ CFU/ gm, respectively. *Clostridium perfringens* could be isolated from cooked chicken meals with mean value reached $1.6x10^1 \pm 0.8x10^1$ and failed to be detected in cooked meat meals. *Staphylococcus aureus* could be only isolated from cooked chicken meals with incidence 5%. *Salmonella* and *E.coli* could not be isolated from any of the examined samples. The two *Clostridium perfringens* isolates were type A while, the isolated *Staphylococcus aureus* strain were enterotoxigenic type D producer . The public health importance of the examined microorganisms as well as the recommended measures to improve the quality and safety of cooked products, were discussed.

Key words: *cooked meat meals, Clostridium perfringens, Staphylococcus aureus, E. coli*

Introduction

Meat and poultry meals are considered the main dishes served in most hospitals due to their nutritional characteristics which is important to the patients. But unfortunately worldwide, foodborne illness is often associated with consumption of meats and poultry and their products sold at retail markets **(Vindigni *et al*., 2007)**. Raw meat is rarely involved in food poisoning than ready to eat meat or its products **(Cliver, 1990)**.Internationally, food poisoning is becoming a very important health problem. *Salmonella species (spp*) are the most important pathogens but due to the extensive effort carried by the health authorities to prevent or eradicate communicable diseases new pathogens are now emerging such as *Escherichia coli (E.coli)*. Meat and chicken are the main items incriminated in food poisoning accidents **(Al-Mazrou, 2004)**. *Salmonella* is pathogenic bacteria that can contaminate food products during or after processing .Ready-to-eat (RTE)food does not undergo any treatment to ensure its safety before consumption, and therefore risk of foodborne disease must be considered if Salmonella is present in the food **(Cabedo *et al*.,2008).** Salmonella serotypes are among the most common bacterial causes of foodborne gastroenteritis in the United States, associated with approximately 1.4 million human illnesses annually **(Arshad *et al.,* 2007)**. In Egypt, *Escherichia coli* was incriminated in cases of gastroenteritis in children **(Marzouk,1985),** epidemic diarrhea in infants, sporadic summer diarrhea in children as well as cases of food poisoning and traveler's diarrhea **(Pyatkin and Krivoshein, 1980, Levine, 1987** and **Schlager *et al.,* 1990).** In a survey summarized the epidemiology of foodborne disease :outbreak in Australia from 1995 to 2000. Of the 214 outbreaks bacterial disease was responsible for 61% of outbreaks. The most frequent etiology of outbreaks was *Salmonella* in 75(35%) outbreaks and *Clostridium perfringens* in 30 (14%). Outbreaks in hospitals and aged care facilities were responsible for 35% of deaths. The most frequently implicated vehicles in the 173 outbreaks with known vehicles were meats 64 (30%). Chicken, the most frequently implicated meat, was associat-

ed with 27 (13%) outbreaks **(Dalton *et al.*, 2004).** *Clostridium perfringens* is an important anaerobic pathogen causing food-borne gastrointestinal (GI) diseases in human and animals. It is thought that *C.perfringens* food poisoning isolates typically carry the enterotoxin gene (cpe) on their chromosome **(Miki *et al.*, 2008)**. Food poisoning by *Staphylococcus aureus* affects hundreds of thousands of people each year. *Staphylococcus aureus* also causes invasive diseases such as arthritis (in poultry) and septicemia (in poultry and humans). Foodborne disease is caused by the ingestion of Staphylococcal enterotoxin(SE). Enterotoxin has also been associated with other *S.aureus* illness in human and domestic animals **(Hazariwala *et al.*, 2002).** Cross contamination of foodborne pathogens from raw meats to ready -to-eat foods has caused a number of foodborne outbreaks **(Ravishankar *et al.*, 2010).**

So, the aim of this research is to evaluate the bacteriological profile of cooked chicken and meat meals served in one of Menoufia hospitals**.**

Material and Methods

A total of forty random samples (20 cooked chicken meals and 20 cooked meat meals) were collected from one of Menufia governorate hospitals. Each sample was kept in separate sterile plastic bag and preserved in ice box then transferred to the laboratory under complete aseptic conditions without delay and examined bacteriologically. All the collected samples are subjected to the following examinations

Preparation of food homogenate: (APHA, 2001)

Twenty five grams of each samples were aseptically taken and mixed with 225 ml sterile peptone water (0.1%) by using a stomacher, then serial dilution prepared up to 10^6 then subjected to the following bacteriological examinations

Total aerobic count: (APHA, 2001).

From each dilution one ml of the food homogenate was transferred, by using a sterile pipette into two separate sterile melted and tempered plates of plate count agar (45 ºC). The inoculated plates were gently shaken in rotatory movement and lift till complete solidification of the agar. The plates were incubated for 24-48 hours at 37 ºC

Total anaerobic bacterial count: (APHA, 2001).

Results and Discussion

Table (1). Statistically analytical results of aerobic bacterial count of examined chicken and meat meals samples (n=20).

Count of C.F.U/g.	Samples					
	Chicken meals			Meat meals		
	Positive samples		Mean±S.E.	Positive samples		Mean±S.E.
	No.	%		No.	%	
Aerobic plate count	20	100	$1.97x10^4 \pm 1.48x10^4$	20	100	$1.39x10^4 \pm 3.12x10^4$
anaerobic plate count	7	35	$3.1x10^3 \pm 2.8x10^3$	5	25	$3.2x10^3 \pm 6.5x10^2$
C. perfringens count	2	10	$1.6x10^1 \pm 0.8x10^1$	----	----	----------
Staph. aureus count	1	5	--------	-----	----	----------

Table (2). Incidence of isolated food poisoning microorganisms in examined samples (n=20).

Samples	*Staph.aureus*		Salmonella		*E.coli*		*C.perfringens*	
	+ve samples		+ve samples		+ve samples		+ve samples	
	No.	%	No.	%	No.	%	No.	%
Chicken meals	1	5	0	0	0	0	2	10
Meat meals	0	0	0	0	0	0	0	0

Table (3). Typing of *C. perfringens* and *Staph. aureus* from chicken meals samples (n=20).

Toxigenic isolates of *C.perfringens*				*Staph. aureus* type D enterotoxin	
Type A		Type D			
No.	%	No.	%	No.	%
2	100	0	0	1	100

The results recorded in Table (1) showed that the mean aerobic plate count was $1.97x10^4 \pm 1.48x10^4$ and $1.39x10^4 \pm 3.12x10^4$ CFU/gm for the examined cooked chicken and meat meals, respectively. Nearly similar results were reported by **Arab (2010)** who recorded $6.3x10^4 \pm 3.5x10^4$/g as a mean value of APC of cooked chicken meal while **El-Kewaiey (2012)** reported the mean value of **APC** as $8.2x10^4 \pm 1.2x10^4$ for chicken nuggets samples and $1.2x10^4 \pm 5.9x10^3$ for chicken luncheon samples. Higher results were obtained by **Elwi (1994)** who recorded that the average **APC** for cooked chicken meat was $5.6x10^5$, in the respect **Hashim (2003)** stated that the mean value of **APC** of ready-to-eat chicken was $1.1x10^5 \pm 5.2x10^3$ and **Ahmed (2004)** who scored $6.5x10^6 \pm 1.12x10^6$ and $3.72x10^6 \pm 0.93x10^6$ as a mean values of **APC** of chicken nuggets and hot wings, respectively. On the other hand lower results recorded by **Abd El-Fattah and Fath El-bab (2005)** who mentioned that the mean value of APC of ready-to-eat nuggets and fajitas was $3.21x10^3 \pm 1.2x10^2$ and $4.24x10^3 \pm 2.1x10^2$ CFU/gm, respectively. Also **El-Taher (2009)** reported that the average APC of cooked chicken was 9.05×10^3.

Also from Table (1) similar result was recorded by **Mohamed (2000)** who stated that the mean APC of meat products was $1.3x10^4$. On the other hand lower results were recorded by **Marouf (1989)**, **El-Taher (2009)** and **Arab (2010)** who recorded $1.7x10^3$, $4.21x10^3$ and $3.85x10^3$ as a mean value of **APC** of cooked meat. While higher results were reported by **Lotfi *et al.* (1990)**, **Elwi (1994)**, **Edris and Mohammed (1995)** and **Ahmed (2004)** they scored $2.1x10^7$, $6.4x10^5$, $3.48x10^5$and $2.18x10^6 \pm 0.81x10^6$ as a mean value of **APC** of cooked meat meals. The high **APC** of some examined cooked meat and chicken samples may be attributed to neglected sanitary measures during their processing, handling and serving of such samples. Furthermore, the high storage temperature of the retail level is probable one of the principle contributing factors to high counts particularly when the initial bacterial load is already high **(El-Khatieb, 1982).**

From the obtained results, cooking cannot destroy all microorganisms; therefore, the holding of cooked foods at ambient temperature for several hours is the primary contributory factor for the growth and multiplication of such organisms **(Bryan *et al.*, 1997).** The temperature of cooking is considered to be adequate to inactivate most of the cells on the chicken surface at the time of cooking. However, it is hypothesized that a percentage of bacteria (16%)

present on a chicken are in areas that do not receive sufficient heating **(FAO/WHO, 2002).** Also the spoilage bacteria can multiply very quickly if the cooked food is stored at room temperature **(WHO, 2003).** The cooking of food of animal origin is the most effective operational step for reducing or eliminating biological contamination. Cooking at proper temperatures for a specified time will kill most harmful bacteria and parasites. Therefore, frequent monitoring of cooking temperature is highly recommended **(USFDA, 2006).**

It is evident from the results given in Table (1) that the mean value of anaerobic count of the examined chicken and meat meals were $3.1x10^3 \pm 2.8x10^3$ CFU/gm and incidence reach 35% for the examined cooked chicken meals. Examined cooked meat meals showed mean value of $3.2x10^3 \pm 6.5x10^2$CFU/ gm with incidence reach 25%.

The present results explained that the anaerobic plate count for the examined cooked chicken and meat samples nearly agreed with **(Abu-Zid, 1998)** who recorded $7.4x10^3 \pm 3.8x10^3$, $10^3 \pm 3.9x10^2$,$1.5x10^4 \pm 6.1x10^3$, $5.5x10^2 \pm 2.4x10^2$, $1.3x10^3 \pm 5x10^2$ and $5.8x10^3 \pm 3.4x10^3$ as a mean value of anaerobic counts for different meat products. While lower results were recorded by **Hashim (2003)** who mentioned that the anaerobic count of different examined ready-to-eat of meat and chicken origin varied from 1.9x10 to $8.9x10^2$ with mean value $1.7x10^2 \pm 1.2x10^2$ for ready-to-eat meat products and 1.6x10 to $2.0x10^2$ with mean value $1.0x10^2 \pm 5.1$ for ready-to-eat chicken products and **Abd El-Fattah and Fath El-bab (2005)** they stated that the mean value of anaerobic bacterial count for ready to eat chicken products (nuggets and fajitas) were less than 10^2 and $0.03 \times 10^2 \pm 0.009 \times 10^2$ CFU/gm, respectively.

High anaerobic count of both cooked chicken and meat samples may be attributed to bad quality of raw meat used, sanitary conditions practiced during preparation and the temperature at time of storage **(Wyatt and Guy, 1980).** In addition to bad microbiological status of additives used during cooking as spices and common salt which are common sources of microbial contamination **(Bauer *et al.,* 1981 and Bernard *et al.,* 1982).**

Tables (1) and (3) explained the incidence of *Clostridium perfringens* in both cooked chicken and meat meals in which 10% of cooked chicken meals showed positive result with mean value reached $1.6x10^1 \pm 0.8x10^1$ and the isolated strain was toxogenic strains type A while *Clostridium perfringens* failed to be detected in cooked meat meals samples.This agreed with **Huerta *et al* (1987)** and **Ezz-Eldin (1992)** who could isolate *C.perfringens* from cooked chicken samples. **Kokubo *et al* (1986)** and **El Naenaeey(1989)**who detected *C. perfringens* in cooked meat products. Also **Edris *et al* (1992)** could isolate both *C.perfringens* type A and D from meat products and both toxigenic and non toxigenic strains of *C. perfringens* could be isolated from meat products by **Abu-Zeid (1998). El-Kateib *et al* (1988)** and **Hashim (2003)** could detect *C. perfringens* in both cooked and ready-to-eat chicken and meat products. *C.perfringens* food poisoning occurred as the failure of efforts done to prevent cross contamination, and to maintain improper control of temperature **(Hashim, 2003). Fapohunda *et al* (1994)** mentioned that *C.perfringens* has a high potential to achieve high numbers in the food if cross contamination of the organism occurs during handling of raw meats. *C.perfringens* type A food poisoning is one of the more common in the industrialized world. This bacterium is also responsible for the rare but severs food borne necrotic enteritis. *C.perfringens* enterotoxin (CPE) has been shown to be the virulence factor responsible for causing the symptoms of *C.perfringens* type A food poisoning **(Brynestad and Granum, 2000).**

From Tables (1) and (3) the incidence of *Staphylococcus aureus* in the examined cooked chicken meals was 5% (only one sample with *S.aureus* count $1x10^3$) but this microorganism could not be isolated from the examined cooked meat meals. The isolated *Staphylococcus aureus* from examined cooked chicken meals was type D. This agrees with **Abd El-**

Fattah and Fath El-bab (2005) and **El-Kewaiey (2012)** who all isolated *Staph.aureus* from ready-to-eat chicken products. On the other hand **Youssef *et al* (1986)** failed to detect coagulase positive *Staphylococci* in the examined cooked meat products. While, **Shalaby and Zaki (2008)** detected enterotoxigenic strains types A, AB, AD and ABCD in different ready-to-eat meat sandwiches. Type A enterotoxin was the most frequently in all the examined samples followed by enterotoxin type D.

From the present *Staph.aureus* produce type D enterotoxin was isolated from the examined cooked chicken meals indicating that cooking usually give time for temperature exposures that would have been lethal for vegetative form of food borne pathogens. On the other hand, holding of food provide time for temperature exposures conductive to microbial growth, particularly in food holds overnight and large populations of aerobic organisms including *S.aureus* and others were recovered from these food. So, time temperature had variable effect on killing the microorganisms but heat stable toxins still not affected **(Pepe *et al.,* 2006).**

Food poisoning caused by *Staph.aureus* through the production of one or more heat stable extracellular toxins, responsible for the symptoms of the disease, time of onset and severity of symptoms depend on the amount of toxin consumed and individual susceptibility. The enterotoxins produced at detectable levels (>0.1ng/gm) in foods occur only when growth reaches approximately 10^6CFU/gm **(Robbins *et al.,* 1974** and **Evenson *et al*., 1988).** *Staph. aureus* plays a great role in bacterial contamination of cooked food. The workers during preparation process may touch cooked foods that are usually eaten without further cooking or heating **(Ahmed, 1991).**

Table (2) summarized the results of isolated food poisoning microorganisms in which declared that *Staph.aureus* and *Cl.perfringens* could be isolated from the examined cooked chicken meals while these microorganisms failed to be detected in the examined cooked meat meals. *Salmonella* and *E.coli* could not be detected either in the examined cooked chicken meals or in the examined cooked meat meals. This agree with **Abd El-Fattah and Fath El-bab (2005)** who failed to isolate Salmonellae from ready-to-eat chicken products. Also **El-Kewaiey (2012)** could not isolate *E.coli* or *Salmonellae* from ready-to-eat chicken products. This disagree with **Doyle and Schoeni (1987)** who could isolate *E.coli* from both meat and poultry samples, **Vonkate Swaran *et al* (1989)** isolated *Salmonellae* from both chicken and meat samples and **Hefnawy and Moustafa (1990)** could detect *E.coli* in 10% of the examined ready-to-eat poultry samples but all examined samples were free from *Salmonella* organisms. **Hashim (2003)** could detect *E.coli* (the true faecal type) in both ready-to-eat chicken and meat products while Salmonellae failed to be detected in both products. On the other hand **Arab (2010)** detected *E.coli* in cooked chicken meals and were could not detect in cooked meat meals. Salmonellae could not be detected either from cooked chicken meals or from cooked meat meals.

In the present study, *E.coli* and *Salmonella* failed to be detected. This may be attributed to the fact that most pathogenic bacteria are destroyed between 72^{o}C to 83^{o}C, so the cooking method should be effectively applied to produce a temperature sufficient to kill all these pathogens **(Murphy *et al.,* 2001).**

Conclusion

We can conclude that, faulty meat handling, poor personnel hygiene, inadequate cleaning methods and lack of facilities for the segregation of raw and cooked meat resulted in a significant increase in the occurrence of food borne illness. So we can summarized the safe food preparation in the ten golden rules drawn by WHO as follows:-

-Choose foods processed for safety.
-Cook food thoroughly.
-Eat cooked foods immediately.
-Store cooked foods properly.
-Reheated cooked food thoroughly.
-Avoid contact between raw foods and cooked foods.
-Wash hands repeatedly.

-Keep all kitchen surfaces clean.
-Protect foods from insects, rodents and other animals.
-Use pure water.

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التقييم البكتريولوجى لوجبات اللحوم و الدواجن المقدمة باحدى المستشفيات بمحافظة المنوفية.

داليا عاطف سالم ، إسلام زكريا ، طاهر عبد الحميد

الملخص العربى

أجريت هذه الدراسة لتقييم الحالة البكتريولوجية لوجبات اللحوم والدواجن التى تقدم باحدى المستشفيات بمحافظة المنوفية وتمت الدراسة على 40 عينة من اللحوم و الدواجن المطهية. أظهرت نتائج الفحص البكتريولوجى أن متوسط العدد الكلى للبكتريا الهوائية لكل من الدواجن واللحم المطهية تمثل $1,97 \times 10^4 \pm 1,48 \times 10^4$ و $1,39 \times 10^4 \pm 3,12 \times 10^4$ على التوالى. كما كان متوسط العدد الكلى للبكتريا اللاهوائية $1,3 \times 10^3 \pm 2,8 \times 10^3$ للدواجن المطهية كما كان $2,3 \times 10^3 \pm 5,6 \times 10^2$ للحوم المطهية . أمكن عزل كلا من الكلوستريديم برفرنجيز و الميكروب العنقودى الذهبى من الدواجن المطهية ولم يتم عزلاهما من اللحوم المطهية .لم يتم عزل ميكروب السالمونيلا و الآى كولى من أى من الدواجن المطهية و اللحوم المطهية . كما تم مناقشة الخطورة الصحية للميكروبات المعزولة .