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DETECTION OF MULTI DRUG RESISTANT FOOD BORN BACTERIA IN READY TO EAT CHICKEN MEAT

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ABSTRACT

One hundred fifty samples were read to eat chicken meaty collected from different restaurants in Luxor city to evaluate bacteriological quality RTE chicken meat. The mean values of *APC*, *Coliform*, *Staphylococcus counts*, *Anaerobic count*, *Yeast count*, *Mould count* (cfu /g). $1.36X10^4 \pm 2.94X10^3$&$8.42x10^2 \pm 3.90x10^2$,4.67±1.15&1.00±0.73&7.67±2.23,2.67±1.26 to chicken shawirma without addition, $5.58X10^3 \pm 1.16X10^3$&$3.34x10^2 \pm 9.82x10$&2.33 ±1.24&1.00±0.75&8.33±2.40&2.67±1.26 for chicken burger without addition and $4.62X10^3 \pm 7.42X10^2$&$1.53x10^2 \pm 4.85x10$&1.33±0.79&3.00±1.37&4.33±1.64,1.67±0.84 for fried chicken, all samples not detected for *Shigella*, respectively.9 *E.coli* (6%) were isolated from samples represented 4(8%),(2)O125:H2,(1)O143:H8and (1)O111:H1from chicken shawirma without addition, 3(6%) O55: H2, O158:H3and O86a:H1 from chicken burger without addition, 2 (4%) O142:H3, O26:H1from fried chicken, Also, 8(5.33%) Coagulase positive *Staphylococcus aureus* were isolated from samples. Represented as 4(8%) isolate from chicken shawirma without addition, 3 (6%) from chicken burger without addition, 1(2%) from fried chicken. 6(4%) of *Salmonella* were isolated from Samples represented as 2(4%) *S. typhimurium* and 1(2%) *S. enteritidis* from shawirma without addition, 1(2%)*S. enteritidis* and 1(2%) *S. Anatum* from chicken burger without addition, 1(2%) *S.Kentucky* from fried chicken. Isolates. Most of *E.coli* isolate sensitive for Colistin sulphate(10ug) and Nalidixic acid(10ug). *St.aureus* isolates sensitive to Ampicillin (10ug) and Vancomycin (15ug). Most of *Salmonella* isolate sensitive for Gentamycin (10ug), Colistin Sulphate(10ug) and Ceftriaxone.

Key words: Multi Drug resistant; Food-borne bacteria; ready to eat Chicken Meat.

INTRODUCTION

Foodborne illness is major health problem associated with ready to eat chicken meat (Tabashsum *et al.*, 2013). Multidrug resistant food borne microorganisms made the food safety situation more vulnerable in public health (Ahmed *et al.*, 2011). Contamination of ready-to-eat foods persists through preparation and cooking due to the quality of raw materials (Dhama *et al.*, 2013). *Staphylococcus spp*. contamination of excessive handling during preparation of the meal (Mensah *et al*., 2002). Foodborne illness is one of the significant public health

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problems worldwide Hereford, microbiological safety of food has become an important concern for consumers, various industries, and regulatory agencies (Bavisetty *et al.,* 2018). Some microorganisms maintain their normal life functions in food and are used in food production, whereas others may cause food spoilage or foodborne diseases. as *Salmonella spp*., *Campylobacter spp*., *Staphylococcus aureus, Escherichia coli* and S*higella spp.* (Saglam and Seker 2016). Approximately 1.8 million people died due to food-borne diarrheal infections each year in developing countries (WHO, 2007).Using of raw ingredients, insufficient workers hygiene, holding for long period, lead to contamination of food with pathogenic microorganisms (Gundogan *et al*., 2005). *E. coli* has become recognized as a serious foodborne pathogen that associated with diseases outbreaks including diarrhea, hemorrhagic colitis and the life-threatening hemolytic uremic syndrome in humans (Hussein, 2007). Consumption of ready-to-eat products is thought to be the major cause of the *Salmonella* outbreaks (Thai *et al.,* 2012). *Shigella dysentriae* contaminated water, food, and unhygienic environment like overpopulated area, malnourished people, and poor waste management area, usually it survives poorly outside human body (Wilson *et al.,* 2005). *Mould* and mycotoxin contamination of food by feed ingredients (Trenholm *et al.,* 2000). Bacteria and fungi are associated with Chicken meat spoilage acquired during slaughter, packaging, transportation or selling and handling include species of *Salmonella, Shigella, Staphylococcus, Escherichia coli, Campylobacter, Penicillium and Cladosporin* (Clarence *et al.,* 2009). Antibiotics are used for control and treatment of bacterial diseases in poultry. There is growing scientific evidence that use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Van Looveren *et al.,* 2001). Recent reports have shown that different types of food and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-producing animals (Anderson *et al.,* 2003). Annual cost of treating infections caused by antibiotic-resistant bacteria is estimated to be $4 to $5 billion (McGowan 2001). Therefore, the present study was carried out for determination of *APC, Coliform* count & *Staphylococcus counts , Anaerobic count*, *Yeast count, Mould count* and identification of *St.aureus, entero pathogenic E. coli, Salmonella, Shigella* for ready to chicken eat meat meals including chicken shawirma, chicken burger without addition, and fried chicken

MATERIAL AND METHODS

1. Collection and transportation of samples:

A total of 150 random samples of ready to eat chicken meat meals including, chicken shawirma, chicken burger each of them without addition, and fried chicken. (50 of each of product) were collected from different restaurants in Luxor City. Each sample was kept in a separate sterile plastic bag, put in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay for bacteriological examination of samples.

2. Preparation of samples (APHA 1992):

Twenty five grams of the samples were taken under aseptic condition to sterile stomacher bag then 225 ml sterile Buffer peptone water were added, the contents were homogenized at Stomacher for 2minutes, the mixture was allowed to settled, for 5 minutes at room temperature. The contents were transferred into sterile flask, thoroughly mixed, 1 ml was transferred into separate sterile test tube containing 9 ml sterile slain, from which ten fold serial dilutions were prepared.

3. Isolation and identification of food-borne bacteria:

The prepared samples were subjected to the following bacteriological examination: Determination of *Aerobic Plate Count* using ISO 4833-1:2013. *Coliform count* using ISO 4832\2006. Determination of total anaerobic bacterial count by Roberts *et al.*, 1995. Determinations of *Staphylococci* count using ISO 6888-1-1999. Enumeration of *yeast* and *Mould* at 25˚C for 5-7 days using ISO 21527-2:2008. Isolation of *E.coli* by ISO, 2001 and Varnam-Evans, 1991. The method for detection of *S.aureus* by ISO 6888-1-1999. The method for detection of *Salmonella* by ISO 65 79, 2002. The method for detection of *Shigella* using ISO 21567: 2004

4. Identified serologically by using diagnostic sera:

Serological diagnosis of *Salmonella* according to Kauffman – white scheme – 1974 for determination of somatic antigen(O) and Flagler antigen(H) using DENKA SEIKEN CO®Japan). (Sero diagnosis of *E.coli* according to

5. Antibiotic sensitivity:

Antibiotic sensitivity tests were done by using disc diffusion test following the method described by (WHO and CDC 2002). Were interpreted with the standard diameters of (NCCLS 2002) and were recorded as sensitive (S), intermediate (I) and resistant (R). The following antibiotics will be used for disc diffusion test: Antibiotics will be used in *Salmonella, E.coli, Shigella* Amoxicillin-Streptomycin-Gentamycin-Nalidixic-Colistin sulphat - Ceftriaxaon Ciprofloxacin- Neomycin-Sulphamethazol. Antibiotic used in *Staph.* Ampicillin- Clindamycin- Oxacillin - Erythromycin- Gentamicin- Vancomycin-Tetracycline-Chloramphenicol.

RESULTS

Table 1: Statistical analytical results of shawirma without addition in the examined samples (n=50).

	APC	*Coliform Count*	*Staph. Count*	*Anaerobic bacteria*	*yeast Count*	*Mould Count*
Minimum	$2.40X10^3$	≤10	≤10	≤10	≤10	≤10
Maximum	$7.80X10^4$	$8.30X10^3$	2.00X10	2.00X10	4.00X10	3.00X10
Mean	$1.36X10^4$	$8.42X10^2$	4.67	1.00	7.67	2.67
SE	$2.94X10^3$	$3.90X10^2$	1.15	0.73	2.23	1.26

Table 2: Statistical analytical results of burger without addition in the examined samples (n=50).

	APC	*Coliform Count*	*Staph. Count*	*Anaerobic bacteria*	*yeast Count*	*Mould Count*
Minimum	≤10	≤10	≤10	≤10	≤10	≤10
Maximum	$3.50X10^4$	$2.50X10^3$	3.00X10	2.00X10	5.00X10	3.00X10
Mean	$5.58X10^3$	$3.34X10^2$	2.33	1.00	8.33	2.67
SE	$1.16X10^3$	9.82X10	1.24	0.75	2.40	1.26

Table 3: Statistical analytical results of fried chicken in the examined samples (n=50).

	APC	Coliform Count	Staph. Count	Anaerobic bacteria	yeast Count	Mould count
Minimum	≤10	≤10	≤10	≤10	≤10	≤10
Maximum	$1.50X10^4$	$8.30X10^2$	2.00X10	3.00X10	3.00X10	2.00X10
Mean	$4.62X10^3$	$1.53X10^2$	1.33	3.00	4.33	1.67
SE	$7.42X10^2$	4.85X10	0.79	1.37	1.64	0.84

Table 4: The incidence of *Staph, E. coli and Salmonella* in samples.

Samples	**Number of samples**	***St.aureus* Isolates 8(5.33%)**	***E. col* isolates 9(6%)**	***Salmonella* isolates 6(4%)**
chicken shawirma	50	4(8%)	4(8%)	3(6%)
chicken burger	50	3 (6%)	3(6%)	2(4%)
fried chicken	50	1(2%)	2(4%)	1(2%)

Table 5: Serological identification of entero pathogenic *E.coli in* Samples.

Samples	***Serotyping of E.coli***	**%**
chicken shawirma	O125 :H2, O143 :H8 O111: H1	8%
chicken burger	O55: H2 ,O158:H3 O86a :H1	6%
Fried chicken	O142:H3 ,O26 :H1	4%

Table 6: Serological identification of *Salmonella in* Samples.

Samples	**Identified Strains**	**Group**	**Antigenic structure**	
			O	H
chicken shawirma	*Salmonella Typhimurium*	*B*	*1,4,5,12*	*i : 1,2*
Fried chicken	*Salmonella Kentucky*	C3	8,20	i : Z6
chicken burger shawirma	*Salmonella Enteritidis*	D1	1,9,12	g,m
chicken burger	*Salmonella Anatum*	E1	3,10	e,h;1,2

Table 7: Show the result of sensitivity test for *Salmonella* spp.

Antibiotic disc	***Salmonella Typhimurium***			***Salmonella Kentucky***			***Salmonella Enteritidis***			***Salmonella Anatum***		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Gentamycin (10ug)	S			S			S			S		
Neomycin (30ug)	S					R	S					R
Streptomycin(10mcg)			R			R		I				R
Amoxicillin(25ug)			R			R	S					R
Sulphamethoxazole(25)			R			R	S					R
Colistin sulphate(10ug)	S			S			S			S		
Nalidixic			R		I				R		I	
Ciprofloxacin(5ug)	S					R	S					R
Ceftriaxaon	S					R	S			S		

Most isolate are sensitive for these antibiotic Gentamycin (10ug), Colistin sulphate (10ug)and Ceftriaxaon but Streptomycin is(R)and Nalidixic (I)

Table 8: Show the result of sensitivity test for *E.coli in* Samples.

E.Coli Isolates	O142:H3			O158:H3			O86a:H1			O26:H1			O55:H7			O125:H2			O143:H8		
Antibiotic disc	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Gentamycin(10ug)	S			S			S				I		S					R	S		
Neomycin(30ug)			R			R			R			R			R			R			**R**
Streptomycin(10ug)			R		I				R			R			R			R			**R**
Amoxicillin(25ug)		I				R			R			R		I				R			**R**
Sulphamethoxazole (25ug	S					R			R			R			R	S					**R**
Colistin sulphate(10ug)	S					R	S			S			S			S			S		
Ciprofloxacin(5ug)	S					R			R			R			R	S					**R**
Nalidixic acid(10ug)	S				I		S			S			S					R	S		
Cefirioxacin	S					S		I				R	S			S					**R**

Most of *E.coli* isolate sensitive for Colistin sulphate(10ug) and Nalidixic acid(10ug)

Table 9: Show the result of sensitivity test for *Staphylococcus* aureus *in* Samples.

Antibiotic disc	***1***			***2***			***3***			***4***		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Erythromycin E (15ug)	S			S				I		S		
Vancomycin VA (15ug)	S			S			S			S		
Tetracycline T(30ug)			R			R			R			R
Ampicillin AM (10ug)	S			S			S				I	
Oxacillin OX(1ug)			R			R			R			R
Gentamycin *Cn (10ug)*			R			R			R		I	
Clindamycin*(10ug)*			R			R			R			R
Chlroamphenicol(30ug)			R			R			R			R

Most of isolate are sensitive to Ampicillin AM (10ug), Erythromycin (15 ug) and Vancomycin VA (15ug) and Gentamycin, Clindamycin and Chloramphenicol are (R)

DISCUSSION

The total aerobic plate count is important for evaluation of sanitary condition of ready to eat chicken meat. Limits suggested for total aerobic bacterial count in ready to eat chicken meat range not less than 10^4 microbes /g (ISO 4833-1:2013). Accordingly the high bacterial count of some examined samples may be attributed to neglected sanitary measures during their handling, preparation and serving. The mean values of *APC* for chicken shawirma without addition, burger without addition and fried chicken were $1.36X10^2$ (cfu/g), $5.58X10^3$(cfu/g) and $4.62X10^3$(cfu/g).These results were Nearly (El Taher-Amna 2009) who found that *APC* 5.38×10^3 (cfu/g) from burger. The mean values of *Coliform* for chicken shawirma without addition, burger without addition and fried chicken were $8.42X10^2$ (cfu/g), $3.34X10^2$ (cfu/g), $1.53X10^2$ (cfu/g). The current results lower than (El-Rayes 2008) who found that the mean value of Coliform was 3.8×10^3/g in ready to eat chicken meat and agree with (Saad *et al*., 2011) who found that the mean value of Coliform was 5.17×10^2 in the examined samples. The mean values of *Staph Count* for chicken shawirma without addition, burger without addition and fried chicken were 4.67 (cfu/g), 2.33 (cfu/g), 1.33 (cfu/g). The low incidence of *Staph.aureus* attributed to exposure of those products to high temperature during processing (Amal, 2004). The mean values of *Yeast and Mould* for chicken shawirma without addition, burger without addition and fried chicken were 7.67, 2.67 (cfu/g) and 8.33, 2.67 (cfu/g) and 4.33, 1.67 (cfu/g). respectively Mould and yeast contamination usually occurred due to handling, deboning, processing, packing and washing with

polluted water and may due to dust, flies, air, workers, equipment and fluctuation of temperature during transportation and storage(Farghaly, 1998). *Salmonella* is responsible for disease in humans. It has been the leading cause of many outbreaks and infections around the world and is considered as one of the major causes of human gastroenteritis (Rasschaert *et al.,* 2005)**.**In these result isolates were 6 (4%) *Salmonella* from 150 examined ready to eat chicken meat samples. The serological examination of identified *Salmonella* isolates showed that *S. typhimurium, S. enteritidis*, *S. Anatum* and *S.Kentucky* were detected. These results was higher than (Al-Mutairi 2011)and less than(Shaltout *et al.*, 2013) and (Harakeh *et al*., 2005) which reported prevalence of *Salmonella* 7.4 %. Most isolate of *Salmonellae* are sensitive for Gentamycin (10ug), Colistin sulphate (10ug), Ceftriaxone which agree with (Gomba *et al.,* 2016), (Lamas *et al.,* 2016) and (Arora *et al.,* 2015) and dis agree with (Stopforth *et al.,* 2006) who said most of isolate of *Salmonellae* are resist for Ceftriaxone. The presence of *Staph aureus* in heat treated food may be due to its contamination from food handlers, inadequate cleaned equipment or post processing contamination (Duffy *et al.,* 2000). 8(5.33%) isolates of *St.aureus* were isolated from 150 examined these results was higher than those obtained by (Shafizi *et al.*, 2016) who recorded *Staph. aureus* was 2.3% and less than (Ali-Sohaila and Abd-Elaziz-Doaa, 2011) who recorded *Staph. aureus* was 30% and agree with, (Diaz-Lopes *et al*., 2011) who recorded 6.3% of *Staph. aureus*. Cooking plays a great role in killing of most of these microorganisms but not all. Presences of heat resistance toxins from some of these bacteria represent a great public health hazard especially in places with great groups of people receiving this food. Also, post cooking recontamination when holding of such meals for a period until serving in unhygienic condition especially at room temperature or insufficient reheating represent of major public health hazard. Food handlers are important source of *Staphylococcal* Spp for food contamination in restaurants and food outlets (Colombari *et al.,* 2007). As a result there is an increased risk of pathogens surviving and transferring not only by cross-contamination, but also by undercooking as observed in this kind of fast-food industry (Nimri-Laila *et al.,* 2014). Most of isolate sensitive for Colistin sulphate and Nalidixic acid. The result agree with (Abdul Matin *et al.,* 2017). *E.coli* play an important role as human pathogens, which give rise to gastroenteritis outbreaks, severe diarrhea in infants, coli-bacillosis in adults, meningitis and enteritis (Youssef *et al.,* 1992). 9(6%) isolates of *E.coli* were isolated from 150 examined ready to eat chicken meat samples. O125:H2, O143:H8 and O111:H1 from chicken shawirma without addition, O55: H2, O158:H3, O86a:H1 from chicken burger without addition, O142:H3, O26:H1. Isolates were positive for fried chicken. These results agreement with (Mohammed *et al.,* 2014), O55:H2 these results nearly in agreement with, (DíazSánchez *et al.* 2012). Most isolate of *E.coli* were sensitive for Colistin sulphate (10ug) which agree with (El-Sukhon *et al.,* 2002) and dis agree with (Obeng *et al*., 2012) who said most of isolate of *E.coli* are resist for Nalidixic acid.

CONCLUSION

This study indicated that RTE Chicken meats are often contaminated with APC, *Coliform*, *Staphylococcus, Yeast,* Mould *Counts and Staph. aureus, Salmonella* and *E.c*oli. There is need for the relevant local authorities to ensure that the food sold to consumers in fast food restaurants is safe, wholesome and fit for human consumption in order to prevent outbreaks of food-borne illnesses. Addition of vegetables lead to increase contamination and count of microorganism, so the samples without addition of vegetables low in contamination and count of microorganism, Shawirma is more contaminated product because low in cooking. Cooking plays a great role in

killing of most of these microorganisms but not all. After Cooking may occur recontamination when holding of such meals for a period until serving in unhygienic condition especially at room temperature or insufficient reheating represent of major public health hazard. Food handlers, Utensils, bad Quality packaging and bad environment are important source of the contamination There should be In addition Procurement of raw materials of the best possible microbiological quality, Prevention of undue contamination of fast foods prior to processing, Regular training to food handlers in all aspects of food hygiene and safety, Quality packaging to keep foods fresh and get rid of health risk factors, Adequate storage, ideal transportation, and hygienic handling to finished product, Microwave oven treatment prior to serving to consumption, Ensuring good quality raw materials, adequate lethality treatment, and effective sanitation of both the equipment and processing environment.

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الكشف عن البكتيريا المقاومة للمضادات الحيوية في لحوم الدواجن الجاهزة للاستهلاك

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تم تجمع مائة وخمسين عينة عشوائية من لحوم الدواجن الجاهزة للاستهلاك من مطاعم مختلفة في مدينة الأقصر لتقييم للفحص البكتريولوجي وكانت القيم المتوسطة للعد الكلي للبكتيريا الهوائية والمجموعة القولونية وعدد المكور العنقودي الذهبي و العد الكلي للبكتيريا اللاهوائية المتجر ثمة و النموات الفطرية الظاهرية بنسبة ١,٣٦x١٠^٤ ±٢,٩٤ x١٠^٣ ،٨,٤٢x١٠^٢ ±٣,٩٠ x١٠^٢ ،٤,٦٧ ±١,١٥ ،١,٠٠ ±٠,٧٣ ،٧,٦٧ ±٢,٢٣ و٢,٦٧ ±١,٢٦للشاورما الدجاج بدون إضافة و٥,٥٨x١٠^٣ ±١,١٦ x١٠^٣ ،٣,٣٤x١٠^٣ ±٩,٨٢ x١٠ ،٢,٣٣ ±١,٢٤ و١,٠٠ ±٠,٧٥ ،٨,٣٣ ±٢,٤٠ و٢,٦٧± ١,٢٦ من بيرجر الدجاج بدون إضافة و ٤,٦٢x١٠^٣ ±٧,٤٢ x١٠^٢ ،١,٥٣x١٠^٢ ±٤,٨٥ x١٠ ،١,٣٣ ± ٠,٧٩،٣,٠٠ ±١,٣٧ ،٤,٣٣ ±١,٦٤ و١,٦٧± ٠,٨٤ للدجاج المقلي. وكانت العينات غير ايجابيه لميكروب الشيجيلا.

وبعد تصنيف العترات الموجودة سير ولوجي تم عزل ٩عينات من ميكروب الاشيريشيا كولاي بنسبة (٦٪) من العينات منهم٤ بنسبة (٨٪) ، (٢) O125: H2، O143: H8 ، O111: H1 من شاورما دجاج بدون إضافة و ٣عينات بنسبة (٦٪) O55: H2، O158: H3، O86a: H1 من بيرجر الدجاج بدون إضافة و ٢عينات بنسبة (٤٪) O142: H3، O26: H1 من دجاج مقلي.

كما تم عزل ٨ عينات بنسبة (٥,٣٣٪) من المكور العنقودي الذهب والتي تمثلت في ٤ عينات بنسبة (٨٪) معزولة عن شاورما الدجاج بدون إضافة ، ٣عينات بنسبة (٦٪) من بيرجر الدجاج بدون إضافة ، ١عينة بنسبة (٢٪) من دجاج مقلي.
كما تم عزل ٦ عينات بنسبة (٤٪) من ميكروب السالمونيلا من العينات والتي تمثلت في ٢ عينه بنسبة (٤٪) سالمونيلاتيفيموريوم و ١عينة بنسبة (٢٪) سالمونيلا انترتيدس من شاورما الدجاج بدون إضافة ، ١عينة بنسبة (٢٪) سالمونيلا انترتيدس وعينة ١ (٢٪) سالمونيلا اناتم من بيرجر الدجاج بدون إضافة ، ١عينة بنسبة (٢٪) سالمونيلا كينتاكي من الدجاج المقلي.

وجد انه معظم ميكروب الاشيريشيا كولاي حساسة للمضادات الحيوية الاتيه كبريتات الكوليستين (١٠ ميكروغرام) وحمض الناليديكسيك (١٠ ميكروغرام)وكانت المكورات العنقودي الذهبي حساسة للأمبيسيلين (١٠ ميكروغرام) وفانكومايسين (١٥ ميكروغرام). معظم عزلات السالمونيلا حساسة للجنتاميسين (١٠ ميكروغرام) ، كوليستين سلفات (١٠ ميكروغرام) و سيفترياكسون