

Bacteriological Evaluation of Prepared Food of Animal Origin in Butcher Area at Hotel's kitchen

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Abstract

To evaluate the hygienic status during preparation of food in fixed and floating hotels from Kitchen area in Luxor, Egypt; one hundred samples were collected; 20 from each raw beef, chicken meat, surface, hand and knife swabs (10 from each, fixed and floating). The meat samples were examined for the Aerobic plate count (APC), Coliforms count (CC) and Coagulase Positive Staphylococci (CPS). As the results indicated that the count varies from satisfactory to unsatisfactory according to the parameter mentioned in Egyptian standards of frozen meat (ES 1522/2005) and (Egyptian standards of frozen poultry) ES 1090/2005, also, the meat samples were examined for the important food borne pathogens (*E.coli*, *Salmonella* spp. and other enteric bacteria). The results indicated higher percentage in beef than poultry for *E.coli* isolation 20% (4/20) and 15% (3/20), respectively; its serotypes are O₂₆: H₁₁, O₄₄:H₁₈, O₇₈, O₁₀₃:H₄ and O₁₂₇:H₆. Detected STEC by multiplex PCR for *Stx1*, *Stx2* and *eaeA* genes; showed different result for each serotype. *Salmonella* isolation was similar in both beef and poultry 10% (2/20), its serotypes were *S. Typhimurium*, *S. Enteritidis* and *S. Montevideo* with applied of multiplex PCR for *invA*, *hliA* and *fimH* as virulence genes. The other enteric bacteria were prevalent in 35% (7/20) of examined meat samples; one *Citrobacter freundii* and also one *Serratia liquefaciens* and *Enterobacter agglomerans* with percentage of (5%) for each, two *Enterobacter aerogenes* and also two *Proteus morabilis* with percentage of (10%) for each. While, from poultry samples enteric bacteria constituted 10% (2/20) as one *Enterobacter aerogenes* and one *Klebsiella pneumonia* with percentage of 5% for each one. This obtained data indicated that the microbiological quality of analyzed raw beef samples had high unsatisfactory result than poultry meat samples, and both could be an important cause of food borne disease. Good manufacturing practices (GMP) for Egyptian hotels should be applicable and food handler's health screening have to be made to control pathogenic microbes which potential for public health risks.

Key words: Butcher area, *Salmonella*, *S.aureus* count, *E.coli* isolation, Aerobic bacterial count, Coliform count, Hotel's kitchen, enteric bacteria.

Introduction

Meats are the main media of bacterial growth due to; it contains high moisture, rich in protein, have fermentable carbohydrate, have favorable pH and other growth factors (Mboto *et al.*, 2012). Many food-borne diseases are asso-

ciated with consumption of meat and poultry. Some pathogens are not previously known (new pathogens), others have newly arisen as food-borne (emerging pathogens), and others have become more potent or associated with other products (evolving pathogens). Many of

these pathogens may cause severe illness, besides gastroenteritis. Outbreaks of enterohemorrhagic *Escherichia coli* have been associated with consumption of contaminated meat (**Mor-Mur and Yste, 2010**).

Presence of microorganisms indicated that the food has been processed in unhygienic condition. Presence of Coliforms and Aerobic bacteria is a good indicator for determining the hygienic quality of meat. In food poisoning the most important bacteria is *S. aureus* count which has been compared in the standards (**İsmail and Belma, 2002**). *S. aureus* plays a great role in bacterial contamination of food, Staphylococcus can be carried on human hands, nasal passage or throats, so workers play a major role of *S. aureus* contamination in different stages of food. Most food-borne illnesses of *S. aureus* outbreaks are a result of production of heat stable enterotoxins in the food which may lead to severe food poisoning outbreaks (**Ahmed, 1991 and FSIS, 2003**).

The microbiological quality of the raw meat, personal hygiene and any contamination during the process will determines the quality of end product in terms of microbial contamination (**Elmali and Yaman, 2005**).

Food-borne illnesses are defined by the world health organization as diseases caused by consumption of contaminated foods or water by infectious or toxic substance. Food-borne illnesses have two broad groups; intoxication and infection. Intoxication is caused by ingestion of toxin produced by pathogens, while infection is caused by ingestion of food containing viable pathogens (**Addis and Sisay, 2015**). Food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The severity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the enterotoxins (**Clarence et al., 2009**).

The pathogenic mechanism of *E. coli* among various serotypes differs depending on intestinal colonization by STEC produce either Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2), or a combination of both toxins. The Shiga-like toxins, or verocytotoxins, are the major virulence fac-

tor which induced death in certain individuals. But, colonization by enteropathogenic *E. coli* (EPEC) causes effacement of microvilli by adherence to the epithelial cell membrane. Presence of the EPEC detecting by the ability of adherence factor plasmid to adhere by an outer membrane protein called intimin that is encoded by the *eae* gene (**Bacon and Sofos, 2003**).

Most of these food-borne diseases are due to mishandling of foods, those ways should be avoided (e.g. improper cooling, inadequate heating/reheating, and poor personal hygiene), education of food handlers and consumers about the importance of food hygiene to improve safety and so prevent many illness (**Leon-Velarde et al., 2004 and Sofos, 2008**). Other ways such as incorrect thaws, inadequate cleaning and sanitation of utensils resulting in higher contamination with microorganisms and lower keeping quality measures, which lead to severe public health hazards (**Hassan et al., 2015**). So, to ensure that the food is microbiologically safe, both the manipulators and the food need to be continually monitored (**Gilling et al., 2001**).

The HACCP system identifies critical control points during food processing where contamination is likely to occur. This allows food industry personnel to focus on the critical areas and put in place controls to prevent contamination. HACCP places primary responsible for the safety of food in food processing industry. The government's role is to verify that industry is carrying out its responsibility, by examining this area and to initiate appropriate regulatory action if necessary. Meat and poultry production and processing facilities must have an HACCP plan in place (**Roberts, 2001**).

Here in the present study, the butcher area in hotel's kitchen have been bacteriologically examined using APC of handler and contact surfaces of food during its preparation as indicator for hygienic status. Also, quality of food by bacteriological identification of APC, CC, CPS and isolation of food poisoning bacteria from meat samples to highlight potential problems of storage and handling since production and detect the most toxic and pathogenic genes.

Materials and Methods

Collection of Samples:

A total of one hundred (100) food samples and contact surface swabs were collected from 20 fixed and floating hotels from Kitchen area in Egypt and used for evaluation of the hygienic status during preparation of food (raw meat and chicken) and isolation of food poisoning microorganisms.

The collected samples include raw meat and raw chicken (40), food contact surfaces (20), chef's hand swab (20) and Knife swabs (20).

Each sample was kept in a separated sterile plastic bag and put in an ice box then transferred to the laboratory under complete aseptic condition without undue delay and examined as quickly as possible. The collected samples were subjected to the microbiological examination to evaluate their quality.

Preparation of samples

Preparation of sample homogenate.

To each 25 grams of the food sample, 225 ml of sterile peptone water were added and thoroughly mixed using sterile homogenizer for 1 – 1.5 minutes, from which tenfold serial dilutions were prepared according to standard methods (ISO 6887-1 and 2: 2017).

Swab samples: Sterile cotton screw capped plastic tubes with 10 ml diluents ready for use and a template which made of metal having an exposed inner area of 10 cm² (2×5 cm) was used to delineate area of sampling.

The template was wrapped in aluminum foil and sterilized in hot air oven at 180°C for 20 minutes. Buffered peptone water was used as rinsing and diluents fluid. The solution was distributed to small heat resistant screw capped tubes, each containing 10 ml of rinsing fluid, and then sterilized in the autoclave at 121°C for 20 minutes. For use, the sterilized template placed firmly against the surface of the food serving establishments and food handlers to limit the examined area.

The sterile cotton swab drawn from the tubes, moistened in rinsing fluid solutions (buffered peptone water), then rolled over the limited area inside the template rolled in one direction and perpendicular to this direction to represent

all area. Finally, cotton swab was aseptically retained into the rinsing fluid screw capped tubes containing 10 ml buffered peptone water. (ISO 18593: 2004).

Bacterial isolation, counting, purification and identification

The prepared samples and swabs were subjected to the following examinations:

Aerobic Plate Count (APC) according to (ISO 4833:2013)

Total Coliforms count (CC) according to (ISO 4832: 2006)

Enumeration of Coagulase Positive *Staphylococci* (CPS) according to (ISO 6888-1: 2003)

Isolation and identification of Enteropathogenic *Escherichia coli* according to (Lee and Arp, 1998).

Isolation and identification of *Salmonellae* according to (ISO 6579-1:2002)

Identification of suspected enteric bacteria according to (MacFaddin, 2000).

Serological identification of *E. coli*:

The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E. coli* antisera sets ("Denka Seiken" Co., Japan).

Serological identification of *Salmonellae*:

Serological identification of *Salmonellae* was carried out according to (ISO 6579-3:2014) and reading with Kauffman – White scheme (Grimont and Weill, 2007) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum ("Denka Seiken" Co., Japan and "Sifin", Germany).

IV. Molecular characterization using multiplex PCR technique:

Demonstration of virulence factors including invasion A (*invA*), hyper-invasive locus (*hilA*) and fimbrial (*fimH*) genes of the isolated *Salmonella species*.

Identification of shiga toxins (*stx1* & *stx2*) and intimin (*eaeA*) genes of *E. Coli* was performed essentially by using primers (Pharmacia Biotech).

DNA Extraction using QIA amp DNA Mini kit (Qiagen, Germany, GmbH).

Oligonucleotide Primer Primers used were supplied from (Pharmacia Biotech) and was

listed in **table (1 and 2)**.

PCR amplification. Primers were utilized in a 25- μ l reaction containing 12.5 μ l of EmeraldAmp5X Taq master (Fermentas), 1 μ l of each primer of 20 pmol concentrations, 4.5 μ l of water, and 6 μ l of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler (Master cycler, Eppendorf, Hamburg, Germany).

Analysis of the PCR Products. Finally, 5 μ l of each amplicon was electrophoresed in 1.5 % agarose gel (Sigma –USA, stained with ethidium bromide and visualized as well as captured on UV transilluminator. A 100 bp DNA ladder (Qiagen, Germany, GmbH) was used as a marker for PCR products.

Table (1). primer sequence of virulence genes used in Salmonella isolates with annealing temperature 58°C:

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>invA</i> (F)	5' GTGAAATTATCGCCACGTTCCGGGCA '3	284	Shanmugasamy <i>et al.</i> (2011)
<i>invA</i> (R)	5' TCATCGCACCGTCAAAGGAACC '3		
<i>hilA</i> (F)	5' CTGCCGCAGTGTTAAGGATA '3	497	Guo <i>et al.</i> (2000)
<i>hilA</i> (R)	5' CTGTGCGCTTAATCGCATGT '3		
<i>fimH</i> (F)	5' GGA TCC ATG AAA ATA TAC TC '3	1008	Menghistu (2010)
<i>fimH</i> (R)	5' AAG CTT TTA ATC ATA ATC GAC TC '3		

Table (2). primer sequence of genes used in *E.coli* isolates with annealing temperature 58°C:

Primer	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>stx1</i> (F)	5' AACTGGATGATCTCAGTGG '3	614	Dhanashree and Mallya(2008)
<i>stx1</i> (R)	5' CTGAATCCCCCTCCATTATG '3		
<i>stx2</i> (F)	5' CCATGACAACGGACAGCAGTT '3	779	
<i>stx2</i> (R)	5' CCTGTCAACTGAGCAGCACTTTG '3		
<i>eaeA</i> (F)	5' GTGGCGAATACTGGCGAGACT '3	890	Mazaheri <i>et al.</i> (2014)
<i>eaeA</i> (R)	5' CCCATTCTTTTTACCGTCG '3		

Results

The results were statically analytical to evaluate the mean values of APC (CFU/gm) of randomly taken samples of meat from 10 fixed hotels were $4.3 \times 10^5 \pm 2.6 \times 10^5$ for thawed beef

and $1.4 \times 10^4 \pm 4.7 \times 10^3$ for thawed poultry, respectively; but for floating boats hotels were $7.4 \times 10^5 \pm 3 \times 10^5$ for thawed beef and $10^5 \pm 8.3 \times 10^4$ for thawed poultry, respectively as shown in Table (3).

Table (3). Statistical analytical results of aerobic plate count (APC) (CFU/g) of meat and chicken samples from fixed hotels and floating boats: (n= 20 of each)

Type of Hotel	Type of Samples (Acceptable limit)	Aerobic Plate Count (APC) (CFU/g)		
		Min.	Max.	Mean ± SE
Fixed	Beef ($\leq 10^6$)	10^3	3×10^6	$4.3 \times 10^5 \pm 2.6 \times 10^5$
	Chicken ($\leq 10^5$)	10^3	5×10^4	$1.4 \times 10^4 \pm 4.7 \times 10^3$
Floating	Beef ($\leq 10^6$)	10^3	2.4×10^6	$7.4 \times 10^5 \pm 3 \times 10^5$
	Chicken ($\leq 10^5$)	10^3	9×10^5	$1 \times 10^5 \pm 8.3 \times 10^4$

SE=Standard error

Table (4) showed that the mean values of Coliforms count (CFU/gm) of randomly taken samples of meat from fixed hotels were $6.6 \times 10^4 \pm 4.8 \times 10^4$ for thawed beef and $7.6 \times 10^2 \pm 4.7 \times 10^2$ for thawed poultry, respectively; but for floating boats hotels were $1.7 \times 10^3 \pm 8.9 \times 10^2$ for thawed beef and $2.2 \times 10^3 \pm 1.9 \times 10^3$ for thawed

poultry, respectively. On the other hands, the result of all examined samples was less than 1×10^1 CFU/g for enumeration of Coagulase Positive *Staphylococci* (CPS).

Table (4). Statistical analytical results of Coliforms count (CC) (CFU/g) of meat and chicken samples from fixed hotels and floating boats: (n= 20 of each)

Type of Hotel	Type of Samples (Acceptable limit)	Coliforms Count (CFU/g)		
		Min.	Max.	Mean ± SE
Fixed	Beef ($\leq 10^2$)	10^2	5×10^5	$6.6 \times 10^4 \pm 4.8 \times 10^4$
	Chicken ($\leq 10^2$)	10^2	5×10^3	$7.6 \times 10^2 \pm 4.7 \times 10^2$
Floating	Beef ($\leq 10^2$)	10^2	9×10^4	$1.7 \times 10^3 \pm 8.9 \times 10^2$
	Chicken ($\leq 10^2$)	10^2	2×10^4	$2.2 \times 10^3 \pm 1.9 \times 10^3$

SE= Standard error

Conclusion: unaccepted samples show higher APC than standard were (2/10) 20% and 4/10 (40%) of beef meat from fixed and floating boat hotels, respectively and (2/10) 20% of chicken meat from floating boat hotels, while all chicken meat of fixed hotels were acceptable. Others unaccepted samples show higher CC than standard were 4/10 (40%) and (3/10) 30% of beef meat from fixed and floating boat hotels, respectively and 2/10 (20%) and (1/10) 10% of chicken meat from fixed and floating boat hotels, respectively.

The food contact surfaces and food handler's swabs results were statically analyzed to evaluate the mean values of APC (CFU/cm²); from fixed hotels were $5 \times 10^2 \pm 2.6 \times 10^2$ for cutting

board surfaces, $8.6 \times 10 \pm 2.9 \times 10$ for knife blade and $4.4 \times 10 \pm 1.6 \times 10$ for chef hand. While, from floating boat hotels were $3.6 \times 10^2 \pm 1.2 \times 10^2$ for cutting board surface, $3.2 \times 10^2 \pm 1.2 \times 10^2$ for knife blade and $4.4 \times 10 \pm 1.4 \times 10$ for chef hand, as shown in table (5).

Table (5). Statistical analytical results of aerobic plate count (APC) (CFU/cm²) of environmental samples from fixed hotels and floating boats: (n= 10 of each).

Type of Hotel	Type of Samples (Acceptable limit)*	Aerobic Plate Count (APC) (CFU/cm ²)		
		Min.	Max.	Mean ± SE
Fixed	Cutting Board surface ($\leq 10^2$)	10	2.8×10^3	$5 \times 10^2 \pm 2.6 \times 10^2$
	Knife blade ($\leq 10^2$)	10	2.9×10^2	$8.6 \times 10 \pm 2.9 \times 10$
	Chef Hand ($\leq 10^2$)	10	1.5×10^2	$4.4 \times 10 \pm 1.6 \times 10$
Floating	Cutting Board surface ($\leq 10^2$)	10	9×10^2	$3.6 \times 10^2 \pm 1.2 \times 10^2$
	Knife blade ($\leq 10^2$)	10	9×10^2	$3.2 \times 10^2 \pm 1.2 \times 10^2$
	Chef Hand ($\leq 10^2$)	10	1.4×10^2	$4.4 \times 10 \pm 1.4 \times 10$

SE=Standard error

*According to Jankowicz, (2002)

Finally, the unaccepted count of swabs samples (cutting board surface, knife blade and chef hand) show higher APC than standard were 70%, 40% and 20% in fixed hotels and 60%, 50% and 20% in floating boat hotels, respectively.

As shown in Table (6) the incidence of *E.coli* isolated from meat samples, was (4) with per-

centage 20% and from poultry samples, was (3) with percentage 15%; also the incidence of *Salmonella* Spp. Isolated from meat samples, was (2) with percentage 10% and from poultry samples, was (2) with percentage 10%.

Table (6). Incidence of *E. coli* and *Salmonella* spp. isolated from the examined food samples from fixed hotels and floating boats:

Samples	No. of Samples	No. of Positive Samples			
		<i>E. coli</i>		<i>Salmonella</i> spp.	
		No.	%	No.	%
Beef	20	4	20	2	10
Chicken	20	3	15	2	10
Total	40	7		4	

Isolation and identification of other isolates belonged to Enterobacteriaceae, found 7/20 from meat samples and 2/20 from chicken samples, which revealed from one *Citrobacter freundii* with percentage of (5%), two *Enterobacter aerogenes* with percentage of (10%), one *Enterobacter agglomerans* with percentage (5%), Two *Proteus morabilis* with percentage (10%), one *Serratia liquefaciens* with percentage of (5%). While, from poultry samples;

were one *Enterobacter aerogenes* with percentage 5% and one *Klebsiella pneumonia* with percentage 5% as shown in table (7).

Table (7). Identification of Enterobacteriaceae microorganism isolated from examined food samples:

Identified Microorganism	Meat (n=20)	%	Chicken (n=20)	%
<i>Citrobacter freundii</i>	1	5	0	0
<i>Enterobacter aerogenes</i>	2	10	1	5
<i>Enterobacter agglomerans</i>	1	5	0	0
<i>Klebsiella pneumonia</i>	0	0	1	5
<i>Proteus mirabilis</i>	2	10	0	0
<i>Serratia liquefaciens</i>	1	5	0	0

Table (8) showed the serotypes of isolated *E.coli* from examined meat samples, 7 strains isolated from samples as two isolates of *E.coli* O26:H11, one isolate of *E.coli* O44:H18, also

one isolate of *E.coli* O78 and *E.coli* O103: H4, while two isolates of *E.coli* O127: H6.

Table (8). Serotypes of isolated *E. coli*:

Serotype	Meat	Chicken	Total (7)
O26:H11	1	1	2
O44:H18	0	1	1
O78	1	0	1
O103:H4	1	0	1
O127:H6	1	1	2

Fig. (1) showed the prevalence of STEC in different serotypes isolated from meat samples by agarose gel electrophoresis of multiplex PCR of *Stx1* (614 bp), *Stx2* (779 bp) and *eaeA* (890 bp) genes which detect that *E.coli* O26 from beef was positive for *stx1*, *stx2* and *eaeA* genes, the other *E.coli* O26 from chicken was positive for *stx2* and *eaeA* genes, while *E.coli* O78 and two isolates O127 were positive for *stx1* gene but *E.coli* O103 was posi-

tive for *stx1* and *stx2* genes. *E.coli* O44 from chicken was positive to *stx2* gene.

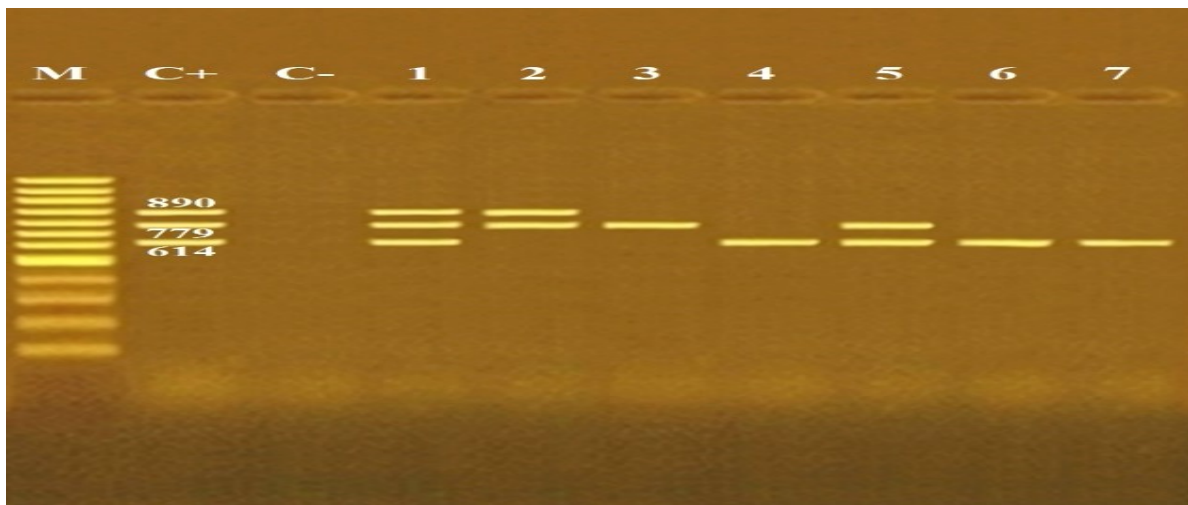


Fig. (1): Agarose gel electrophoresis of multiplex PCR of *Stx1* (614 bp), *Stx2* (779 bp) and *eaeA* (890 bp) genes for characterization of Enteropathogenic *E. coli*.

Lane M: 100 bp ladder as molecular size DNA marker, **Lane C+:** Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes, **Lane C-:** Control negative, **Lane 1** (O26): Positive *E. coli* for *stx1*, *stx2* and *eaeA* genes, **Lane 2** (O26): Positive *E. coli* for *stx2* and *eaeA* genes, **Lane 3** (O44): Positive *E. coli* strain for *stx2* gene, **Lanes 4** (O78), **6 & 7** (O127): Positive *E. coli* strains for *stx1* gene, **Lane 5** (O103): Positive *E. coli* strain for *stx1* and *stx2* genes.

Table (9) revealed that the serotyping of four *Salmonella* spp. was *S. Typhimurium*, *S. Enteritidis* and *S. Montevideo* as 2, 1 and 1 isolate, respectively. Those isolates, prevalence and characterization from meat samples by agarose gel electrophoresis of multiplex PCR for *invA* (284 bp), *hliA* (497 bp) and *fimH* (1008 bp) as virulence genes appeared *S.*

Typhimurium and *S. Enteritidis* were positive for *invA*, *hliA* and *fimH* genes. But *S. Montevideo* was positive for *invA* and *hliA* genes only as shown in Table (10) and Fig. (2).

Table (9). *Salmonella* serovars isolated from meat and chicken samples:

Serovars	Meat	Chicken	Total (4)
<i>S. Typhimurium</i>	1	1	2
<i>S. Enteritidis</i>	1	0	1
<i>S. Montevideo</i>	0	1	1

Table (10). Prevalence of virulence genes in *Salmonella* spp. isolated from food samples:

Type of Shiga toxin	<i>Salmonella</i> strains		
	<i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>S. Montevideo</i>
<i>invA</i>	+	+	+
<i>hliA</i>	+	+	+
<i>fimH</i>	+	+	-

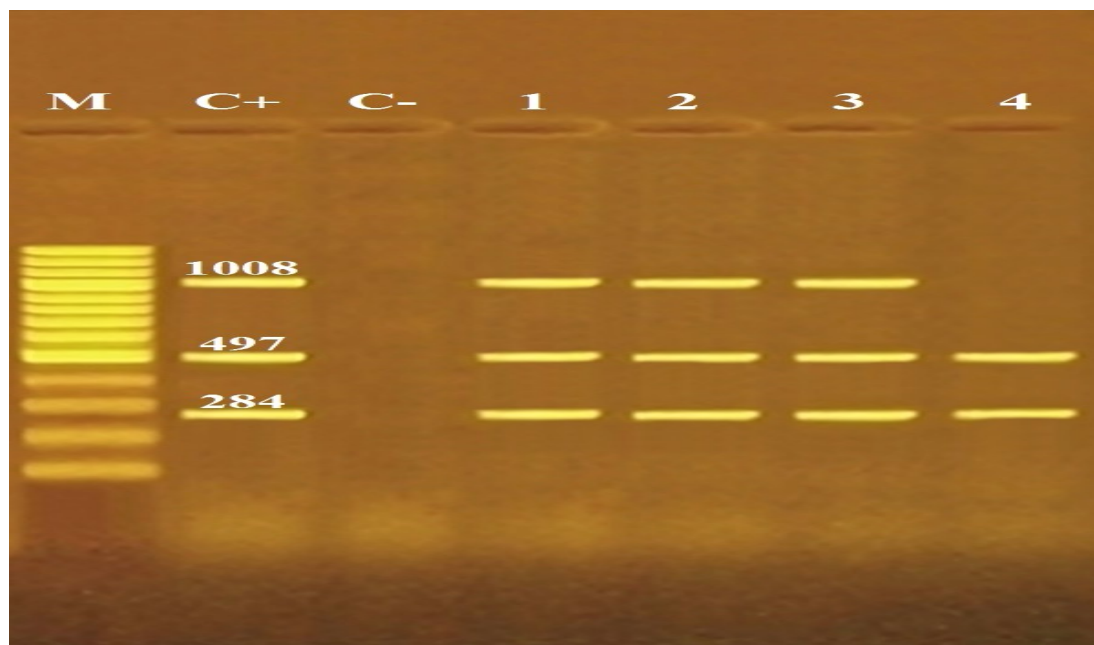


Fig. (2): Agarose gel electrophoresis of multiplex PCR of *invA* (284 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulence genes for characterization of *Salmonella* strains.

Lane M: 100 bp ladder as molecular size DNA marker, **Lane C+:** Control positive *S. Typhimurium* for *invA*, *hilA* and *fimH* genes, **Lane C-:** Control negative, **Lanes 1, 2 (*S. Typhimurium*) and 3 (*S. Enteritidis*):** Positive strains for *invA*, *hilA* and *fimH* genes, **Lane 4 (*S. Montevideo*):** Positive strain for *invA* and *hilA* gene.

Discussion

Aerobic plate count (APC) is the most reliable index of meat quality, sanitary processing and shelf- life of meat products (ICMSF, 1980). Coliforms count was greatly considered to be suitable indicator for fecal contamination (Mousa *et al.* 2001). High count of *S. aureus* in a food indicates its contamination from food handlers and inadequately cleaned equipments (ICMSF, 1996). In the present study, the mean values of APC (CFU/gm) of randomly taken samples of meat from 10 fixed hotels (Table 3) were $4.3 \times 10^5 \pm 2.6 \times 10^5$ for thawed beef and $1.4 \times 10^4 \pm 4.7 \times 10^3$ for thawed poultry, but for floating boats hotels were $7.4 \times 10^5 \pm 3 \times 10^5$ for thawed beef and $10^5 \pm 8.3 \times 10^4$ for thawed poultry. On the other hands, the mean values of Coliform count (CFU/gm) of randomly taken samples of meat from fixed hotels (Table 4) were $6.6 \times 10^4 \pm 4.8 \times 10^4$ for thawed beef and $7.6 \times 10^2 \pm 4.7 \times 10^2$ for thawed poultry, but for floating boats hotels were $1.7 \times 10^3 \pm 8.9 \times 10^2$ for thawed beef and $2.2 \times 10^3 \pm 1.9 \times 10^3$ for thawed poultry. The results obtained of all examined samples were less than 1×10^1 (<10) CFU/g for enumeration of Coagulase Positive *Staphylo-*

cocci (CPS). Thomas *et al.* (2015) showed higher result when collect 260 raw beef samples (55 Slaughterhouse, 55 butchers shop (restaurants/ hotels), 150 from different types of markets) in analysis of microbiological quality; the mean aerobic plate count in beef was slightly higher in butchers shop (restaurants/hotels) (2.82×10^6). Similarly, the coliform count in meat samples was higher from restaurants/hotels (3.73×10^4 CFU/g), and Sharma and Chattopadhyay (2015) found APC of meat from different parts of carcasses ranged from 10^6 to 10^7 CFU/g but chicken around 10^6 CFU/g.

The mean values of APC (CFU/cm²) of food contact surfaces and food handler's swabs; from fixed hotels (Table 5) were $5 \times 10^2 \pm 2.6 \times 10^2$ for cutting board surfaces, $8.6 \times 10 \pm 2.9 \times 10$ for knife blade and $4.4 \times 10 \pm 1.6 \times 10$ for chef hand. While, from floating boat hotels were $3.6 \times 10^2 \pm 1.2 \times 10^2$ for cutting board surface, $3.2 \times 10^2 \pm 1.2 \times 10^2$ for knife blade and $4.4 \times 10 \pm 1.4 \times 10$ for chef hand. Similar results were obtained by Montville and Schaffner (2004) who found variation of log normal dis-

tribution parameters (log CFU/g) in different types of surface swabs from meat contact surfaces showed higher mean microbial levels tended to be plastic (e.g., juice dispenser tips, pastry brushes, plastic cutting boards) which suggested that most of these items were commonly encountered wet, which provided a good environment for bacterial growth and provided a higher bacterial transfer rate than dry surfaces. Also, **Abdel-Shakour *et al.* (2014)** concluded that surface swabs were satisfactory with standard when used chlorine tablets as safety material while unsatisfactory handler swabs with same place as a result of using gel hand and others with very high count (6×10^3 CFU/g) of PCA but others by using normal disinfectant are satisfactory. While, contact surfaces of ready to eat food were shown high APC (3.3×10^3 CFU/g and 9.7×10^2 CFU/g).

Food-borne illness is a major international problem and leads to great socioeconomic impact. The contamination of the food with pathogens and its persistence, growth, multiplication and/or toxin production has emerged as an important public health concern. Most of these problems could be controlled with the efforts on the part of the food handlers, whether in a processing plant, a restaurant, and others (**Mensah *et al.*, 2002**). In the current study, the incidence of *E.coli* from meat samples (Table 6), was (4 isolates) with percentage 20% and from poultry samples, was (3 isolates) with percentage 15%. Also, the incidence of *Salmonella* spp. from meat samples was (2 isolates) (10%) and from poultry samples, was (2 isolates) (10%). Isolation and identification of other isolates belonged to Enterobacteriaceae, (Table 7) were 7/20 from meat samples and 2/20 from chicken samples, which identified in meat samples as one *Citrobacter freundii* with percentage of (5%), two *Enterobacter aerogenes* with percentage of (10%), one *Enterobacter agglomerans* with percentage (5%), Two *Proteus morabilis* with percentage (10%), one *Serratia liquefaciens* with percentage of (5%). While, from poultry samples; were one *Enterobacter aerogenes* with percentage 5% and one *Klebsiella pneumonia* with percentage 5%. Higher results were reported by, **Zhao *et al.* (2001)** who reported high prevalence of *E.*

coli (38.7%) and lower prevalence of *Salmonella* spp. 4.2% from 212 chicken meat samples. Also, **Milhem *et al.* (2016)** examined beef meat samples from major abattoirs and found 35 isolates (29.9%) were *Escherichia coli* and 2 isolates (1.7%) were *Citrobacter Freundii*. Also, **Gwida *et al.* (2014)** identify 27/50 (54.0%) and 8/50 (16.0%) from raw beef and chicken meat samples, respectively, as *E. coli* and *Proteus* spp. from chicken meat (78.0%) and beef meat (58.0%) while, *Klebsiella* spp. were isolated from 3 samples of raw beef (6.0%) and 13 samples (26.0%) of raw chicken meat but *Citrobacter* spp. (13.3%).

Badr *et al.* (2016) isolated *Salmonella* with percentage 3.8% (4/104) from chicken meat but *E. coli* was 35.6% (37/104). While, **Adeyanju and Ishola (2014)** reported 47.2% (25/53) from *E.coli* isolation and **Dhaher *et al.* (2011)** reported rate of *Salmonella* of 24.76%. **Phillips *et al.* (2001)** detected *E. coli* on 10.3% of carcasses and 5.1% of boneless beef samples, In Australia. Also, **Sumner *et al.* (2003)** isolated *E. coli* with percentage 18.8% from beef carcasses. In Croatia, **Miokovic *et al.* (2004)** found *E. coli* in 6% of the beef samples. **Iroha *et al.* (2011)** reported bacterial contamination in fresh meat that is the most common bacteria were respectively *E. coli* followed by *Klebsiella pneumonia*. Other study reported by **Omorodion and Odu (2014)** about Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria, showed that *K. pneumoniae* (16.7%) was the most predominant pathogens. This was followed by *Enterobacter* spp (13.9%) and *C. freundii* (13.9%). Other studies showed different isolation and percentage as, **Sharma and Chattopadhyay (2015)** isolated pathogenic microorganisms in higher percentages; *E. coli* (98%), *Enterococcus faecalis* (90%), *Salmonella* spp. (2%), *Bordetella* spp. (1%). Other organisms that were isolated in this study were *Klebsiella pneumoniae* (98%), *Enterococcus aerogenes* (90%), *Citrobacter* spp. (52%), *Proteus* spp. (50%), *Klebsiella oxytoca* (35%) from raw meat. Also, **Tanimoto *et al.* (2005)** found that *Enterococcus faecalis* and *Enterobacter aerogenes* were present in 90% of the samples in raw chicken meat of Japan and **Kim *et al.***

(2005) showed prevalence 98% of *Klebsiella pneumoniae* in the chicken samples and **Simjee et al. (2002)** reported *E. faecalis* Isolation from raw chicken meat. Also, **Abdel-Shakour et al. (2014)** isolated *Citrobacter freundii* (meat and chicken), *Enterobacter aerogenes* (meat and chicken), *Enterobacter agglomerans* (chicken), *Klebsiella pneumonia* (meat and chicken), *Proteus mirabilis* (meat and chicken) and *Serratia liquefaciens* (chicken)

Seven *E.coli* strains from meat and chicken samples were serotyped (Table 8) as two isolates of *E.coli* O26:H11, one isolate of *E.coli* O44:H18, also one isolate of *E.coli* O78 and *E.coli* O103: H4, while two isolates of *E.coli* O127: H6. On virulence genes identification detected that *E.coli* O26 isolate was positive for *stx1*, *stx2* and *eaeA* genes, other *E.coli* O26 isolate was positive for *stx2* and *eaeA* genes, *E.coli* O78 and (no. = 2) O127 isolates were positive for *stx1* gene but *E.coli* O103 isolate was positive for *stx1* and *stx2* genes. **Yassin and EL-Gammal (2016)** detected *E. coli* in 12% of 50 examined samples serologically identified as O78, O103:H2, O1:H7 and O125:H21. **Abdel-Shakour et al. (2014)** isolated *E.coli* O111:K58, O26:K60, O55:K59 from raw beef meat. Close to the obtained data, **Younis et al. (2017)** reported that 11.66% (14/120) of chicken meat samples were *E. coli*, the most predominant serotypes were O78 and O128: H2 (21.5%, each), followed by O121: H7 and O44: H18 with molecular method detected that 2 strains (25%) harbored *stx1*, 3 strains (37.5%) *stx2*, and 3 strains (37.5%) both *stx1* and *stx2*, while 1 (12.5%) strain carried *eaeA* gene. Particularly, only O26 serotype had all tested virulence genes (*stx1*, *stx2*, and *eaeA*). In another study conducted by **Rashid et al., 2013** found that *E. coli* isolates had *stx1* (10.5%), *stx2* (7%), both *stx1* and *stx2* (1.5%), and (8%) as virulence genes. Other investigators detected both *stx1* and *eaeA* genes in all strains, but no strains had *stx2* (**Park et al., 2015**).

Several genes have been used to detect *Salmonella* in natural environmental samples as well as food and fecal samples. Virulence chromosomal genes including; *invA*, *invE*, *himA* and *phoP* are target genes for PCR amplification of

Salmonella species (**Jamshidi et al., 2009**). The *invA* gene has ability as specific primers sets to confirm the isolates as *Salmonella* at the genus level **Shanmugasamy et al. (2011)**. On Serotyping of *Salmonella* spp. found *S. Typhimurium*, *S. Enteritidis* and *S. Montevideo* as 2, 1 and 1 isolate, respectively. with percentage of 50%, 25% and 25%, respectively. Those isolates, prevalence and characterization from meat samples by agarose gel electrophoresis of multiplex PCR for *invA* (284 bp), *hliA* (497 bp) and *fimH* (1008 bp) as virulence genes appeared *S. Typhimurium* and *S. Enteritidis* were positive for *invA*, *hliA* and *fimH* genes. But *S. Montevideo* was positive for *invA* and *hliA* genes only (Table 9). Also, **Molla and Mesfin (2003)** reported *Salmonella* in chicken meat (15.4%), mainly *S. Typhimurium*. While, in Kafr elshiekh Governorate **Yassin and EL-Gammal (2016)** detected *Salmonella* spp. with a percentage of 18% Serotypes as *S. Typhimurium* (6%), *S. Enteritidis* (4%), *S. Kentucky* (4%), *S. Molade* (2%) and *S. Infants* (2%), and **Abdel-Shakour et al. (2014)** isolated *Salmonella* spp. as *S. Enteritidis* in raw meat While, *S. Munester*, *S. Enteritidis* and *S. Typhimurium* in raw chicken. Also, **Saad et al. (2015)** detected *S. Enteritidis*, *S. Typhimurium* and *S. Anatum* 33%, 50% and 17% from raw chicken samples, respectively then applied multiplex PCR methods for detection of virulence factors (*invA*, *hil A*, *fimH* and *Stn* genes) of *S. Typhimurium* and *S. Enteritidis*. On the other hand, **Javadi and Safarmashaei (2011)** failed to isolate *Salmonellae* spp. from marketed broiler meat.

Conclusions and Recommendations:

Unsatisfactory of this sample refers to bad manufacturing or preparation practices which may be cross contamination from other contaminated foods, bad personal hygiene or improper cleaning and sanitation. So it must be used good healthy measures like, periodic cleaning for tools and surfaces contact to the meat during skinning or defeathering and slicing of the meat in order to reduce the potential contamination. Programs like good manufacturing practices (GMP), good hygienic practices (GHP), HACCP and ISO 22000 systems must be applied especially in hotels to prevent and control foodborne pathogens with regular food

safety and hygiene workshops and training for food handlers that commensurate with their roles as well as and food handler's health screening were made.

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