Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Bacteriological Evaluation of some meat products from different markets in El-Behera Governorate

Saber Ali Saad [*], Eman Mahrous[**], Mohamed Abdelgawad Hassan [*] and Amal Mohamed El-Sayed[***]

[*]Damanhur branch, Animal Health Research Institute, ARC, Egypt;
[**]Bacteriology Department, Animal Health Research Institute, Agriculture Research Center (ARC), Egypt
[***]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Aswan University, Egypt

Received in 30/1/2023
Received in revised from 14/2/2023
Accepted in 22/3/2023

Keywords:
C. perfringens
E. coli
Salmonella
S. aureus
Serovars and virulence genes

ABSTRACT

The study assessed the bacterial quality of some meat products; minced meat, Luncheon, and Sausage. One hundred and Fifty random samples of meat products including 50 samples each of minced meat, luncheon and sausage were randomly collected from different market at El Behera Governorate for bacteriological evaluation. In addition, detection of virulence genes in some isolated strains. The obtained results revealed that prevalence of *S. aureus, E. coli, Salmonella and C. perfringens* was (40, 24, 20 and 30%) ،((1, 2, 0, 2) (28, 10, 18 and 36%) in the examined minced meat, luncheon and sausage, respectively. Isolated *salmonella* were belonged to *S. Typhimurium* and *S. Enteritidis* . Serogrouping of the isolated *E. coli* revealed O_{111}, O_{26}, O_{146}, O_{126}, O_{114}, O_{158} and untyped strains. Some isolates were tested for virulence genes and (*Pvl* and *Sea*) and (*invA* and *Stn*) were detected in all tested *S. aureus* and Salmonella strains, respectively. *eaeA* was detected in all tested *E. coli* strains while *Stx2* was detected in only one strain. Enterotoxin gene (*cpe*) and alpha-toxin (*cpa*) were detected in all tested *C. perfringens* isolates. The presence of *S. aureus*, *E. coli*, *Salmonella*, and *C. perfringens* indicating unhygienic measures during processing and handling of meat products. Therefore, strict application of hygienic practices should be adopted to improve the quality of such meat products .

Corresponding author: Eman Mahrous, Bacteriology Department, Animal Health Research Institute, Agriculture Research Center (ARC), Egypt
E-mail address: eman_mahrous12@yahoo.com
DOI: 10.21608/EJAH.2023.294692

INTRODUCTION

Foodborne diseases (FBD) represent global public health issues resulting in considerable morbidity and mortality in all age groups **(He et al., 2023**). The most frequent bacterial causes of foodborne diseases worldwide are *Salmonella*, *vibrio parhaemolytica, listeria monocytogenes, S. aureus* and some other pathogens (**Gallo et al. 2020**). Meat and meat products are an important source for protein, basic amino acids, vitamins, fats, minerals and other nutritive components for human (**Abuelnaga et al. 2021)**. In contrast, it can serve as an ideal media for the growth of different organisms due to unhygienic practices during meat processing (**Alahakoon et al. 2015**).

E. coli is one of the major normal intestinal inhabitants in human and mammals; it is harmless to the host and can cause diseases only in the immune-compromised host or when it breaches the gastrointestinal barriers (**Ibrahim et al. 2018**).

Meanwhile, some *E. coli* strains can acquire specific virulence and represent primary pathogens with enhanced potential to cause many disease conditions (**Morshdy et al. 2018**).

All over the world, Staphylococcal food poisoning is one of the most common foodborne diseases; resulting from ingestion of preformed staphylococcal enterotoxins in food by enterotoxigenic strains of *S. aureus* (**Lika et al. 2021**).

Salmonella is a vital microorganism responsible for FBDs mainly found in raw food of animal origin **(Sun et al. 2021**). *Salmonella* annually causes 200 million to over 1 billion infections worldwide with 93 million cases of gastroenteritis and 155.000 deaths (**Chlebicz et al. 2018**).

C.perfringens bacteria are one of the most common causes of food poisoning. CDC estimates that the bacterium causes nearly 1 million foodborne illnesses in the United States every year. *C. perfringens* makes spores, which are inactive forms of the bacterium that help it to survive heat, dryness, and other environmental conditions. *C. perfringens* spores can transform into active bacteria, which multiply in the food then produce a toxin (poison) that causes diarrhea (**CDC, 2022**). Therefore, the present study aimed to assess the bacterial contamination of some meat products such as Luncheon, minced meat and Sausage with further molecular analysis of isolates' virulence genes. In addition, evaluation of the suitability of these products for human consumption.

MATERIAL AND METHODS

Collection of Samples

A total of One Hundred and Fifty random meat product samples including minced meat, luncheon and sausage (50 samples of each) were collected from different supermarkets at Damanhur city, each sample was collected in a separate sterile plastic bag and transferred in an ice box for bacteriological examination.

Bacteriological Examination of Meat Products

Sample preparation

The samples preparation was done according to **APHA (2001).** Twenty Five grams of each sample were aseptically transferred into sterile blender flask containing 225ml of sterile peptone water 1% and homogenized at 14000rpm for 2.5min.

Aerobic Plate Count

Aerobic Plate Count (APC) was determined using the standard plate count following **(ISO 4833-1, 2013).**

S*taphylococcus aureus* count

Using Baird-Parker Agar Plates according to **Quinn et al. (2011).** Suspected colonies were picked up for further morphological and biochemical identification according to (**FDA**, **2001**).

Detection of *C. perfringens*

Detection of *C.* perfringens was done according to **(ISO 7937, 2004)** using Tryptose Sulfite Cycloserine (TSC) media at 37±1˚C under anaerobic conditions for 20±2h.

Isolation and identification of *E. coli* according to Quinn et al. (2011)

A loopful from enriched broth was streaked onto MacConkey's agar and Eosin Methylene Blue agar (EMB) and incubated at 37ºC for 24h. The metallic green colonies were picked up and identified biochemically.

2Serological identification of ***E. coli*** **isolates according to Quinn et al. (2011)**

The somatic (O) antigen of *E. coli* was detected by slide agglutination test as well as Flagellar (H) antigen was serotyped**.** Anti-O-sera were purchased from DENKA SEIKEN CO LTD Tokyo, Japan.

Isolation and identification of *Salmonella* according (ISO, 2002).

Salmonella Shigella agar and XLD agar plates were used. Suspected colonies were identified morphologically by Gram-stain and biochemically. All *salmonella* isolates were subjected to serological typing by slide agglutination test using standard polyvalent and monovalent salmonella antisera (Seiken, Japan).

Detection of virulence genes in isolated strains by PCR

DNA extraction:

DNA extraction from three isolates from *Staph. aureus*, *Salmonella species* and *E. coli* and two isolates of *C. perfringens* was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200µl of the sample suspension was incubated with 10µl of proteinase K and 200µl of lysis buffer at 56ºC for 10min. After incubation, 200µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in table (A).

PCR amplification: For PCR, primers were utilized in a 25-µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)**, 1µl of each primer of 20 pmol concentration, 4.5µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (AppliChem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products were loaded in each gel slot. Gene ruler 100bp ladder (Fermentas, Thermo Scientific, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Statistical analysis

Data were expressed as mean ± SEM using Statistical data analysis and carried out using SPSS 17.0 for windows (SPSS Inc, Chicago, IL, USA) according to **Feldman (2003).**

RESULTS

Aerobic Plate Count

Aerobic mesophilic bacteria were detected in all examined meat products samples with mean values of $1.70\times10^{6}\pm0.13\times10^{6}$, $4.38\times10^{4}\pm 0.38\times10^{4}$ and $7.90\times10^{5}\pm0.15\times10^{5}$ Cfu/g, in examined minced meat, luncheon and sausage; respectively (Table 1).

S*taphylococcus aureus* determination

S. aureus was detected at level of 40, 20 and 30% in examined minced meat, luncheon and sausage with mean values of $3.15\times10^{4}\pm 0.47\times10^{4}$, $5.20\times10^{2}\pm 0.41\times10^{2}$ and $1.85\times10^{3}\pm0.32\times10^{3}$ Cfu/g; respectively (Table 2).

Isolation and identification of *E. coli:*

The prevalence of *E. coli* in examined minced meat, luncheon and sausage were 36, 8 and 30%; respectively (Table 3).

Isolation and identification of *Salmonella:*

The prevalence of *Salmonella* in minced meat, and sausage were 2 and 3; respectively. Salmonella were identified serologically into

S. Typhimurium and *S. Enteritidis* (Table 4).

Detection of *C. perfringens*

C. perfringens were detected in examined minced meat, luncheon and sausage at a percentage of 28, 18 and 36 with mean values of $2.6\times10^3\pm0.24\times10^3$,$1.75\times10^2\pm0.24\times10^2and2.59\times10^3\pm0.24\times10^3$ Cfu/g; respectively (Table 5).

Serological identification of *E. coli* strains.

Isolated *E. coli* strains were classified into serogroup , O_{111}, O_{26}, O_{146}, O_{126}, O_{114}, O_{158} and untyped strains (Table 6).

Detection of virulence genes in tested by PCR

All tested *S. aureus* strains harbored *pvl* gene at 433bp gene and *sea* at 102bp by PCR (Fig 1). All tested *E. coli* Strain *harbored eaeA* gene (248bp) detected tested *E.* while *Stx2* gene (779bp) detected only in one isolated *E. coli* strain (Fig 2). stn gene and *invA* were detected at 617 and 284bp in three *Salmonella* isolates (Fig 3). All tested *C. perfringens* strains possess enterotoxin gene (*cpe*) at 247bp and alpha toxin (*cpa*) at 402bp by PCR (Fig 4).

Table A. Primers sequences, target genes, amplicon sizes and cycling conditions.

Bacteria	Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles) Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Reference
S. aureus	*Pvl*	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A GCA TCA AST GTA TTG GAT AGC AAA AGC	433	94˚C 5min.	94˚C 30sec.	55˚C 4sec.	72˚C 45sec.	72˚C 10min.	Park et al. (2008)
	Sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	94˚C 5min.	94˚C 30 sec.	57˚C 30sec.	72˚C 30sec.	72˚C 7min.	Mehrotra et al. (2000)
Salmonella	*invA*	GTGAAATTATCGCCAC-GTTCGGGCAA TCATCGCAC-CGTCAAAGGAACC	284	94˚C 5min.	94˚C 30sec.	55˚C 30sec.	72˚C 30sec.	72˚C 7min.	Oliveira et al. (2003)
	Stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5min.	94˚C 30sec.	59˚C 40sec.	72˚C 45sec.	72˚C 10min.	Murugkar et al. (2003)
E. coli	*Stx2*	CCATGACAACGGACAG-CAGTT CCTGTCAACTGAGCAGCAC-TTTG	779	94˚C 5min.	94˚C 30sec.	58˚C 40sec.	72˚C 45sec.	72˚C 10min.	Dipineto et al. (2006)
	eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248	94˚C 5min.	94˚C 30sec.	51˚C 30sec.	72˚C 30sec.	72˚C 7min.	Bisi-Johnson et al. (2011)
C. perfringens	*Alpha*	GTTGATAGCGCAG-GACATGTTAAG CATGTAGTCATCTGTTCCAG-CATC	402	94˚C 5min.	94˚C 30sec.	55˚C 40sec.	72˚C 40sec.	72˚C 10min.	Yoo et al. (1997)
	Enterotoxin (cpe gene)	ACATCTGCAGATAGCTTAG-GAAAT CCAGTAGCTGTAATT-GTTAAGTGT	247	94˚C 5min.	94˚C 30sec.	55˚C 30sec.	72˚C 30sec.	72˚C 7min.	Kaneko et al. (2011)

Table 1. Statistical analytical results of aerobic plate count in examined meat products samples (N=50 each).

Products	Positive samples		Min	Max	Mean ±SEM
	No	%			
Minced meat	50	100	1.6×10^5	2.92×10^6	$1.70\times10^6\pm0.13\times10^6$
Luncheon	50	100	6.0×10^3	9.8×10^4	$4.38\times10^4\pm0.38\times10^4$
Sausage	50	100	6.0×10^5	9.5×10^5	$7.90\times10^5\pm0.15\times10^5$

Table 2. Statistical analytical results of *S. aureus* in examined meat products samples (N=50 each).

Products	Positive samples		Min	Max	Mean ±SEM
	No	%			
Minced meat	20	40	1.0×10^4	1.0×10^5	$3.15\times10^4\pm0.47\times10^4$
Luncheon	10	20	3.2×10^2	6.8×10^2	$5.20\times10^2\pm0.41\times10^2$
Sausage	15	30	5.0×10^2	5.0×10^3	$1.85\times10^3\pm0.32\times10^3$

Table 3. Prevalence of *E. coli* in examined samples of meat products (N=50 each).

Products	Positive samples	
	No	%
Minced meat	18	36
Luncheon	4	8
Sausage	15	30

Table 4. Prevalence of *Salmonella* serovars isolated from examined meat products samples (N=50 each).

Products	Positive samples	Salmonella serotyping for 5 Salmonella strains	
		S. Enteritidis	***S. Typhimurium***
Minced meat	2	1	1
Luncheon	0	0	0
Sausage	3	1	2

Table 5. Prevalence of *C. perfringens* in examined samples of meat products (N=50 each).

Products	Positive samples		Min	Max	Mean ±SEM
	No	%			
Minced meat	14	28	1.3×10^3	4.0×10^3	$2.6\times10^3\pm0.24\times10^3$
Luncheon	9	18	9.0×10	3.2×10^2	$1.75\times10^2\pm0.24\times10^2$
Sausage	18	36	1.2×10	4.0×10^3	$2.59\times10^3\pm0.24\times10^3$

Table 6. *E. coli* Serogrouping from examined samples

Products	O_{111}	O_{26}	O_{146}	O_{126}	O_{114}	O_{158}	Untyped
Minced meat	1	1	1	0	1	1	2
Luncheon	0	1	0	0	0	0	0
Sausage	0	1	1	1	0	0	1

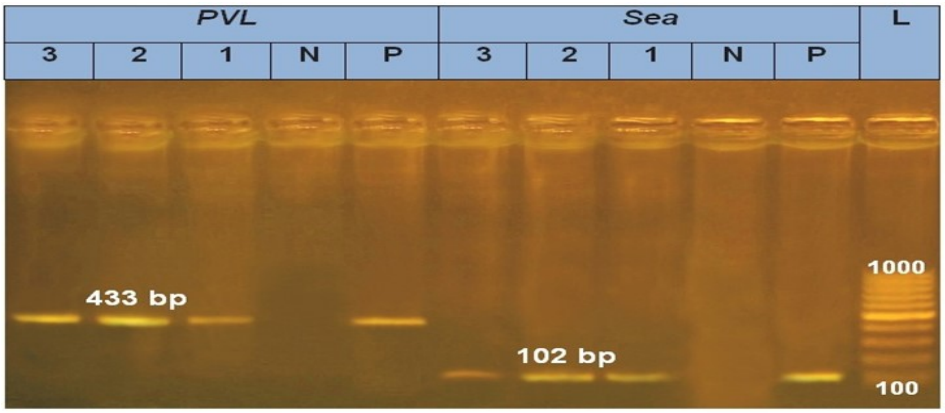

Fig (1): Agarose gel showing polymerase chain reaction amplification products of *pvl* (433bp) and *Sea* gene (102bp) in three *S. aureus* strain, L:100-1000bp DNA ladder, pos: control positive for sea and *pvl*, Neg: control negative for sea and *pvl*, lane 1-3: positive strain for *pvl*, lane 1-3: positive strain for sea

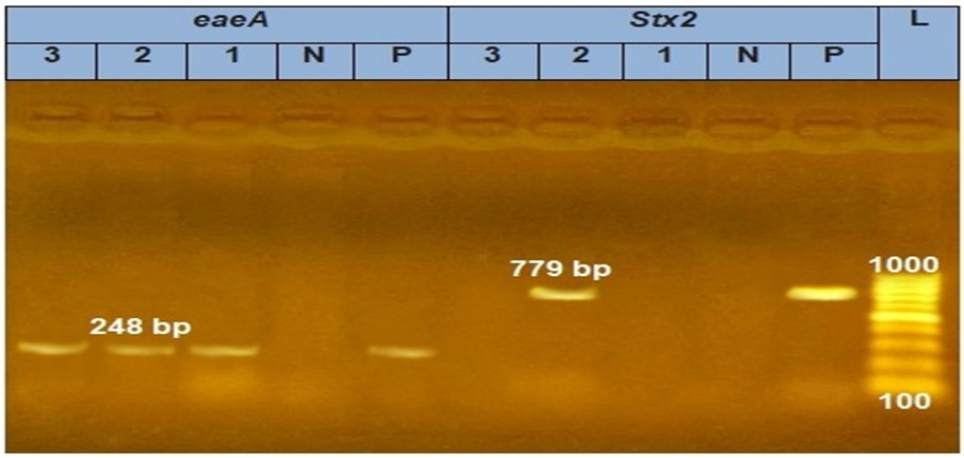

Fig (2): Agarose gel showing polymerase chain reaction amplification products of *stx2* (779bp) and *eaeA* gene (248bp) in three *E. coli* strain, L:100-1000bp DNA ladder, pos: control positive for *eaeA* and S*tx2*, Neg: control negative for *eaeA* and *Stx2*, lane 1-3: positive strain for *eaeA* , lane 2: positive strain for *Stx2*.

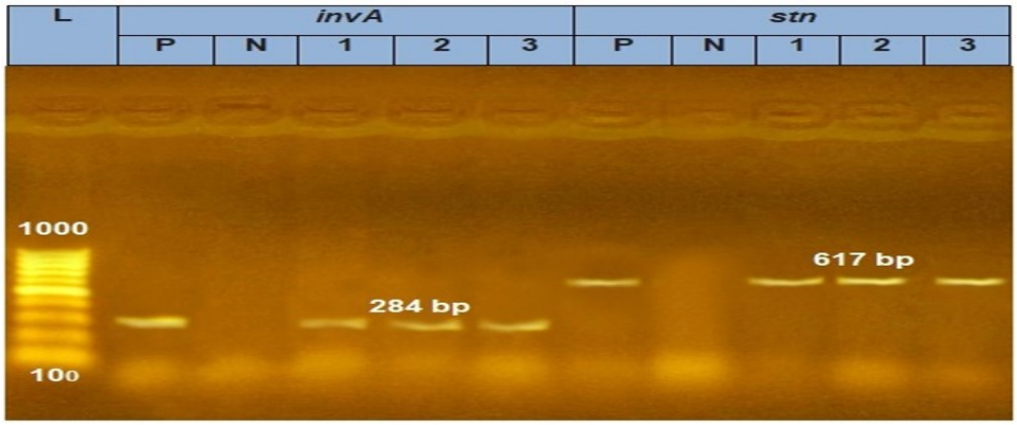

Fig (3): Agarose gel showing polymerase chain reaction amplification products of *stn* gene (617bp) and *invA* (284) in three *Salmonella Typhimurium*, L:100-1000bp DNA ladder, pos: control positive for *stn* and *invA*, Neg: control negative for stn and *invA*, lane 1-3: positive strain for *invA* , lane 1-3: positive strain for *stn*.

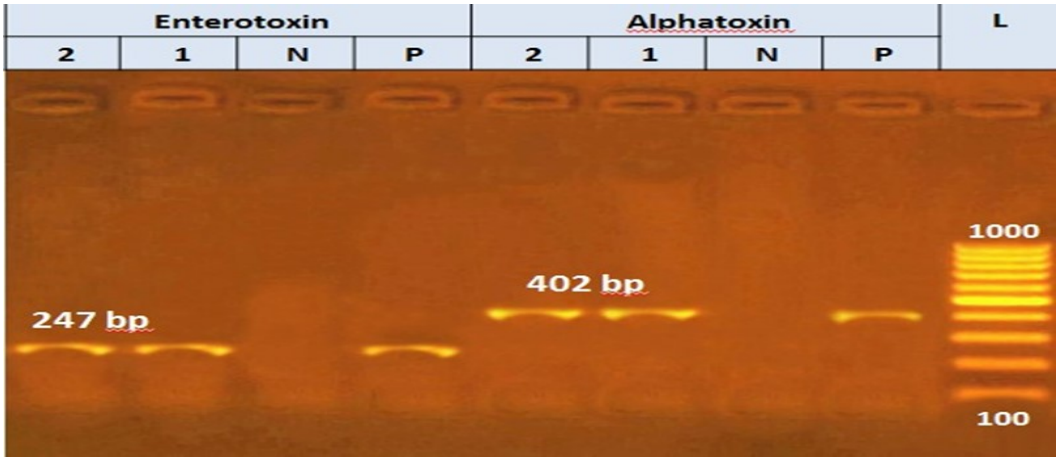

Fig (4): Agarose gel showing polymerase chain reaction amplification products of Alpha toxin (*cpa*) at 402bp and enterotoxin (*cpe*) gene at 247bp in two *Clostridium perfringens* strain, L:100-1000bp DNA ladder, pos: control positive for *cpa* and *cpe*, Neg: control negative for *cpa* and *cpe*, lane 1-2: positive strain for *cpa* , lane 1-2: positive strain for *cpe*.

DISCUSSION

Meat is favored by millions of people worldwide as a main source of animal protein. In the contrast, it is a suitable medium for the growth of different microorganisms, so it acts as a hygienic risk problem to the consumer (**Elsayed et al. 2018**). Meat and meat products are considered a major cause of foodborne pathogens human (**Abuelnaga et al. 2021**). That's due unhygienic practices of manufacture, storage and handling from production sites to the consumers. Therefore, it is crucial to use microbiological criteria to ensure the quality of those products (**Abuzaid et al. 2020**).

The obtained results in table (1) were higher than **Hassanien et al. (2018)** who detected aerobic bacterial count in examined sausage, minced meat and luncheon as $3.3x10^5$, $3.3x10^5$ and $3.3x10^5$ Cfu/g; respectively. In addition, **Shaltout et al. (2016)** reported that mean value of aerobic plate count in examined minced meat, sausage and luncheon were 8.03×10^4 $\pm0.12\times10^4$, 6.74×10^4 $\pm0.28\times10^4$ and 5.85×10^4 $\pm0.24\times10^4$ Cfu/g; respectively. The total bacterial count reflects the bacterial contamination

level and hygienic quality of meat products **(Salem et al. 2018).**

Concerning data in table (2), lower results obtained by **Hassanien et al. (2018)** as $0.2x10^2$, $1x10^2$ and $3x10^2$ Cfu/g in minced meat, luncheon and sausage, respectively. In addition, lower prevalence of *S. aureus* was detected by **Shaltout et al. (2016)** as 6, 20 and 25% in luncheon, sausage and minced meat, respectively.

Higher results of *S. aureus* in sausage were reported by **Abd El-Latef (2014)** as $2.8x10^4$ Cfu/g. *S. aureus* can be carried in nasal passage and on the hands. Most outbreaks of food borne disease were due to contamination following poor handling and production of heat stable toxins *S. aureus.* Refrigeration, proper cooking and sanitary food handling are recommended measure to prevent disease outbreak related to *S. aureus* **(Morshdy et al. 2023).**

All tested *S. aureus* strains harbored *pvl* gene at 433bp and *sea* at 102bp by PCR (Fig 1). Similar results were obtained by **Morshdy et al. (2018) and Saif et al. (2019)** who detected Sea gene in all tested *S. aureus* strains isolated form meat samples.

Prevalence of *E. coli* shown in table (3) was similar to **El-Shabrawy (2015)** who detected *E. coli* in 8% of luncheon samples. **Shaltout et al. (2016**) and **Eltanani and Arab (2021)** reported lower prevalence of *E. coli* in minced meat (22.9 & 16%) and in sausage (17.1 & 20%) respectively. Moreover, lower prevalence (5.7%) in luncheon was reported by **(Shaltout et al. 2016).**

In addition, **Abd El-Tawab et al. (2015)** cited that the prevalence of *E. coli* in sausage was 9%. Meanwhile, higher prevalence of *E. coli* in luncheon was 36 and 28% reported by **Ashraf (2016)** and **Ramadan (2015)**.

Escherichia coli were identified serologically as O26, O126, O111, O158, O146, and untyped *E. coli* as shown in Table 6. *E. coli* O26 was the most prevalent serogroup in the current study like **Abuelnaga et al. (2021)** but **Moawad et al. (2017)** in Egypt identified *E. coli* O158 mainly in beef meat.

Detection of virulent genes in some *E. coli* isolates obtained in Fig (2) and agreed with **Abd El-Tawab et al. (2019)** who detected *eaeA* gene (248bp) in all tested *E. coli* strains from meat products. In contrary, **Hassan et al. (2020)** reported that *E. coli* isolates from minced meat and sausage were negative for *Stx2* gene.

Prevalence of Salmonella in table (4) agreed with **Edris et al. (2011)** who didn't detect *Salmonellae* in the examined luncheon samples.

In contrary, **Eltanani and Arab (2021)** isolated *Salmonella* from luncheon at prevalence rate 12%. Similar prevalence of *Salmonella* in minced meat 2.8% was reported by **Filliol et al. (2008)** while, higher prevalence in minced meat 24% was reported by **Eltanani and Arab (2021).** Similar prevalence of *Salmonella* in sausage 4% was reported by **Abd El-Atty and Meshref (2007)** while, higher results 24% were reported by **Hassanien et al. 2018)**.

In the present study, *Salmonella Typhimurium* was the prevalent serovar (table, 4).

In Egypt, similar results obtained by **Moawad et al. (2017)** and **Aboelnaga et al. (2021)** who indicated that, *S. Typhimurium* was the most dominant serovar in the examined meat and meat samples. In addition, *invA* and *Stn* gene were detected in all tested isolates at 617 and 284bp PCR amplified fragment, respectively (Fig, 3). The results were agreed with **Ezzat et al. (2020)** who mentioned that all isolated *Salmonella* from meat products harbored *invA* gene at 284bp. In addition, **Sallam et al. (2014)** confirmed the presence of *Stn* gene in all identified *Salmonella* isolates form meat products.

Tabulated *C. perfringens* prevalence in table (5) was lower than that obtained by **Khalafalla et al. (2020)** in sausage and luncheon samples as 60 and 52%; respectively. In addition, **Kamber et al. (2007)** and **Younis et al. (2018)** reported higher prevalence in minced meat which was 55 and 93.3%; respectively.

Prevalence of *C. perfringens* in minced meat may be attributed to poor hygienic measures during processing as well as spores

could be separately added to meat products during cutting, wrapping and handling (**Younis et al. 2018).**

The *C. perfringens* ability to produce alpha toxin (*cpa*) and enterotoxins (*cpe*) which are responsible for possible food poisoning has remained of public health relevance **(Farag et al. 2023).** In addition, Fig (4) declared that two tested *C. perfringens* strains possess enterotoxin gene (*cpe*) at 247bp and alpha toxin (*cpa*) at 402bp by PCR. These results agreed with **Younis et al. (2018)** who found that all tested *C. perfringens* type A possess *cpa* at 204bp and *cpe* at 247bp.

CONCLUSION

Results in the present study proved that meat products were highly contaminated indicating improper handling during processing.

Consequently, strict maintenance of good practices during processing, maintaining the cold chain during transport, distribution and carcass commercialization is very important to ensure both public health and food quality. In addition, obligatory implementation of strict food safety regulations in restaurants that sell meat products should be imposed and checked by regulatory agencies in Egypt for food safety.

REFERENCES

Abd El-Atty N, Meshref A. 2007. Prevalence of Salmonella and E. coli O157 in some foods Beni-Suef Vet. Med. J., 5th Scientific Conference pp 73-78.

Abd El-Latef, A. 2014. Microbial load of some meat products. M.V.SC, meat Hygiene. Alexandria University.

Abd El-Tawab A, Maarouf A, El-Sayed A 2019. Bacteriological and molecular studies on antibiotic resistant Escherichia coli isolated from meat and its products in Kaliobia, Egypt. Benha Veterinary Medical Journal, 36 (2):335-344.

Abd El-Tawab A, Maarouf A, El-Hofy F, El-Said A. 2015. Bacteriological studies on some foodborne bacteria isolated from chicken meat and meat products in Kaliobia Governorate, Benha Vet Med J., 29 (2): 47-59.

Alahakoon U, Jayasena D, Ramachandra S, and Jo C. 2015. Alternatives to nitrite in processed meat: Up to date. Trends in Food Science and Technology, 45(1): 37-49. DOI: https://www.doi.org/10.1016/j tifs.2015.05.008

Abuelnaga ASM, Abd El-Razik KhAE-H, Hassan Soliman MM, Sultan Ibrahim H, Abd-Elaziz MMM, Elgohary AH, Hedia RH, and Elgabry EA-E. 2021. Microbial Contamination and Adulteration Detection of Meat Products in Egypt. World Vet. J., 11 (4): 735-744. DOI:https:// dx.doi.org/10.54203/scil.2021.wvj94

Abuzaid KEA, Shaltout F, Salem R, and El-Diasty EM. 2020. Microbial aspect of some processed meat products with special reference to aflatoxins. Benha Veterinary Medical Journal, 39(2): 24-28. DOI: https://www.doi.org/10.21608/ bvmj.2020.44886.1274

APHA "American Public Health Association" 2001. Compendium of methods for microbiological examination of foods, 4th Edition 365- 366. 800. 1st, NW Washington DC 2000. 1-3710. USA

Ashraf A. 2016. Microbial evaluation of heat-treated meat products. M.V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Alex Univ., Egypt.

Bale JA, de Pinna EM, Threlfall EJ, and Ward LR (2007). Kauffmann-White scheme: *Salmonella* identification - serotypes and antigenic formulae. London: health protection agency. Available at: https:// www.worldcat.org/title/kauffmann-white-scheme-2007-salmonella-identification-serotypes-and-antigen-formulae/ oclc/228193783?referer=di&ht=edition

Bisi-Johnson M, Obi C, Vasaikar S, Baba K, Hattori T. 2011. Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens 3:9.

CDC. 2022. Prevent illness from *C. perfringens*. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)

Chlebicz A and Slizewska K. 2018. Cam-

pylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic foodborne diseases: a review. Int J Environ Res Public Health. 15:863. doi: 10.3390/ ijerph15050863

Dipineto L, Santaniello A, Fontanella M, Lagos K, Fioretti A Menna L. 2006. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens, Letters in Applied Microbiology, (43): 293–295.

Edris A, Shaltout F, Salem G El-Toukhy, E. 2011. Prevalence and isolation of salmonellae from some meat products. Benha Veterinary Medical Journal Special, 1: 194 -200.

Elsayed ME, Algammal AM, El-Diasty EM, Abouelmaatti RR, and Abbas SM (2018). Prevalence of *Aspergillus* spp and Aflatoxins in luncheon, minced meat and sausage. Global Animal Science Journal-GASJ, 6(2): 17-23. DOI: https:// www.doi.org/10.5281/zenodo.1476984

El-Shabrawy H. 2015. Bacteriological and chemical evaluation of some locally manufactured beef burger. M. V. SC. Thesis Meat Hygiene, Fac. Vet. Med., Benha Univ., Egypt.

Eltanani G, Arab W 2021. Quality Assurance of Some Meat Products. Alexandria Journal of Veterinary Sciences. 69 (1): 70-75.

Ezzat M, Helal I, Hagar G. 2020. Prevalence of Virulent Genes in Salmonella Isolated from Some Raw Meat Products, SCVMJ, 25 (2): 339-355.

Farag EF, Hassan MA, Basiony AA and Mahrous E. 2023. Role of Clostridium perfringens and Escherichia coli in the occurrence of diarrhea in newborn rabbits. International Journal of Veterinary Science 12(2): 218-223. https://doi.org/10.47278/ journal.ijvs/2022.178

FDA (Food Drug Administration) 2001. Food borne pathogenic microorganisms and natural toxins. Center for food safety and applied nutrition.US, FDA, Washington, DC.

Feldman D, Ganon J, Haffman R and Simpson J. 2003.The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.

Filliol I, Hassar M, Cohen N, Karraouan B, Badri S, Carle l, Brahim H, Karib H. 2008. Microbial Quality Control of Raw Ground Beef and Fresh Sausage in Casablanca (Morocco). J. Environ. Health.

Gallo M, Ferrara L, Calogero A, Montesano D, Naviglio D. 2020. Relationships

between food and diseases: what to know to ensure food safety. Food Res Int.137:109414. doi: 10.1016/ j.foodres.2020.109414

Hassan M, Reham A, Amera M. 2020. Assessment of chemical quality of local frozen cattle's liver, Benha Veterinary Medical Journal, (38): 97-100.

Hassanien F, Fahim S, Hashim F, Lotfy L, El-Nagar M. 2018. Quality assurance of some meat products. Journal of Dairy, Veterinary & Animal Research. 1-9.

He Y, Wang J, Zhang R, Chen L, Zhang H, Qi X and Chen J. 2023. Epidemiology of foodborne diseases caused by Salmonella in Zhejiang Province, China, between 2010 and 2021. Front. Public Health 11:1127925. doi: 10.3389/ fpubh.2023.1127925

Ibrahim, HM, Hassan M., Amin RA, Shawqy NA, Elkoly RL. 2018. The bacteriological quality 0f some chicken meat products. Benha Vet. Med. J. (35): 50-57

ISO, International Standard Organization" 2002. International Organization for Standardization. No.6579. Microbiology of Food and Animal Feeding Stuffs. Horizontal Methods for detection of Salmonella species.

ISO, "International Standard Organization" (2004). Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of Clostridium perfringens -colony count technique", Ref. No. ISO, 7937:2004.

International Organization for Standardization "ISO" 2013. International Organization for Standardization. No.4833-1. Microbiology of the food chain —Horizontal method for the enumeration of microorganisms —Part 1: Colony count at 30 °C by the pour plate technique.

Kamber U, Gokce I, Elmali, M. 2007. *Clostridium perfringens* and its toxins in minced meat from Kars, Turkey, Food Additives and Contaminants, (24): 673-678.

Kaneko I, Miyamoto K., Mimura K, Yumine N, Utsunomiya H, Akimoto S, McClane

B .2011. Detection of Enterotoxigenic *Clostridium perfringens* in meat samples by using molecular methods, Applied and Environmental Microbiology, pp. 7526–7532.

Khalafalla F, Barakat D, Abdel-Atty N. 2020. Bacteriological quality of canned meat marketed in Beni-Suef, Egypt, Benha Veterinary Medical Journal, (39): 154-158.

Lika E, Puvaca N, Jeremic D, Stanojevic S, Shtylla T, Cocoli S, Frutos R. 2021. Antibiotic susceptibility of Staphylococcus species iso-lated in raw chicken meat from retail stores. Antibiotics. 10,904.

Mehrotra M, Wang G, Johnson W. 2000. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance, Journal of Clinical Microbiology, 38(3): 1-9.

Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafez HM, and El-Adawy H. 2017. Occurrence of Salmonella enterica and Escherichia coli in raw chicken and beef meat in Northern Egypt and dissemination of their antibiotic resistance markers. Gut Pathogens, 9(1): 1-13. DOI: https://www.doi.org/10.1186/s13099-017-0206-9

Morshdy A, Hussein M, Tharwat A, Fakhry A .2018. Prevalence of enterotoxigenic and multi-drug resistant Staphylococcus aureus in ready to eat meat sandwiches. Slov. Vet. Res., 55(20): 367–374.

Morshdy A, Darwish W, Mohamed FM and Mahmoud A. 2023. Bacteriological Quality of Retailed Chicken Meat Products in Zagazig City, Egypt. Journal of Advanced Veterinary Research. 13(1) 47-51.

Murugkar H, Rahman H, Dutta P. 2003. Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. Indian J Med Res., (117): 66-70.

Oliveira S, Rodenbusch C, Cé M, Rocha S, Canal C. 2003. Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection', letters in applied microbiology, 36(4): 217–221.

Park H, Woo S, Jung Y, Lee E, Cha J, Park H, Lee S. 2008. Detection of Virulence Genes of Staphylococcus aureus and Staphylococcus epidermidis Isolated from Suprapubic Urine from Infants with Fever, Journal of Bacteriology and Virology, 38 (4):189 – 196.

Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S and Fitz Patrick ES. 2011. Veterinary Microbiology and Microbial Disease. 2nd Edition, Wiley-Blackwell, Chichester. Wiley, Hoboken

Ramadan, M. 2015. Contamination of meat products with human pathogens. M.V. Sc. Thesis (Meat hygiene), Fac. Vet. Med., Alex. Univ., Egypt.

Saif M, Saad S, Hasanine F, Shaltout F, Zaghloul M. 2019. Molecular detection of enterotoxigenic Staphylococcus aureus in ready-to-eat beef products, Benha Veterinary Medical Journal, (37): 7-11. Salem AM, Shawky NA, Abo-Hussein LM 2018. Microbiological Profile of Some Meat Products in Menofia Markets. BVMJ -34(2): 1-7

Sallam K, Mahmoud A, Mohammed A, Tomohiro T 2014. Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt. Food Control, (38): 209-214.

Shaltout F, Maarouf A, El-Kewaiey I, Heweidy A (2016). Prevalence of some foodborne microorganisms in meat and meat products, Benha Veterinary Medical Journal, 31 (2):213-219. Sun T, Liu Y, Qin X, Aspridou Z, Zheng J, Wang X. 2021. The prevalence and epidemiology of Salmonella in retail raw poultry meat in china: a systematic review and meta-analysis. Foods.10:2757. doi: 10.3390/ foods10112757

Yoo H, Lee S, Park K, Park Y. 1997. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR, Journal of Clinical Microbiology, 35(1): 228-232.

Younis G, Radwan M, Elkenany R, Elderieny M. 2018. Detection of alpha toxin and enterotoxins of *Clostridium perfringens* isolated from minced meat by real time polymerase chain reaction (PCR), African Journal of Microbiology Research, 12(23): 546 -555.