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Research Article

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Food Born Pathogen Contamination of Some Meat Products in Damanhur City, Egypt

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Abstract

The study assessed the chemical and microbial quality of some meat products (Liver, Luncheon, minced meat, and Sausage) in addition to detecting some virulence genes associated with some isolated strains. Two hundred random samples of meat products including 50 samples each of (minced meat, Liver, luncheon and sausage) were randomly collected from different butchers at Damanhur city, El Behera Governorate for some chemical (TVN and TBA) and microbiological evaluation (APC, Staph. aureus, E.coli, Salmonella, Bacillus cereus, and Clostridium perfringens), in addition, detection of virulence genes in some isolated strains. The obtained results revealed that mean values of TVB-N and TBA was higher in liver than other meat products. In regard to microbiological evaluation, results revealed that incidence of staph. aureus, E.coli, Salmonella, Bacillus cereus and clostridium perfringens were (40, 24, 20 and 30%), (36, 10, 8 and 30%), (2, 4, 0, 4%), (16, 20, 24 and 34%) and (28, 10, 18, and 36%), in the examined minced meat, liver, luncheon and sausage, respectively. Salmonella could not be detected in examined luncheon samples. Regarding virulence genes (Pvl and Sea) and (invA and Stn) were detected in 100% of isolated Staph. aureus and Salmonella strains, respectively. eaeA was detected in 100% of isolated E.coli strains while Stx2 was detected in only one strain. Isolated strains of Clostridium perfringens were positive for enterotoxin gene (cpe) and alpha-toxin (cpa) at percent 100%. The presence of aerobic bacteria like Staphylococci aureus, E. coli, bacillus cereus, Salmonella, and Clostridium perfringens may be due to poor hygienic measures during processing and handling of meat products. So, consumption of these products could be associated with possible risk of infection, suggesting the need for the institution of strict hygienic measures during handling of meat products.

Keywords: TVB-N, TBA, E. Coli, Salmonella, Staph Aureus.

Introduction

Meat and meat products over time have been considered as the heart of convenience food trend [1]. Meat products such as sausage, luncheon, minced meat and burger are highly demanded, because they are more attractive for consumers than fresh meat due to their good taste and high nutritive value also, it is easy prepare within short time. Meat products contamination can occur during preparation, handling and storage practices [2]. Bacterial contamination of meat products can be measured using the aerobic plate count, total *Escherichia coli.* and *Staphylococci*. This gives a good idea on possible hygienic measures put in place during production of meat product [3]. *E. coli* gives reliable information on possible fecal contamination [4].

The major cause of food born disease burden in most developing countries is contamination of food with *Salmonella* species,

Staph. aureus and *E. coli*. These pathogens are transmitted mainly through consumption of contaminated food products with them. The presence of these pathogens in raw meat or meat products has public health implications [5]. *Globally Staph*. *aureus* is food borne pathogen due to their ability to produce extracellular toxins responsible for its virulence. These toxins cause sudden abdominal discomfort resulting in vomiting, nausea and abdominal cramps [6]. There are five major classes of Staphylococcal enterotoxins (SEs) recognized globally include SEA, SEE, SEB, SEC and SED. Another class worth noting is the toxic shock syndrome toxin (TSST-1) which is responsible for the toxic shock syndrome experienced in humans during infection of *Staph. aureus* [7]. Some strains of *Staph. aureus* produced Panton-Valentine leukocidin (PVL) causes necrotizing pneumonia and associated with various kinds of soft tissue and skin reactions [8]. *Staph. aureus* has been recognized as an indicator of inefficiency during thermal processing of food products, including meat products. *Staph. aureus* indicate poor hygiene during food production or preparation. They also indicate improper cooling of food products after preparation [9].

The main sources of *Salmonella* infections were found in meat products so, it is very important to make surveillance for its level of contamination in meat products to prevent severe diseases [10]. The enteropathogenicity of *Salmonella* is dependents on different virulence genes include fimbrial related gene (*spvC*), the enterotoxin (*stn*) and invasion (*invA*) gene which is responsible for Salmonella invasion and adhesion to the epithelium of the intestine and *spvC* is responsible for the exaggerated systemic spreading of *Salmonella* after inactivation of macrophage. The stn gene is responsible for severe and life-threatening diarrhea associated with *Salmonella* infection [11]. *E. coli* cause foodborne diseases as it has virulence factors includes intimin (eaeA), Shiga toxin (stx)1 & 2. [12].

All strains of *C. perfringens* produce alpha (α) toxin and encoded by the cpa gene. Some strains have the ability to produce *C. perfringens* enterotoxin encoded by cpe gene, which is responsible for food poisoning [13]. All *C. perfringens* strains produces alpha toxin, a necrotizing toxin causing liver damage and acute pulmonary (The center for food security and public health 2004). The *C. perfringens* ability to produce spores confer them with ability to survive adverse conditions during food processing. Contamination of food products by *C. perfringens* spore may occur before or during food processing or following unhygienic handling of food products [14]. In Egypt, there is a lack of information about problems related to the consumption of meat meals and the incidence of food-borne diseases. Therefore, the present study aimed to assess the microbial and chemical quality of some meat products (Luncheon, Liver, minced meat and Sausage) in addition to detection of some virulence genes associated with some isolated strains.

Material and Methods

Collection of Samples

A total of 200 random meat product samples including minced meat, Liver, luncheon and sausage (50 samples of each) were collected from different supermarkets at Damanhur city. Samples were kept for the subsequent examinations in a separate sterile plastic bag and transferred in an ice box as soon as possible to the laboratory under aseptic conditions.

Determination of Chemical Parameters

• The Determination of Total Volatile Nitrogen (TVB-N) (mg/100g) was done using Conway's test as outlined by [15].

• The Determination of Thiobarbituric acid number (TBA) (mg malonaldehyde /kg sample) as described by [16].

Bacteriological Examination of Meat Products

Sample Preparation

The samples preparation was done according to 25 grams of each sample were aseptically transferred into sterile blender flask containing 225 ml of sterile peptone water 1% and homogenized at 14000 rpm for 2.5 minutes [17].

Aerobic Plate Count

Aerobic Plate Count (APC) was determined using the standard plate count following [18].

Staph. Aureus Determination

Staph. aureus determination was done using Baird-Parker Agar Plates according to [19]. Suspected colonies of *Staph aureus* were picked up onto slants of nutrient agar for further purification and morphological identification using Gram-stain; biochemically and coagulase activities according to [18].

Isolation and Identification of Enteropathogenic *e. Coli*

Isolation and identification of enteropathogenic E. coli was done according to [20].

Isolation and Identification of *Salmonellae*

Isolation and identification of *Salmonellae* was done according to [21]. Suspected colonies of *Salmonella* isolates with red and with black centers on XLD agar were identified morphologically by Gram-stain and biochemically according to [19]. All *salmonella* isolates were subjected to serological typing by slide agglutination test in animal health research institute, Damanhur branch, Egypt according to using standard polyvalent and monovalent *Salmonella* antisera (Seiken, Japan) [22].

Isolation and Identification of *Bacillus Cereus*

Isolation and identification of *B. cereus* was done according to using PEMPA agar and incubated at 37ºC for 24 hours. Peacock blue colored colonies (3-5mm) surrounded by blue zone of egg yolk hydrolysis against green/greenish yellow background were presumed to be *B. cereus* [23].

Detection of Clostridium Perfringens

Detection of *C. perfringens* was done according to using Tryptose Sulfite Cycloserine (TSC) media at 37 ± 1 ◦C under anaerobic conditions for 20 ± 2 h. Presumptive C. perfringens appeared as

black colonies surrounded by opaque white zones approximately 2–4 mm due to lecithinase activity [24].

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, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56OC for 10 min. After incubation, 200 µl 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 100 µl 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), 1 µl of each primer of 20 pmol concentration, 4.5 µl 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 100 bp 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 was expressed as mean ± SEM using Statistical data analysis was carried out using SPSS 17.0 for windows (SPSS Inc, Chicago, IL, USA).

Results

Determination of Chemical Parameters

The mean values of TVN in examined minced meat, liver, luncheon and sausage were 11.90±0.80, 14.23±0.81, 11.31±0.67 and 13.40±0.92 mg/100 gm; respectively while, the respective mean of TBAs were 0.56±0.04, 0.79±0.05, 0.54±0.05 and 0.40±0.02 mg MD/kg (Table 1).

Table 1: Total Volatile Nitrogen and Thiobarbituric Acid in Examined Meat Products Samples

Products	TVB-N (mg/100 gm)	TBA (mg MD/kg)
	Mean ±SEM	Mean ±SEM
Minced meat	11.90±0.80	0.56±0.04
Liver	14.23±0.81	0.79±0.05
Luncheon	11.31±0.67	0.54±0.05
Sausage	13.40±0.92	0.40±0.02

Total volatile nitrogen (TVB-N) and thiobarbituric acid (TBA). Value was considered significantly different at $p<0.05$.

Aerobic Plate Count

Aerobic mesophilic bacteria were detected in all examined meat products samples with mean values of $1.70\times10^{6}\pm0.13\times10^{6}$, $5.68\times10^{5}\pm0.42\times10^{5}$, $4.38\times10^{4}\pm0.38\times10^{4}$ and $7.90\times10^{5}\pm0.15\times10^{5}$ Cfu/g, in examined minced meat, liver, luncheon and sausage; respectively (Table 2).

Table 2: Statistical Analytical Results of Aerobic Plate Count in Examined Meat Products Samples

Products	No. of examined samples	Positive samples		Min	Max	Mean ±SEM
		No	%			
Minced meat	50	50	100	1.6×10^{5}	2.92×10^{6}	$1.70\times10^{6}\pm0.13\times10^{6}$
Liver	50	50	100	1.2×10^{5}	1.2×10^{6}	$5.68\times10^{5}\pm0.42\times10^{5}$
Luncheon	50	50	100	6.0×10^{3}	9.8×10^{4}	$4.38\times10^{4}\pm0.38\times10^{4}$
Sausage	50	50	100	6.0×10^{5}	9.5×10^{5}	$7.90\times10^{5}\pm0.15\times10^{5}$

Staph. aureus Determination

Staph. aureus was detected at level of 40, 24, 20 and 30% in examined minced meat, liver, luncheon and sausage with mean values of $3.15\times10^{4}\pm0.47\times10^{4}$, $3.33\times10^{3}\pm0.44\times10^{3}$, $5.20\times10^{2}\pm0.41\times10^{2}$ and $1.85\times10^{3}\pm0.32\times10^{3}$ Cfu/g; respectively (Table 3).

Table 3: Statistical Analytical Results of Staph. Aureus in Examined Meat Products Samples

Products	No. of examined samples	Positive samples		Min	Max	Mean ±SEM
		No	%			
Minced meat	50	20	40	1.0×10^4	1.0×10^5	$3.15\times10^4\pm0.47\times10^4$
Liver	50	12	24	1.5×10^3	6.0×10^3	$3.33\times10^3\pm0.44\times10^3$
Luncheon	50	10	20	3.2×10^2	6.8×10^2	$5.20\times10^2\pm0.41\times10^2$
Sausage	50	15	30	5.0×10^2	5.0×10^3	$1.85\times10^3\pm0.32\times10^3$

Staph. aureus Determination

Staph. aureus was detected at level of 40, 24, 20 and 30% in examined minced meat, liver, luncheon and sausage with mean values of $3.15\times10^4\pm0.47\times10^4$, $3.33\times10^3\pm0.44\times10^3$, $5.20\times10^2\pm0.41\times10^2$ and $1.85\times10^3\pm0.32\times10^3$ Cfu/g; respectively (Table 3).

Table 3: Statistical Analytical Results of Staph. Aureus in Examined Meat Products Samples

Products	No. of examined samples	Positive samples		Min	Max	Mean ±SEM
		No	%			
Minced meat	50	20	40	1.0×104	1.0×105	3.15×104±0.47×104
Liver	50	12	24	1.5×103	6.0×103	3.33×103±0.44×103
Luncheon	50	10	20	3.2×102	6.8×102	5.20×102±0.41×102
Sausage	50	15	30	5.0×102	5.0×103	1.85×103±0.32×103

Isolation and Identification of Enteropathogenic *e. Coli*

The incidences of enteropathogenic *E. coli* in examined minced meat, liver, luncheon and sausage were 36, 10, 8 and 30%; respectively (Table 4)

Table 4: Incidence of Enteropathogenic *e. Coli* in Examined Samples of Meat Products

Products	No. of examined samples	Positive samples	
		No	%
Minced meat	50	18	36
Liver	50	5	10
Luncheon	50	4	8
Sausage	50	15	30

Isolation and Identification of *Salmonellae*

The incidence of *Salmonella* in minced meat, liver and sausage were 2, 4 and 4%; respectively (Table 5).

Table 5: Incidence and Serotyping Of *Salmonella* Species Isolated From Examined Meat Products Samples

Products	No. of examined samples	Positive samples		Salmonella serotyping for 5 Salmonella species			
		No	%	*S. Enteritidis*		*S. typhimurium*	
				No	%+	No	%+
Minced meat	50	1	2	1	20	0	0
Liver	50	2	4	1	20	1	20
Luncheon	50	0	0	0	0	0	0
Sausage	50	2	4	1	20	1	20

%+ acc. to No of isolated strains

Isolation and identification of *Bacillus cereus*

Bacteriological examination of meat products samples revealed that *B. cereus* species were found in 16, 20, 24 and 34 % of examined minced meat, liver, luncheon and sausage with mean values of $3.57\times10^3\pm0.54\times10^3$, $3.20\times10^3\pm0.38\times10^3$, $3.08\times10^3\pm0.46\times10^3$ and $1.61\times10^4\pm0.11\times10^4$ Cfu/g; respectively (Table 6).

Table 6: Incidence of *Bacillus Cereus* in Examined Samples of Meat Products.

Products	No. of examined samples	Positive samples		Min	Max	Mean ±SEM
		No	%			
Minced meat	50	8	16	4.0×10^2	5.0×10^3	$3.57\times10^3\pm0.54\times10^3$
Liver	50	10	20	1.4×10^3	5.0×10^3	$3.20\times10^1\pm0.38\times10^3$
Luncheon	50	12	24	1.0×10^2	6.0×10^3	$3.08\times10^3\pm0.46\times10^3$
Sausage	50	17	34	8.0×10^3	2.5×10^4	$1.61\times10^4\pm0.11\times10^4$

Detection of Clostridium *Perfringens*

Clostridium perfringens were detected in examined minced meat, liver, luncheon and sausage at incidence rate 28, 10, 18 and 36% with mean values of $2.6\times10^3\pm0.24\times10^3$, $2.16\times10^3\pm0.26\times10^3$, $1.75\times10^2\pm0.24\times10^2$ and $2.59\times10^3\pm0.24\times10^3$ Cfu/g; respectively (Table 7).

Table 7: Incidence of *Clostridium Perfringens* in Examined Samples of Meat Products

Products	No. of examined samples	Positive samples		Min	Max	Mean ±SEM
		No	%			
Minced meat	50	14	28	1.3×10^3	4.0×10^3	$2.6\times10^3\pm0.24\times10^3$
Liver	50	5	10	1.4×10^3	2.8×10^3	$2.16\times10^3\pm0.26\times10^3$
Luncheon	50	9	18	9.0×10	3.2×10^2	$1.75\times10^2\pm0.24\times10^2$
Sausage	50	18	36	1.2×10	4.0×10^3	$2.59\times10^3\pm0.24\times10^3$

Detection of Virulence Genes in Isolated Strains by Pcr

The present investigation showed that all three tested *Staph. aureus* strain 100% harbored pvl gene at 433 bp gene and sea at 102 bp by PCR (Fig 1). PCR results obtained in Fig 2 showed that eaeA gene (248 bp) detected in 100 % of all isolated *E. coli* Strain while Stx2 gene (779 bp) detected only in one isolated *E. coli* strain. stn gene and invA were detected at 617 and 284bp in three *Salmonella* isolates (Fig 3). The two tested *C. perfringens* strains possess enterotoxin gene (cpe) at 247 bp and alpha toxin (cpa) at 402 bp by PCR (Fig 4).

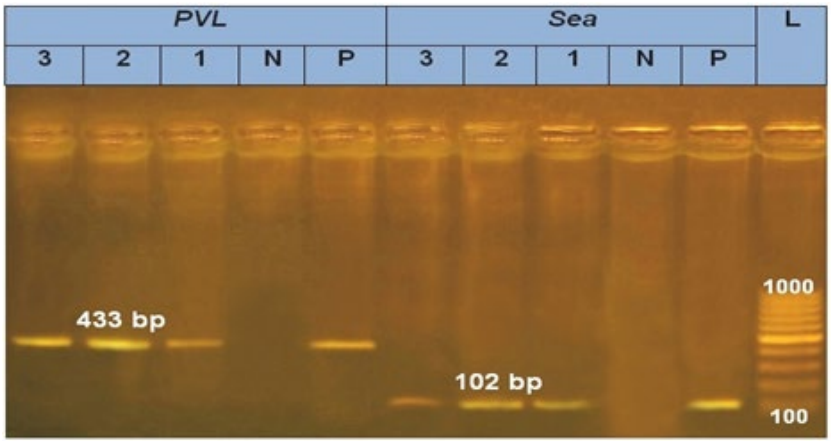

Figure 1: Agarose gel showing polymerase chain reaction amplification products of pvl (433 bp) and sea gene (102 bp) in three staph. aureus strain, l:100-1000 bp dna ladder, pos: control positive for sea and pvl, neg: control negative for sea and pvl, lane 1-3: positive strain for pvl, lane 1-3: positive strain for sea.

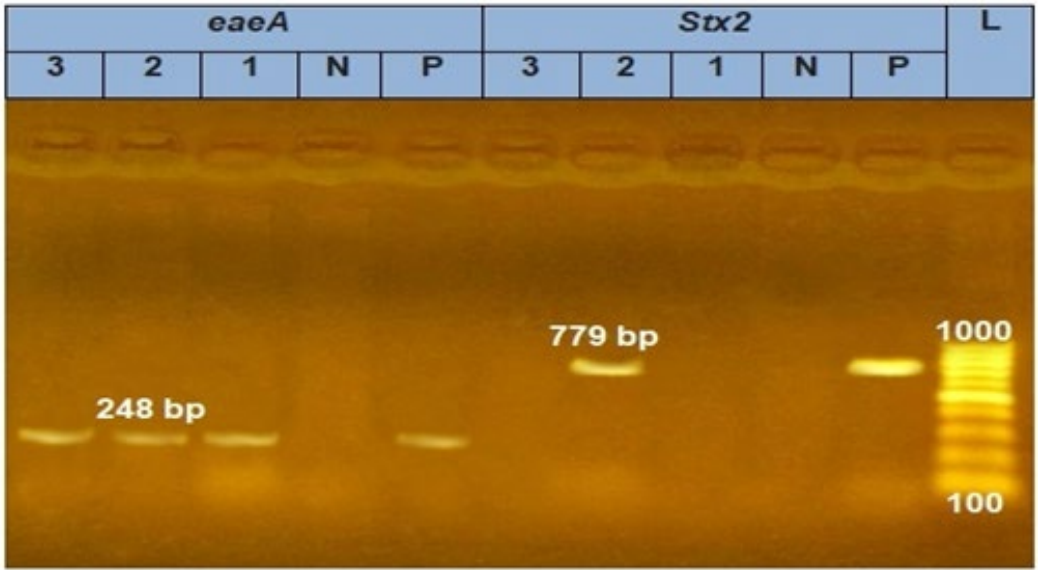

Figure 2: Agarose gel showing polymerase chain reaction amplification products of stx2 (779 bp) and eaeA gene (248 bp) in three E.coli strain, L:100-1000 bp DNA ladder, pos: control positive for eaeA and Stx2, Neg: control negative for eaeA and Stx2, lane 1-3: positive strain for eaeA , lane 2: positive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

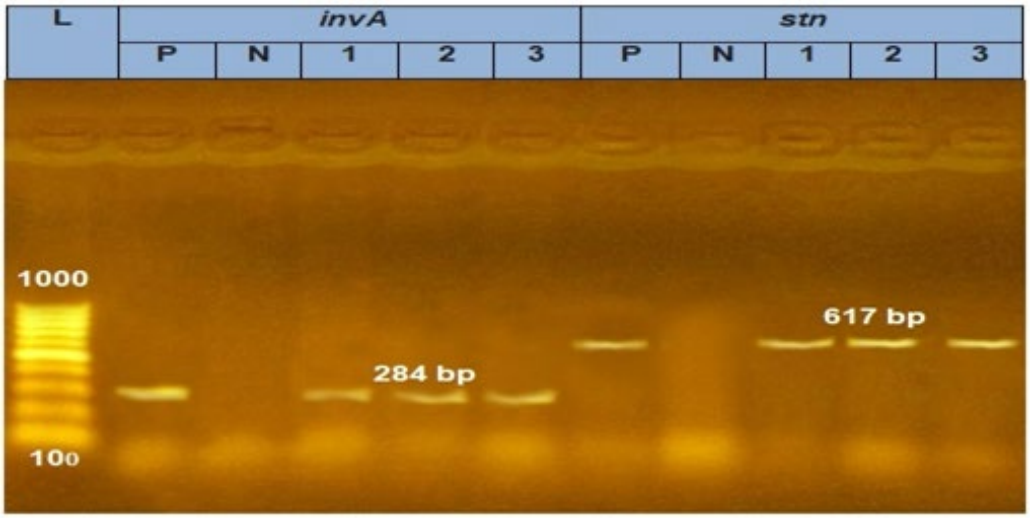

Figure 3: Agarose gel showing polymerase chain reaction amplification products of stn gene (617 bp) and invA (284) in three *Salmonella* isolates, L:100-1000 bp DNA ladder, pos: control positive for stn and invA, Neg: control negative for stn and invA, lane 1-3: positive strain for invA , lane 1-3: positive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

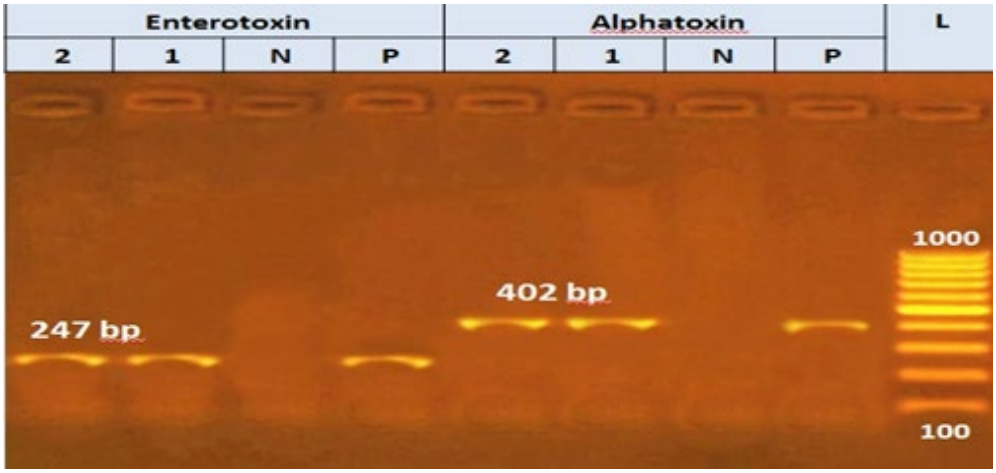

Figure 4: Agarose gel showing polymerase chain reaction amplification products of Alpha toxin (cpa) at 402 bp and enterotoxin (cpe) gene at 247 bp in two clostridium perfringens strain, L:100-1000 bp DNA ladder, pos: control positive for cpa and cpe, Neg: control negative for cpa and cpe, lane 1-2: positive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

Discussion

The contamination of meat products and meat with microorganisms from meat handlers, which may have carried the pathogenic microorganism during the processes of packing, manufacturing and marketing. Poor hygiene during production processes, refrigeration or the retail and storage of foods or improper cooking may lead to food poisoning or meat borne illness causing increase in disease burden and consequent death in most developing countries [25]. Disease burden from improper food handling has swallowed billions of dollars in social costs and medical care [25]. TVB-N value has been used for assessing the degree of deterioration of meat. On the other, TBA is a good indicator of the quality of meat, since it is able to assess malondialdehyde concentrate on which considered as a secondary product of lipid peroxidation and a good indicator for assessing the lipid oxidation level [26].

The TVN high level in the different meat product may be due to post processing circumstances. These circumstances include failure in freezing during marketing, storage and distribution [27]. Bad handling during processing and the use of old meat may have been responsible for the recorded high TBA values in the present studies. The above-mentioned factors may have resulted in the excessive oxidation of fat in the different meat products. Poor technology in the different factories handling meat products may be another possible explanation for increase in TBA level in the different meat products. Reported a mean TVN values of 13.06 ±0.04 in the liver sample they examined which was almost close to the values of TVN we observed in the present study [27]. Reported a mean TVN value of 12.60 in minced meat which was slightly higher than what we observed in the present study [28]. Higher TVN value was also reported by who reported 16.23, 24.69 and 22.1 for sausage, minced meat and luncheon; respectively [29]. Lower results of TBA (0.16 ± 0.01) in liver was reported by [27]. reported 0.17 ± 0.02 in liver samples; reported 0.45 and 0.25 in sausage and luncheon; respectively. Higher TBA in minced (0.7) was reported by and in sausage (0.68) by [28-30]. Mean values of TVN in all the examined meat products we sampled were within the safe acceptable limit (should not exceed 20 mg/100 gm) as recommended by for TVN in frozen liver, minced meat and sausage [31]. In addition, according to same specifications stated that TBA mean value in the examined meat products were within the acceptable limit (should not exceed 0.9 mg malonaldehyde/kg of sample) as recommended by [31].

The obtained result in Table (2) revealed that aerobic mesophilic bacteria were detected in all examined meat products samples with mean values of $1.70\times10^{6}\pm0.13\times10^{6}$, $5.68\times10^{5}\pm0.42\times10^{5}$, $4.38\times10^{4}\pm0.38\times10^{4}$, and $7.90\times10^{5}\pm0.15\times10^{5}$ Cfu/g, in examined minced meat, liver, luncheon and sausage; respectively. Lower results of aerobic bacterial count in examined sausage, minced meat and luncheon was mentioned by 3.3x105, 3.3x105 and 3.3x105 Cfu/g; respectively [29]. In addition, 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 [32]. The total aerobic counts reflect the bacterial contamination level and hygienic quality of meat products [33].

Recorded data in table (3) showed that *Staph. aureus* was detected at level of 30, 24, 20 and 40% in examined sausage, liver, luncheon and minced meat with mean values of $1.85\times10^{3}\pm0.32\times10^{3}$, $3.33\times10^{3}\pm0.44\times10^{3}$, $5.20\times10^{2}\pm0.41\times10^{2}$ and $3.15\times10^{4}\pm0.47\times10^{4}$ Cfu/g; respectively. Lower level of Staph. aureus was reported by $0.2x10^{2}$ Cfu/g in minced meat; $0.1x10^{2}$ Cfu/g in luncheon and $0.3x10^{2}$ Cfu/g in sausage [29]. Higher results of Staph. aureus in sausage was reported by 2.8x104 Cfu/g. Lower incidence of Staph. aureus in luncheon, sausage and minced meat 8,6, 20 and 25.7 % was reported by [32, 34]. Staph. aureus can be carried in nasal passage and on the hands. Most outbreaks of food borne disease due to contamination following poor handling and production of heat stable toxins Staph. aureus. Refrigeration, proper cooking and sanitary food handling are recommended measure to prevent disease outbreak related to Staph. aureus [35].

The present investigation showed that all three tested *Staph. aureus* strains 100% harbored pvl gene at 433 bp and sea at 102 bp by PCR (Fig 1). could detected Sea gene in one *Staph. aureus* strain isolated from meat products [36]. Results from the present study are line with the finding of who recorded detection of enterotoxigenic *Staph. aureus* isolates in meat samples by using multiplex PCR [37]. The enterotoxin genes detection in given strain of *Staph. Aureus* by using PCR is very important for the identification of staphylococcal related food poisoning [38]. Results demonstrated in table (4) revealed that the incidences of enteropathogenic E. coli in examined minced meat, liver, luncheon and sausage were 36, 10, 8 and 30%; respectively. Presence of *E. coli* is a good marker for possible contamination of food product with fecal material [4]. Lower incidence of *E. coli* in minced meat (22.9 & 16 %), in sausage (17.1 & 20%) were reported by and respectively [32, 39].

In addition, cited that the incidence of E. coli in sausage was 9% [40]. Nearly similar incidence of E. coli in luncheon 8% was reported by while, higher incidence of E. coli in luncheon was 36 and 28% reported by and [41-43]. Lower incidence (5.7%) in luncheon was reported by [32]. The practices of manufacture and storage, handling from production sites to the consumers and effectiveness of hygienic measures may be responsible for the observed variation reported by the different authors. PCR results obtained in Fig (2) showed that eaeA gene (248 bp) detected in 100 % of all isolated E. coli Strain while, Stx2 gene (779 bp) detected only in one isolated E. coli strain. These results agreed with who reported that eaeA gene (248 bp) was detected in 100% of isolated E. coli strains from meat products. In contrary, these results disagreed with who reported that E. coli isolates from minced meat and sausage were negative for Stx2 at 779 bp [30, 44]. Abovementioned results in table (5) showed that the incidence of Salmonella in minced meat, liver and sausage were 2, 4 and 4%; respectively while, Salmonella was not detected in examined luncheon samples. Salmonella species isolated from minced meat were serologically identified into Sal. enteritidis 100; from liver into Sal. enteritidis (50%) and Sal. typhimurium (50%) finally from sausage into Sal. enteritidis (50%) and Sal. typhimurium (50%).

Our finding agreed with who reported that Salmonellae failed to be detected in the examined luncheon samples [45]. the same author could isolate Salmonella from sausage at incidence rate 10% and serologically identified in Sal. typhimurium (40%), Sal. typhi (40%) and Sal. enteritidis (20%) while, isolated Salmonella from minced meat at incidence rate 6% and serologically identified into Sal. typhi 33.3% and S. typhimurium 66.2%. In contrary, could isolate Salmonella from luncheon at incidence rate 12% [39]. Nearly similar incidence of Salmonella in minced meat 2.8% was reported by while, higher incidence in minced meat 24% was reported by [39, 46]. Nearly similar incidence of Salmonella in sausage 4% was reported by while, higher results 24% were reported by [29].

In the present study PCR assay for detection of invA and Stn gene in three Salmonella isolates revealed that, two genes detected in all isolates (100%) at 617 and 284 bp PCR amplified fragment (Fig, 3). The results were agreed with who mentioned that all seven isolated Salmonella from meat products (100%) harbored invA gene at 284 bp. In addition, confirmed the presence of Stn gene in all identified Salmonella isolates form meat products [47, 48]. cited that, PCR is a specific, fast highly reliable and sensitive procedure used for characterization of Salmonella when compared to traditional microbiological techniques [49]. Using primers of invA specific for Salmonella in PCR significantly decreases possibility of false negative results. Amplification of invA gene is considered as an international standard for detection of Salmonellae identification. It contains sequences only for Salmonella and proved to be a suitable PCR target, with an important diagnostic application [50]. The stn gene is a very important Salmonellae virulent genes which assist Salmonellae in expressing its virulence in the host and manifestation of its pathogenicity (mainly diarrhea) associated with Salmonellae infection [51].

As shown in Table (6) bacteriological examination of meat products samples revealed that B. cereus were found in 16, 20, 24 and 34 % of examined minced meat, liver, luncheon and sausage with mean values of $3.57\times10^3\pm0.54\times10^3$, $3.20\times10^3\pm0.38\times10^3$, $3.08\times10^3\pm0.46\times10^3$ and $1.61\times10^4\pm0.11\times10^4$ Cfu/g; respectively. Nearly similar incidence of B. cereus in minced meat 17.14 % and in luncheon 20 % was reported by [52]. Higher incidence of B. cereus in minced meat 76% and luncheon 44% were reported by [53]. In contrary, could not isolate B. cereus from minced meat. B. cereus had been implicated in many disease outbreaks related to food poisoning [54]. These outbreaks are mostly characterized by abdominal pain and diarrheal syndromes however, there are many cases of food poisoning with similar signs caused by Staph. aureus and C. perfringens [55]. B. cereus survival in meat and milk products which attributed to its spore formation ability therefore, increasing its possible viability [56]. Tabulated results in table (7) showed that C. perfringens were detected in examined minced meat, liver, luncheon and sausage at incidence rate 28, 10, 18 and 36% with mean values of $2.6\times10^3\pm0.24\times10^3$, $2.16\times10^3\pm0.26\times10^3$, $1.75\times10^2\pm0.24\times10^2$ and $2.59\times10^3\pm0.24\times10^3$ Cfu/g; respectively.

Higher incidence of Clostridia obtained by who cited that the incidence of Clostridia in sausage and luncheon examined samples were 60 and 52%; respectively [57]. In addition, and reported higher incidence of Clostridia in minced meat which was 93.3 and 55 %; respectively [58, 59]. Presence of large number of Clostridia in meat products may be responsible for lowered quantity of meat products, with consequent public health related hazards and economic losses [60]. The variation in the incidence of Clostridia in minced meat may be attribute to poor hygienic measures during processing. Clostridia spores could be separately added to meat products during cutting, wrapping and handling of meat [58]. 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 [61]. PCR results in Fig (4) declared that two tested C. perfringens strains possess enterotoxin gene (cpe) at 247 bp and alpha toxin (cpa) at 402 bp by PCR. These results agree with who found that all tested C. perfringens

type A (33, 100%); respectively possess alpha cpa at 204 bp and cpe at 247 bp [58].

Conclusion

The present study supported the fact meat products are of possible public health concern. The presence of aerobic bacteria like Staph. aureus, E. coli, bacillus cereus, Salmonella and C. perfringens may be due to poor hygienic measures during processing and handling of meat products. So, consumption of these products could be associated with possible risk of infection. Therefore, efficient heat treatment, adequate cleaning and sanitization of utensils may assist reducing this contamination and implement hazard analysis may lead to an improvement in hygiene.

Implications for Policy and Practice

Meat products such as minced meat, sausage, luncheon and burger are highly demanded because in most cases they are considered more attractive for consumers than fresh meat due to their good taste and high nutritive value. Despite the importance of meat products in human nutrition, their contamination with various types of foodborne pathogens can occur. The presence of aerobic bacteria like Staph. aureus, E. coli, bacillus cereus, Salmonella and C. perfringens may be due to poor hygienic measures during the processing and handling of meat products. So, consumption of these products could be associated with the possible risk of infection, suggesting the need for the legislation and institution of strict hygienic measures during the handling of meat products by policymakers.

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