



Microbial Contamination and Adulteration Detection of Meat Products in Egypt

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ABSTRACT

Meat products as rich sources of several nutrients are easily contaminated by microorganisms, which are widely predominant in the environment. Moreover, meat products could also be adulterated with equine, pig, chicken, and mouse meat, and their existence is risky for consumers. A total of 140 meat product samples (minced beef meat, beef burger, sausage, beef luncheon, frankfurter, kofta, and bastirma; 20 samples from each product) were gathered from various supermarkets in Cairo governorate, Egypt. Those samples were subjected to bacteriological and mycological examination together with a novel multiplex PCR method to detect bovine, donkey, horse, pork, chicken, and mouse species in the processed meat in a single reaction step. The results revealed that minced meat had the highest total colony count and *Staphylococcus aureus* counts were the highest in sausage samples. *Escherichia coli* mean values were the highest in luncheon samples and minced meat indicated the highest *Salmonella* count. However, beef burger counts were the highest for both yeast and mold count. For multiplex PCR results, only 16.4% of meat products samples were negative for bovine species. Moreover, 88.5% and 9.2% were respectively positive to chicken and mouse species and negative to equine and pig species. Thus, microbial contamination reported in the current study could raise attention toward the implementation of food safety standards in both factories and markets. Moreover, and multiplex PCR-based assay executed in the current study is suggested since it could afford sensitive and specific detection of mitochondrial cytochrome-b (mtcyt-b) DNA in processed meat products samples to detect and prevent troubles related to wellbeing and safety.

Keywords: Adulteration, Bacteriological, Meat products, Multiplex PCR, Mycological

INTRODUCTION

Meat and meat products are nutritive food for human beings as they are rich in protein, basic amino acids, vitamins, fats, minerals, and other nutritive constituents (Biesalski, 2005). However, meat products can serve as a suitable culture medium for the growth of different organisms because of high moisture, high consistent of nitrogenous compounds, great supply of minerals, a few fermentable carbohydrates as glycogen, and the ideal pH that support the growth of most microbes (Alahakoon et al., 2015).

Raw meat contains different pathogenic organisms, such as *Salmonella* spp. *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*). This makes raw meat a real danger to human health since it can easily cause food-borne diseases as a result of bad handling and improper control measures against those pathogens (Nørrung et al., 2009). *Staphylococcus aureus* is an important source of worldwide food intoxication. Different types of foods may be contaminated by this microorganism and develop numerous forms of enterotoxins due to inappropriate processing of meat products (Balaban and Rasooly, 2000).

Mycotic contamination of meat products may originate from meat or bad quality flavoring materials, particularly spices, and improper hygienic procedures during various processing steps and storage of the products (Gourama and Bullerman, 1995). Fungal contamination of meat products is considered a major public health hazard as fungi can cause three different types of illnesses, namely mycosis, mycotoxicosis, and allergy (Abuzaid et al., 2020).

Accomplishing food safety is a worldwide health objective and food-borne manifestations that have attracted major attention in global health. Hence, the determination of microbial pathogens in food is the key in the identification and prevention of problems related to wellbeing and safety (Gokulakrishnan and Vergis (2015). False or inadvertent mislabeling of meat products, which can not be distinguished using conventional strategies is still predominant over the world. Some individuals may not like to consume the meat of equine and pork due to ethical, religious, or compassionate reasons. So, these clusters of consumers demand methods to detect types of meat (equine, pork, chicken, and mouse) in the food (Haunshi et al., 2009).

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The species of meat used in prepared, cooked, or compounded blends is not continually plausible to be distinguished by schedule examination. However, denaturation of meat proteins during heat treatment or any other technological processes and variation of protein compositions, even in the same species, reduce the chance of success for these methods. Besides, these strategies may be insufficient to segregate between species which are in near connection and are not reasonable for schedule utilize, as the confinement of species-specific proteins is troublesome and time-expending (Kesmen et al., 2010). DNA analysis is now a famous approach for meat species identification. Nowadays, DNA is preferred for species identification due to its great constancy, compared to proteins. Applications of polymerase chain reaction (PCR) have been augmented because of their easiness, speed, and specificity (Kesmen et al., 2007). Multiplex-PCR is an effective technique that can instantaneously amplify the template mix, diminish the recognition charge, and eliminate the failure of single PCR of detecting only one meat species at a time (Abuelnaga et al., 2021).

The current study aimed to detect microbial load and identify foodborne pathogens in different meat product samples to prevent manifestations related to health and safety. Moreover, it investigated the application of a novel multiplex PCR (M-PCR) as a sensitive and specific method to detect adulteration in meat products sold in different supermarkets in Cairo, Egypt with equine, pig, chicken, and mouse meat.

MATERIALS AND METHODS

Ethical approval

The current study was conducted on meat product specimens and no invasive procedures were performed on animals, no ethical approval was required.

Samples

A total of 140 meat product samples included 20 samples from each product of minced beef meat, beef burger, sausage, beef luncheon, frankfurter, kofta, and bastirma were gathered from different supermarkets in Cairo area, Egypt from June 2020 until June 2021. The samples were microbiologically examined (identification and counting of microbes) and also underwent multiplex PCR for the detection of species adulteration. Meat specimens from different chosen species (bovine, equines, pig, chicken, and mouse) were utilized as a positive control when testing the meat product specimens. Meat product samples which were negative to bovine Spp. or positive to the other tested species were considered adulterated.

Preparation of samples for microbiological examinations

To begin 10 g from each specimen were blended with 90 ml of 0.1% sterile peptone water and were mixed for 2-4 minutes and were left to stand for approximately 5 minutes at room temperature; at that point 10 times serial dilutions were executed for counting microbes under complete aseptic condition (Datta et al., 2012).

Bacteriological examinations of meat products

The total bacterial count was done by utilizing a standard plate count agar medium. In this regard, 1 ml from each of the already prepared serial dilutions (10^2 to 10^6) was inoculated in duplicate plates under aseptic circumstances and they were incubated at 37°C for one day. Later, the counted colonies were calculated as cfu/g and recorded (Datta et al., 2012).

Determination of total *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* counts

Total *E. coli*, *S. aureus*, and *Salmonella* counts were performed. To this end, 1 ml from each of the previously prepared serial dilutions was inoculated into duplicate plates of E.M.B, Mannitol salt, *Salmonella Shigella* agar plates and were kept at 37°C for a day. Colonies were counted and these colonies were identified using API 20E kit (Bio Merieux) and the identification was done according to the manufacturer's directions in order to detect the biochemical profile of the isolated microbes (Datta et al., 2012)

Serological identification

The somatic (O) antigen of *E. coli* was detected by slide agglutination test as described by Edwards and Ewing (1972), and Flagellar (H) antigen serotyping was performed according to Davies and Wray (1997). Anti-O-sera was purchased from DENKA SEIKEN CO LTD Tokyo, Japan. *Salmonella spp.* was serotyped as reported by Bale et al. (2007).

Mycological examination of meat products

Pour plating

At the stage, 1 ml of the prepared dilutions (10^2 to 10^6) was added into a petri dish in duplicate. Pour into each Petri dish 10 to 20 ml molten SDA (cooled to 42-45°C). Blend the media and dilutions by whirling clockwise and anti-clockwise and permit solidification (Soliman et al., 2019)

Isolation and identification of fungi

Inoculated Petri dishes were left to solidify at ambient temperature. The plates were inverted to anticipate spreaders and incubated at 25°C for 3-5 days. The yeast colonies, which were in a dull white, creamy, yellow, pink, regular, and irregular shape, were counted independently using a colony counter and yeast count/gram was calculated and recorded, and for mold, the plates were incubated at 25°C for 5-7 days at an inverted position. During the incubation period, the plates were inspected daily for star-shaped mold growth, and colonies were counted and recorded (APHA, 1992). Identification of isolated molds was performed according to Samson et al. (2010).

Multiplex polymerase chain reaction

DNA extraction from meat samples

DNA was extracted from meat samples using the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700, Thermo Fisher Scientific Inc., USA) Following manual instructions with some modifications where 25 mg of tissue samples are used and DNA was eluted in 50 µL of the elution buffer included in the kit.

Primer design

Species-specific PCR primers targeting mitochondrial Cytochrome-b gene of bovine, equines (donkey and horse), pig, and chicken, and the 12S ribosomal RNA mitochondrial gene of the mouse have been developed as shown in Table 1. All primers were obtained from Vivantis Technologies, Malaysia.

Multiplex Polymerase Chain Reaction

The 25µl reaction mixture was prepared using 12.5µl of 2x COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK), 0.5 µL (20 pmol) of each primer, and 1 µL of target DNA. The M-PCR cycles began with Initial Denaturation at 95°C for 2 minutes, followed by 39 cycles of denaturation at 95°C for 20 seconds, and annealing for 30 sec at 56°C (Table 1). After that extension was at 72°C for 45 seconds and a single final extension was at 72°C for 10 minutes (GS-96 gradient thermocycler, Hercuvan, Malaysia). Electrophoresis was performed on agarose gel (1.5%) at 100V for 1h with the use of a 50 bp ladder plus (Cat No. M7115 BIOMATIK, Canada) and examined using InGenius3 gel documentation system (Syngene, UK).

Statistical analysis

Data were analyzed descriptively (mean, maximum, maximum, and standard error) using SPSS 14.

Table 1. The primer pairs used in specific PCR identification of bovine, Donkey, pig, chicken, and mouse meats

Species	Sequence (5'-3')	PCR product	Anneal. temp.	Reference
Bovine	GCCATATACTCTCCTTGGTGACA GTAGGCTTGGGAATAGTACGA	271bp	56°C	Ilhak and Arslan (2007)
Donkey	ATCCTACTAACTATAGCCGTGCTA CTATCCGACACACCCAGAAGTAAAG	145bp	56°C	Kesmen et al. (2007)
Horse	CTATCCGACACACCCAGAAGTAAAG GATGCTGGGAAATATGATGATCAGA	153bp	56°C	Kesmen et al. (2010)
Pig	CCCAGCCCCCTCAAACATCTCA ATGTACGGCTGCGAGGGCGGTAA	525bp	56°C	Khairalla et al. (2005)
Chicken	CTCGCCCTACTTGCCTTCC TAGGACGCAACGCAGGTGT	256bp	56°C	Haunshi et al. (2009)
Rat and Mouse	AATCCAACCTTATATGTGAAAATTCATTGT TGGGTCTTAGCTATCGTCGATCAT	96bp	56°C	Martín et al. (2007)

RESULTS

Meat products samples were microbiologically tested and the results were presented as mean values ± standard error (SE) counts /g for total colony count (TCC), *S. aureus*, *E.coli*, and *Salmonella* (Table 2). Table 3 shows the incidence of *S. aureus*, *E.coli*, and *Salmonella* in meat product samples. *Salmonella spp* were serologically identified as *S. Typhimurium*, *S.Kentucky*, *S. Enteritidis*, *S. Blegdam*, and *S. Agama*. The distribution of those serovars in different meat product samples is presented in Table 4. *Escherichia coli* was serologically identified as *O 26*, *O 126*, *O 111*, *O158*, *O 146*, and untyped *E.coli*. The presence of different serovars in various meat product specimens is presented in Table 5.

The mycological examination of meat products (minced beef meat, beef burger, sausage, beef luncheon, frankfurter, kofta, and bastirma) revealed that the incidence of yeast contamination was 80% , 75%, 55%, 60%, 65%, 60%, and 50%, respectively, and 50%, 65%, 77%, 60%, 70%, 50%,80%, for molds, respectively. Fungal counts for yeast and molds are presented in Table 6.

Multiplex PCR results, the identification of bovine, donkey, horse, pig, chicken, and mouse tissues in the meat products, specificity of the species-specific primers, and peak PCR conditions were intended. The primers provided unique species-specific fragments of 271, 145, 153, 525, 256, and 96 bp, respectively (Figures 1 and 2). In the present study, molecular examination of meat product samples revealed that only 16.4% of meat products samples were negative to bovine Spp. Moreover, 88.5% and 9.2% were positive to chicken and mouse Spp. respectively, and negative to equine and pig Spp. as shown in Table 7.

Table 2. Bacterial counts of different meat products in Egypt during 2020-2021

Samples	TCC	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella</i>
Minced beef meat	$1.8 \times 10^6 \pm 6.6 \times 10^5$	$1.1 \times 10^3 \pm 5.6 \times 10^2$	$9.7 \times 10^2 \pm 3.8 \times 10^2$	$4.8 \times 10^2 \pm 1 \times 10^2$
Beef burger	$4.1 \times 10^5 \pm 2.8 \times 10^5$	$6.8 \times 10^2 \pm 1.7 \times 10^2$	$1.5 \times 10^2 \pm 6.4 \times 10$	$2 \times 10^2 \pm 4.4 \times 10$
Sausage	$1 \times 10^6 \pm 4.2 \times 10^5$	$1.2 \times 10^3 \pm 3.7 \times 10^2$	$8.1 \times 10^2 \pm 2.6 \times 10$	$4.3 \times 10^2 \pm 1 \times 10^2$
Beef luncheon	$8.6 \times 10^5 \pm 3.7 \times 10^5$	$8.2 \times 10^2 \pm 3.6 \times 10^2$	$1.3 \times 10^3 \pm 4 \times 10^2$	$2.2 \times 10^2 \pm 7 \times 10$
Frankfurter	$1.7 \times 10^4 \pm 8 \times 10^3$	$2.4 \times 10^2 \pm 6 \times 10$	$2 \times 10^2 \pm 5.7 \times 10$	$8.1 \times 10 \pm 0.5 \times 10$
Kofta	$6.1 \times 10^5 \pm 3.1 \times 10^5$	$4.4 \times 10^2 \pm 9.5 \times 10$	$4.5 \times 10^2 \pm 1 \times 10^2$	$2.7 \times 10^2 \pm 10 \times 10^2$
Bastirma	$2.3 \times 10^4 \pm 8.5 \times 10^3$	$3.9 \times 10^2 \pm 1 \times 10$	$3.4 \times 10^2 \pm 9.9 \times 10$	$5.5 \times 10 \pm 1.7 \times 10$

TCC: Total colony count

Table 3. Incidence of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* in meat product samples in Egypt during 2020-2021

Samples	<i>Staphylococcus aureus</i>		<i>E.coli</i>		<i>Salmonella</i>	
	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*
Minced beef meat	7	35	9	45	4	20
Beef burger	5	25	2	10	3	15
Sausage	8	40	6	30	4	20
Beef luncheon	6	30	11	55	3	15
Frankfurter	2	10	4	20	2	10
Kofta	4	20	5	25	3	15
Bastirma	3	15	4	20	2	10
Total (140)	35	25**	41	29.3**	21	15**

* Twenty samples of each meat product were examined the percent was calculated according to the number of each meat product (n = 20). ** The percent was calculated according to the number of all meat product samples (140). No. of +ve: Number of positive.

Table 4. *Salmonella* serovars isolated from meat products in Egypt during 2020-2021

Meat product samples	<i>Salmonella Typhimurium</i>		<i>Salmonella Kentucky</i>		<i>Salmonella Enteritidis</i>		<i>Salmonella Blegdam</i>		<i>Salmonella Agama</i>	
	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*
Minced beef meat	2	50	0	0	1	25	1	25	0	0
Beef burger	1	33.3	1	33.3	1	33.3	0	0	0	0
Sausage	2	50	1	25	0	0	1	25	0	0
Beef luncheon	2	66.6	1	33.3	0	0	0	0	0	0
Frankfurter	0	0	1	50	0	0	0	0	1	50
Kofta	0	0	1	33.3	1	33.3	0	0	1	33.3
Bastirma	0	0	1	50	1	50	0	0	0	0

* The percent was calculated according to the number of each serovar of meat product samples.

Table 5. *Escherichia coli* serovars isolated from meat products in Egypt during 2020-2021

Meat product samples	<i>O 26</i>		<i>O 126</i>		<i>O 111</i>		<i>O 158</i>		<i>O 146</i>		<i>O 114</i>		untyped	
	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*	No. of +ve	%*
Minced beef meat	2	22.22	1	11.11	2	22.22	1	11.1	1	11.11	1	11.1	1	11.11
Beef burger	1	50	0	0	0	0	1	50	0	0	0	0	0	0
Sausage	2	33.3	1	16.7	1	16.7	0	0	0	0	2	33.3	0	0
Beef luncheon	2	18.8	2	18.18	1	9.1	1	9.1	2	18.18	1	9.1	2	18.18
Frankfurter	1	25	1	25	2	50	0	0	0	0	0	0	0	0
Kofta	1	20	2	40	0	0	0	0	2	40	0	0	0	0
Bastirma	1	25	0	0	0	0	1	25	0	0	1	25	1	25

*The percent was calculated according to the number of each serotype for each meat product

Table 6. Total yeast and mold counts in different meat product samples in Egypt during 2020-2021

The examined samples	Total yeast (count /gm)	Total molds (count /gm)
Minced beef meat	$1.3 \times 10^3 \pm 1.4 \times 10^2$	$2.5 \times 10^2 \pm 1.6 \times 10^2$
Beef burger	$1.4 \times 10^3 \pm 9 \times 10$	$1.3 \times 10^3 \pm 9.2 \times 10^2$
Sausage	$4.7 \times 10^2 \pm 4.1 \times 10^2$	$3.4 \times 10^2 \pm 2.1 \times 10^2$
Beef luncheon	$1.6 \times 10^3 \pm 1 \times 10^3$	$2.3 \times 10^2 \pm 1.3 \times 10^2$
Frankfurter	$1.1 \times 10^2 \pm 1.8 \times 10$	$0.6 \times 10 \pm 0.2 \times 10$
Kofta	$1.8 \times 10^3 \pm 1.3 \times 10^3$	$0.5 \times 10 \pm 0.1 \times 10$
Bastirma	$4 \times 10 \pm 0.5 \times 10$	$0.4 \times 10 \pm 0.07 \times 10$

The results were expressed as mean \pm Standard Error

Table 7. Number and percentage of adulterated meat samples

Sample	Bovine (-ve)		Equine (+ve)		Pig (+ve)		Chicken (+ve)		Mouse (+ve)	
	No	%*	No	%*	No	0%*	No	%*	No	%*
Minced beef meat	10	50%	0	0%	0	0%	20	100%	3	15%
Beef burger	5	25%	0	0%	0	0%	20	100%	0	0%
Sausage	8	40%	0	0%	0	0%	18	90%	4	20%
Beef luncheon	0	0%	0	0%	0	0%	18	90%	1	5%
Frankfurter	0	0%	0	0%	0	0%	16	80%	0	%
Kofta	0	0%	0	0%	0	0%	19	95%	5	25%
Bastirma	0	0%	0	0%	0	0%	13	65%	0	0%
Total	23/140	16.4%**	0/140	0%**	0/140	0%**	124/140	88.5%**	13/1140	9.2%**

*: Twenty samples of each meat product were examined and the percentage was calculated according to the number of each meat product (n = 20). **: The percent was calculated according to the number of all meat product samples (n=140). +ve: Positive means adulterated

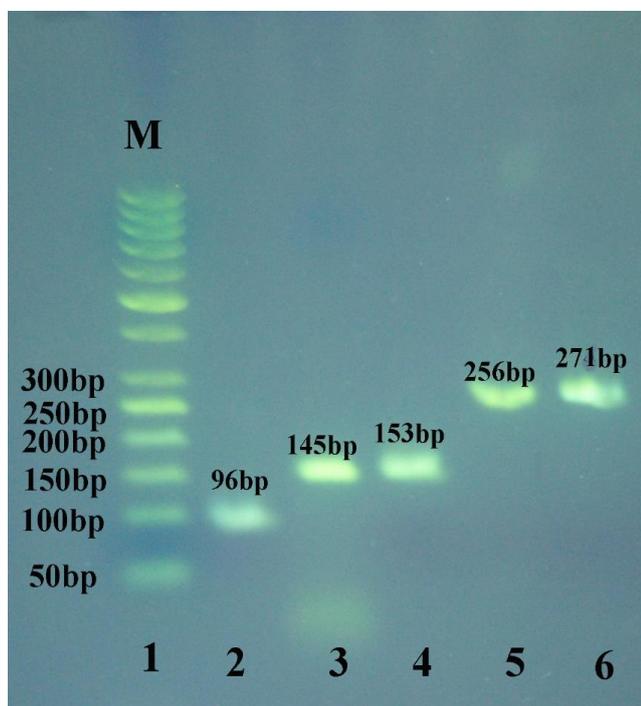


Figure 1. The PCR product of control meat (mouse, donkey, horse, chicken, and bovine meat) amplified with species-specific primers. **1:** Molecular marker (50 bp), **2:** Mouse meat; **3:** Donkey meat; **4:** Horse meat; **5:** Chicken meat; **6:** Bovine meat.

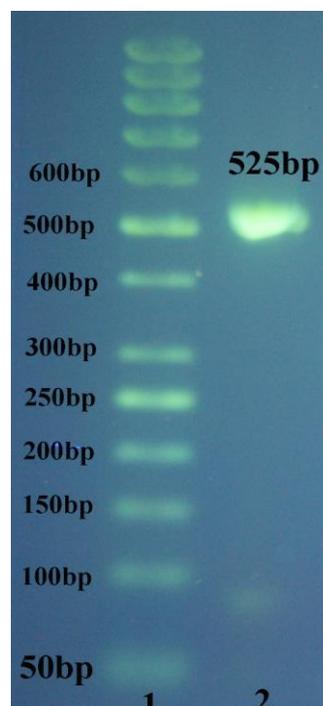


Figure 2. The PCR product of control meat (pig meat) amplified with pig-specific primers. **1:** Molecular marker (50 bp), **2:** Pig meat.

DISCUSSION

Meat is favored by millions of people worldwide as a major supply of animal protein but it is considered a favorable 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 constituted as major causes of most known food poisoning outbreaks. Therefore, it is crucial to use microbiological criteria to detect the quality of those products (Abuzaid et al., 2020).

As indicated in Table 2, the total colony count results obtained in the current study were similar to those reported by Erdem et al. (2014) 9×10^6 CFU/g in minced meat in Istanbul. Shaltout et al. (2016) declared that the mean counts in luncheon, minced meat, kofta, and sausage were 4.2 ± 0.1 , 4.8 ± 0.1 , 5.8 ± 0.1 , and 6.1 ± 0.1 log CFU/g in Egypt, respectively, Abuzaid et al. (2020) calculated the mean of total bacterial counts of 80 types of meat products in Egypt and the counts were $11 \times 10^6 \pm 5.4 \times 10^6$, and $2.04 \times 10^6 \pm 0.12 \times 10^6$ cfu/g for sausages and Kofta, respectively. However, lower counts were obtained by Salem et al. (2010) as they found that the total bacterial count was 5.61×10^5 CFU/g in minced meat in Egypt, and also Mousa et al. (2014) obtained lower results as they stated that the total aerobic bacterial counts were 8.20×10^2 , 6.29×10^2 , 5.4×10^2 , and 8.28×10^2 in the beef burger, luncheon, bastirma, and sausage, respectively in Egypt. Higher results were recorded by Gönülalan and Köse (2003) that was 5.3×10^9 in minced meat of China and Ahmed (2009) who reported that the mean value of APC was 1.26×10^6 for kofta and $11 \times 10^6 \pm 5.4 \times 10^6$ for Sausage in Egypt.

The total colony count results of *S. aureus* were similar to those obtained in Egypt by Morshdy et al. (2013) as *S. aureus* count of minced meat was 4.3×10^2 /g and also Shaltout et al. (2016) found that *S. aureus* counts were 2.5 ± 0.1 , 2.2 ± 0.1 , 2.5 ± 0.2 , and 2.6 ± 0.1 in luncheon, minced meat, kofta, and sausage, respectively. Higher values were obtained in Jordan by Al- Kour (2001) 4.13×10^3 /g in minced meat and Elmossalami (2003) 1.8×10^3 /g in sausages in Egypt.

Regarding the Coliform count presented in Table 2, the results were nearly similar to those obtained in Egypt by Salem et al. (2010) as 5.12×10^3 CFU/g in minced meat in Egypt and by Shaltout et al. (2016) as 2.6 ± 0.1 , 3.1 ± 0.1 , 2.6 ± 0.1 and 2.9 ± 0.01 log CFU/g in luncheon, minced meat, kofta, and sausage, respectively. Higher results were obtained by Erdem et al. (2014) in Istanbul as they recorded a mean count of 4.5×10^7 cfu/g in minced meat and Abuzaid et al. (2020) examined 80 specimens of meat products in Egypt for coliforms and mean counts were $6.7 \times 10^3 \pm 0.3 \times 10^3$ and $1.2 \times 10^3 \pm 0.16 \times 10^3$ for sausages and kofta, respectively. Lower results were obtained by Mousa et al. (2014) which were 2.92×10^2 , 3.5×10^2 , 4.19×10^2 , and 7.64×10^2 in the beef burger, luncheon, bastirma, and sausage, respectively, in Egypt.

The incidence of *S. aureus*, *E. coli*, and *Salmonella* in meat product samples is shown in Table 3. Similar results were obtained by Ragab et al. (2016) as they detected *E. coli* in 50 % of the examined minced meat samples and 30% of both kofta and beef burger samples in Egypt. Mousa et al. (2014) also recorded the incidence of *S. aureus* was 68%, 80%, 60%, and 88% in beef burger, luncheon, bastirma, and sausage, respectively, in Egypt. Higher results were obtained in Argentina by Stagnitta et al. (2006) as they isolated *E. coli* from 62.2%, 56%, and 24% in sausages, hamburgers, and minced beef, respectively. Variable results were obtained by Mousa et al. (2014) in Egypt as they stated that the incidence of *Salmonella* spp. was 20%, 26%, 6%, and 40% in the beef burger, luncheon, bastirma, and sausage, respectively.

In the present study, *Salmonella Typhimurium* was the most predominant serovar as clearly shown in Table 4 and Moawad et al. (2017) in Egypt obtained similar results indicating *Salmonella Typhimurium* as the most isolated serovar from both fresh and frozen beef meat.

Escherichia coli was serologically identified as O26, O126, O111, O158, O146, and untyped *E. coli*, and the distribution of those serovars in different meat product samples was shown in Table 5. *E. coli* O26 was the most prevalent serovar in the current study but Moawad et al. (2017) in Egypt identified *E. coli* type O158 mainly in beef meat.

The defilement of meat and meat products with various species of fungi and aflatoxins is considered a major public health hazard due to the dynamic increase in the utilization rates of these products. The exposure of meat and meat products to mycotic defilement due to the lack of hygienic conditions during slaughtering, processing, and storage, as well as the added spices and some food additives, constituted the principal source of toxigenic molds and mycotoxins leading to either food deterioration or foodborne mycotoxicosis (Al-Yazeed et al., 2015).

In the current study, the mycological examination revealed that the incidence of yeast contamination was 80%, 75%, 55%, 60%, 65%, 60%, 50% in minced beef, beef burger, sausage, beef luncheon, frankfurter, kofta, and bastirma, respectively. Similar results were also obtained in Egypt by Soliman et al. (2019) as they revealed that the recovery of yeasts from luncheon and hamburger were 65% and 77.5%, respectively. However, Ismail et al. (2013) obtained lower results as they isolated the yeasts from only 28% of the examined Luncheon samples.

For molds, the incidence of molds in minced beef meat, beef burger, sausage, beef luncheon, frankfurter, kofta, and bastirma were 50%, 65%, 77%, 60%, 70%, 50%, 80%, respectively, and similar results in Egypt were obtained by Al-Yazeed et al. (2015) as they mycologically examined 200 meat and meat products samples (50 of frozen meat specimens and 30 of each of minced meat, bastirma, hamburger, luncheon, and sausage) and found that the highest incidence of molds contamination was 86.66% in bastirma, and sausage samples, followed by 66.66% in hamburger, 60% in luncheon, 50% in minced meat. Soliman et al. (2019) also stated that the isolation of molds from luncheon and hamburger were 60% and 67.5%, respectively. Abd El-Tawab et al. (2020) assured that molds were isolated from 50% of the examined minced beef specimens, and Abuzaid et al. (2020) also found that the incidence of mold in the examined

samples of Kofta and sausage were 62.5% and 82.5%, respectively. However, the incidence was lower than the incidence of mold in kofta reported by Hussein (2008) as 93.3%. Higher results were also obtained by Ismail et al. (2013) as they isolated the molds from 92% of the examined luncheon samples and also Abd El-Tawab et al. (2020) revealed that molds were isolated from 80% of the examined sausage samples. Higher results were also obtained in Argentina by Stagnitta et al. (2006) as the counts of molds and yeasts were detected in 100% of the 515 samples.

In the current study, the mycological results of total yeast and mold count (mean \pm SE) in different meat product samples are shown in Table 6. In Egypt, Soliman et al. (2019) obtained similar results as they reported the mean of total yeast count/gm \pm standard error for luncheon and beef burger as $2.9 \times 10^3 \pm 7.2 \times 10^2$ and $4.3 \times 10^3 \pm 1.1 \times 10^3$, respectively. Abuzaid et al. (2020) examined 80 specimens of meat products represented by sausages and Kofta and the total yeast count was $0.52 \times 10^3 \pm 0.08 \times 10^3$ and $0.47 \times 10^3 \pm 0.07 \times 10^3$, respectively.

For molds, Elsayed et al. (2018) obtained similar results and found that the mean mold count was $1.3 \times 10^2 \pm 2.1 \times 10$ cfu/g, $2.8 \times 10^2 \pm 4.3 \times 10$ cfu/g, and $6.9 \times 10^2 \pm 1.2 \times 10^2$ cfu/g in luncheon, minced meat, and sausage in Egypt, respectively. A higher mold count was obtained by Abuzaid et al. (2020) as they examined 80 specimens of meat products, including sausages and Kofta with the total mold count of $1.1 \times 10^3 \pm 0.14 \times 10^3$ and $1.4 \times 10^3 \pm 0.27 \times 10^3$ in Egypt, respectively. Higher counts were obtained in Libya by Naas et al. (2009), as they mentioned that examined fresh beef sausage samples had a mold count of $2.3 \times 10^6 \pm 2.7 \times 10^5$ cfu/g. However, lower results were recorded by Mousa et al. (2014) as the total fungal counts were 4.7×10^2 , 1.21×10^2 , 1.22×10^2 , and 1×10^2 in beef burger, luncheon, bastirma, and sausage in Egypt, respectively.

The present paper describes the development and application of a novel multiplex PCR method to detect bovine, donkey, horse, pork, chicken, and mouse species in processed meat in a single reaction step as described by Xu et al. (2008). In the Egyptian markets, the addition of an undeclared meat species (such as chicken in beef products) and/or unacceptable meat type (pork and donkey, Zahran and Hagag, 2015; Yacoub and Sadek, 2017) or the replacement of one valuable species by another inexpensive one are common examples of meat adulteration (Ahmed et al., 2011; Zahran and Hagag, 2015).

The results of the current study indicated that 88.5% of meat samples (124/140) were positive for chicken spp. (Table 7). The current findings showed a high incidence of species fraudulence, particularly, 100% of minced beef meat and beef burger and 95% of kofta exhibited poultry contaminants. In Egypt, poultry meat is cheaper than beef, which may cause such adulteration. This instance can be considered a distinctive illustration of fraudulent replacement of a high value and more expensive meat species by inferior, lower value, and cheaper species, indicating an economic fraud (Abuelnaga et al., 2021).

The obtained values of the current study were higher than those reported by Abd El-Aziz et al. (2018) indicating 78% of kofta and hawawshy samples were found to be adulterated with chicken DNA. Moreover, this also was higher than those of Mehdizadeh et al. (2014) and Omran et al. (2019) that demonstrated that 94.4% of all hamburgers and 87.5% of all examined commercial meat products contained undeclared chicken meat. Away from price, religious customs are one of the distinguished subjects that should be taken into account. For example, it is forbidden for Muslims to consume the flesh of pork and its derivatives. Something else, some permitted meat is not alluring for Egyptian customers, as donkey and horse meat, that may be sold without any offered costs, and thus there's a critical chance of blending them in Egyptian nourishments (Ali et al., 2015). The adulteration rate with donkey meat in the current study (0%) was parallel to that of El-Shazly et al. (2016) and less than that reported value in Egypt by Abd El-Nasser et al. (2010) in minced meat (7%) and sausage (8%), Zahran and Hagag (2015) in beef meat (5%), Abd El-Razik et al. (2019) in beef meat (6.25%), Omran et al. (2019) in commercial meat products as beef burger, oriental beef sausages, kofta and beef luncheon from various regions of Upper Egypt (12.5%). In the present work, the result for equine meat was (0%) and this may be due to the strict control on food in Cairo, the Capital of Egypt.

Moreover, the adulteration rate of horse meat in the current study (0%) was less than that reported rates in Egypt by several investigators (El-Shewy, 2007; Abd El-Nasser et al., 2010; Jaayid, 2013), and the adulteration rate of beef meat (2.08%) as reported by Abd El-Razik et al. (2019). The results indicated that all the meat samples were negative to pig spp. which was parallel to the findings of El-Shazly et al. (2016) and Galal-Khallaf (2021) but lower than reported values by Meyer et al. (1996), Partis et al. (2000), and Yosef et al. (2014). Moreover, Abd El-Nasser et al. (2010) found that 35.7% and 41.7% of examined minced meat and sausage samples were adulterated with pork meat. Recently, soybean protein has been extensively used as a substitute for red meat in manufacturing burgers (up to 60%) as soybean has functional features, dietary value, along with its cheap price (Soares et al., 2013). The presence of soybeans constitutes commercial fraud in hamburgers collected from markets in Tehran City, Iran as indicated by the DNA extraction (Tafvizi and Hashemzadegan, 2016).

The findings of the present work also revealed that 50%, 25%, and 40% of minced beef meat, beef burger, and sausage were negative to bovine spp., respectively, although it was labeled as beef meat. These meat samples were supposed to be adulterated with soybeans. The adulteration rate with mouse meat in the current study (9.2%), especially

in kofta (25%) and sausage (20%), may be linked to the unhygienic measures during the processing steps or with the addition of contaminated soybean with mouse (rat) offal.

CONCLUSION

Although Meat products are rich in various nutritive substances, they can easily be attacked by different microorganisms, which are widely distributed in the environment. Processed meats are more susceptible to microbial contamination during different stages of processing. Hence, it is of utmost importance to screen the microbial quality of meat products to deliver better quality and safety. The highly sensitive, specific, and rapid M-PCR method developed in this work is strongly suggested to be used as a screening assay for adulteration detection and abuses of labeling necessities for meat products as this PCR method is valuable to detect the adulteration in products submitted to denaturing technologies.

DECLARATIONS

Authors' contribution

Azza S. M. Abuelnaga designed the plan of the study, participated in performing the experiments, and analyzed the data. Khaled Abd El-Hamid Abd El-Razik participated in designing the plan of the study, performing the experiments, and writing the manuscript. Mona MH Soliman took part in performing the experiments and writing the manuscript. Hala S. Ibrahim; Mona, M.M. Abd-Elaziz and Amany H. Elgohary performed the experiments and participated in analyzing the data. Riham H. Hedia and Elgabry E. A. participated in analyzing the data and writing the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

Consent to publish

The authors agreed to publish the article.

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