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Prevalence of *Pseudomonas aeruginosa*, *Escherichia coli*, and Their Virulence Genes in Adulterated Meat Products

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Abstract | Meat product adulteration is a prevalent issue that has been a significant concern for humanity as it reduces the quality of food by either adding low-quality nutrients or removing useful components from it, which can affect consumers who rely on meat as a primary source of these nutrients. Food adulteration can be intentional (for the financial benefit of producers, processors, and retailers), or incidental (happens during production, handling, and storage). This study used the sensitive and specific polymerase chain reaction (PCR) technique to identify the various meat species in meat products marketed as 100% beef and sold in Aswan City, Egypt, with distinct microbiological analysis that highlighted the detection of *Pseudomonas aeruginosa* and *Escherichia coli*. Ninety samples of meat in total (15 of each minced beef, sausage, burger, shawarma, and hawawshi) were obtained from several markets in Aswan City, Egypt, and exposed to bacterial analysis and PCR methods. The fraud samples exhibited a higher overall bacterial count than pure bovine meat. *P. aeruginosa* and *E. coli* were identified in 41.1% and 30% of the examined samples, with a high occurrence rate in minced beef samples. All *P. aeruginosa* isolates examined by PCR carried the *rpoB* gene, and 70% and 40% showed positive *lasB* and *exoS* virulence genes. Meanwhile, *E. coli* isolates showed *hylA*, *stx1*, and *stx2* virulence genes in 50%, 80%, and 20% of them. Furthermore, this investigation's PCR technique detected fraudulence in chicken, camel, pig, and equine meat in 100% of the beef samples, with rates of 63.9%, 19.4%, 8.3%, and 13.9%, respectively. This research suggests that quality control labs and inspection services can swiftly ascertain the contamination or adulteration of various meat products using these detection techniques. Issues such as contamination during DNA extraction, the presence of inhibitors, and suboptimal PCR conditions can affect the accuracy of results and are considered limitations of PCR.

Keywords: Adulteration, Contamination, Food safety, Meat products, Species

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INTRODUCTION

Meat is nutrient-dense and has a high nutritional value since it provides essential protein, lipids, vitamins, and minerals that are necessary for a balanced diet. The significance of value in meat and meat products has increased due to increased consumer awareness (Elbarbary *et al*., 2024). Meat is one of the most unpreserved foods when it involves preparing or storage because of its high protein and moisture content. It can spoil for a variety of reasons, including microbial infection (Lei *et al*., 2023). The application of various processing techniques, preservation methods, and technologies can have a significant impact on the nutrient content and safety of meat through physical and chemical changes Physical alterations are changes in tissue structure that affect the sensory features of the product, such as volume, appearance, color, texture, aroma, and taste. Dehydration reduces surface moisture in meat, protein denaturation increases moisture and fat retention, and incorporated additives enhance the functional properties of proteins. The molecular interactions that occur when applying thermal treatment, adding food additives, or prolonging storage cause the chemical alterations in meat (Gómez *et al*., 2020).

Food hazards are prevalent in the food industry and can enter the food chain through a variety of pathways, thereby escalating the risk of food contamination. A variety of sources, including human activities, ventilation systems, vermin, building design, and waste management, can contaminate food products through chemical, physical, and biological hazards. In order to ensure a safe and clean processing environment, it is necessary to conduct a thorough study of the ways that food gets contaminated and the mitigation strategies that can be used. Many dangerous bacteria, like *Salmonella* spp., *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (*P. aeruginosa*), are found in raw meat. This makes uncooked meat a real threat to human health because it can cause foodborne illnesses if it is not handled properly and there are not enough precautions taken to stop these bacteria from spreading (Nørrung *et al*., 2009).

Some foodborne illnesses caused by bacterial contamination are associated with poor handling practices during meal processing in food industries or food service establishments. Foodborne risk increases due to poor hygiene conditions during processing, storage, and distribution. In addition, cross-contamination due to the transfer of microorganisms from objects or surfaces to the food is an important issue. Indeed, a pathogenic microorganism can remain viable on cutting boards and food contact surfaces, demanding the rigorous cleaning and disinfection of kitchen utensils, equipment, and surfaces (Alves *et al*., 2021). *E. coli*, a prominent member of the Enterobacteriaceae family, has been associated with food poisoning incidents caused by undercooked meat. These bacteria appear to primarily infect beef. Diarrhoea outbreaks in infants and young children have been associated with Entero-hemorrhagic *E. coli* (EHEC). The usual symptoms of an EHEC infection are watery diarrhoea at first, followed by bloody diarrhoea later on (El-Sheikh *et al*., 2024). *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic Gram-negative bacterium that causes hospital infections, including bacteremia, and gastrointestinal and systemic infections (Spagnolo *et al*., 2021). The bacterium may be responsible for food decomposition, which is defined by the production of slime and malodour, pigment secretion, and off-flavours (Kolbeck *et al*., 2021). This bacterium poses a serious global problem of food rotting, particularly in underdeveloped nations where inadequate preparation and chilling methods are prevalent. Reports link *P. aeruginosa* to an increasing number of foodborne infections (Bantawa *et al*., 2021).

Another major issue facing meat consumers is the adulteration of meat species, which poses a hazard to public health and facilitates the spread of dangerous foodborne infections. To achieve their financial objectives, manufacturers will occasionally replace the main beef constituents with less expensive alternatives. According to Spink *et al*. (2019), these activities are harmful to clients, go against societal and religious values, and endanger public health. Furthermore, some people abstain from eating horse and pork flesh for ethical, spiritual, or humanitarian reasons. Consequently, these consumer groups look for ways to distinguish between different types of meat (such as camel, hog, chicken, and horse) in beef products (Haushi *et al*., 2009), which may be a threat to food safety or negatively affect the nutritional performance of foods. While food fraud is an intentional act for economic gain, a food safety incident is an unintentional act with unintentional harm, and a food defense incident is an intentional act with intentional damage (Tibola *et al*., 2018). Meat adulteration has economic consequences, including financial losses for both consumers and producers. Ethically, it involves deceiving consumers about the true nature of the meat they purchase. In addition to human health impacts, food malpractices also have negative socioeconomic impacts, including revenue loss and increased health costs (Willis *et al*., 2023). Furthermore, food fraud and adulteration always cause immense financial losses, as most consumers simply switch immediately to other products, categories, or brands and may not return to purchasing the original items (FAO, 2017).

Nowadays, the most well-known technique for identifying meat species is DNA analysis. PCR is an effective, sensitive, and specific method for identifying different meat species in meat products (Abuelnaga *et al*., 2021). However, PCR can face limitations such as difficulties in distinguishing

Table 1: Oligonucleotide primers used for species detection

cytb gene	Primers sequences	bp	
Bovine	GCCATATACTCTCCTTGGTGACA GTAGGCTTGGGAATAGTACGA	271	Ilhak and Arslan, (2007)
Porcine	GCCTAAATCTCCCCTCAATGGTA TGAAAGAGGCAAATAGATTTTCG	212	
Equine	GACCTCCCAGCTCCATCAAACATCTCATCT TGATGAAA CTCAGATTCACTCGACGAGGGTAGTA	439	Mane *et al.* (2009)
Camel	AGCCTTCTCTTCAGTCGCACAC GCCCATGAAAGCTGTTGCT	208	Chen *et al.* (2005)
Chicken	CTCCCATAGACAGCTCC CCCCAAAAAGAGAAGGAA	371	Bellis *et al.* (2003)

between closely related species or detecting very low levels of DNA, requires rigorous validation to avoid false results, and requires careful optimisation of primers and conditions to ensure accurate species identification. Another limitation of DNA barcoding is that industrial processes tend to degrade DNA and cut it into small fragments (≤ 200 bp), which prevents its application on processed meat samples (Cavin *et al*., 2018). To prevent health and safety-related manifestations, food processing facilities must implement preventive measures and systems against potential food contamination hazards. In that regard, different management systems that address food safety issues, such as HACCP, good manufacturing practices (GMP), and hygienic design practices, have been developed and are widely used today. The current study sought to determine the microbial load as well as the virulence genes of certain foodborne pathogens in various samples of beef products. Additionally, it looked into the use of PCR as a sensitive and specific way to find adulteration in beef products sold in Aswan City, Egypt's various supermarkets, with chicken, camel, pig, and horse meat.

MATERIAL AND METHODS

Samples Collection

Ninety beef samples total-fifteen each of sausage, burgers, shawarma, minced beef, and hawawshi-were purchased randomly from retail markets in the Aswan Governorate, Egypt. As quickly as feasible, all samples were gathered, put in an icebox container, handled aseptically, and sent straight away to the Faculty of Veterinary Medicine, Aswan University's Meat Hygiene Laboratory for analysis.

Detection of Meat Species in Beef Products by Conventional PCR Technique

Genomic Tissue DNA Extraction

According to *the Quick*-gDNA™ MiniPrep kit (Cat. No. D3024, Zymoresearch, USA), DNA was taken out of each sample by the PCR using specific primers targeting the *cytb* gene of different species (Table 1). The PCR amplification procedure was approved in 25 µL with the following ingredients: 12.5 µL *COSMO* PCR RED Master Mix (Code No. W1020300X, Willowfort, United Kingdom), 0.5 µL each primer (20 pmol), 11 µL free nuclease water, and 0.5 µL template. The PCR reactions required a preheating stage (5 min at 94 °C), 35 cycles of denaturation (94 °C for 45 sec), annealing temperature 50 °C/45 sec (beef), 54 °C/45 sec (camel), 48 °C/45 sec (pig), 56 °C/45 sec (equine), and 46 °C/30 sec (chicken), extension (72 °C for 30-60 sec), and final extension (72 °C for 7 min). A reference to each animal tissue provided by the Scientific Hospital of the Faculty of Veterinary Medicine at Aswan University as a positive sample and a DNA-free sample as a negative sample. 1.5% agarose gel electrophoresis was visualized using a documentation system that utilized the Qiagen 100 bp DNA Ladder (USA).

Bacteriological Examination

Using a SewardTM StomacherTM Model 400 Circulator Lab Blender, 110 V, aseptically, 25 g of each beef sample was placed into stomacher bags together with 225 mL of peptone water, and the mixture was then mixed for two minutes at 200 rpm. For 24 h, homogenates were serially diluted at 0.1% (nonselective preenrichment) in peptone water at 37 °C. For the subsequent analysis, one mL of each previously made serial dilution was separately inoculated into three correctly labeled duplicate Petri dishes.

Total Aerobic Count (TAC)

By incubating the plates at 35 °C for 48 h, the pour plating technique was employed with plate count agar (M091A, HiMedia) and one ml of each serial dilution that had been previously prepared (Khairy *et al*., 2024). In plates with 25–250 colonies, every colony was counted, and the results were noted.

E. coli Isolation and Identification

Eosin methylene blue (Oxoid, CM 69) agar was scattered with a loopful of the incubated broth, and the mixture was then incubated for 24 h at 37 °C (ISO, 2018). According to MacFaddin (2000), certain colonies with a metallic sheen

Table 2: Oligonucleotide primers for detection of bacterial virulence genes

Bacteria	Target gene	Primers sequences	bp	Reference
E. coli	*hylA*	ACGATGTGGTTTATTCTGGA CTTCACGTGACCATACATAT	165	Fratamico *et al*. (1995)
	stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	Dhanashree and Mallya (2009)
	stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	
P. aeruginosa	*rpoB*	CAGTTCATGGACCAGAACAACCCG ACGCTGGTTGATGCAGGTGTTC	759	Benie *et al*. (2017)
	ExoS	CTTGAAGGGACTCGACAAGG TTCAGGTCCGCGTAGTGA AT	504	Strateva (2008)
	LasB	GGA ATG AAC GAG GCG TTC TC GGT CCA GTA GTA GCG GTT GG	300	

and a dark centre were taken from each plate, streaked onto nutrient agar plates, and incubated at 37 ˚C for 24 h to facilitate further identification.

Pseudomonas aeruginosa Isolation and Identification

A loopful from the incubated broth was scattered on *Pseudomonas* selective agar media enhanced with glycerol (M085, HiMedia) and incubated at 25 ˚C for 24-48 h, and greenish yellow pigment colonies were picked up, streaked onto nutrient agar plates, and incubated at 25 ˚C for 24 h for additional identification, according to Quinn *et al*. (2002).

Determination of Some Food Poisoning Virulence Genes

The isolates of confirmed phenotypically studied bacteria were chosen to be analyzed for the recognition of virulence genes of the detected beef-borne pathogens using conventional PCR. DNA was taken out using a Gene^{JET} Genomic DNA Purification Kit (Cat. No. K0721, Thermo Scientific, USA). Using specific oligonucleotide primers synthesized by Willowfort Company (United Kingdom), shown in Table 2. The PCR amplification was performed in a total amount of 25 µL with the following components: 12.5 µL of Emerald Amp Max PCR Mastermix (Takara, Japan), 1 l of each primer (20 pmol), 4.5µL of free nuclease water, and 6 µL of the template. The PCR reactions for *hylA*, *stx1*, and *stx2* required a 5 min at 95 ˚C preheating stage, following 35 cycles of denaturation (95 ˚C for 20 sec), annealing temperature (58 ˚C for 40 sec), extension (72 ˚C for 90 sec), and final extension (72 ˚C for 5 min). While *rpoB*, *exoS*, and *lasB* genes, preheating stage (5 min at 94 ˚C), following 35 cycles of denaturation (945 ˚C for 60 sec), annealing temperature (58 ˚C for 60 sec *rpoB*, 60 ˚C for 60 sec *exoS,* and *lasB*), extension (72 ˚C for one min), and a final extension (72 ˚C for 5 min). A reference sample of *E. coli* and *P. aeruginosa* supplied by the Animal Health Institute in Giza, Egypt, was used as a positive sample, and a PCR reaction deprived of any DNA was used as a negative sample. Agarose gel electrophoresis 1.5% was visualized using a documentation system that utilized the Qiagen 100 bp DNA Ladder (USA).

Statistical Analysis

Significant variances among the adulterated and normal samples for bacterial contamination levels were examined using a *t*-test. In addition, the significant differences between the different sources of adulterated samples were tested using a one-way ANOVA (PROC ANOVA) with a significance level set at $p < 0.05$. Results were expressed as means ± SE. If a significant effect was found, Tukey's test was used to compare the means in pairs.

RESULTS AND DISCUSSION

PCR was designed to amplify partial genes with varying amplicon sizes to identify various meat species (Table 1). The obtained results demonstrated the successful amplification of the target *cytb* gene sequences. The genomic DNA of beef, chicken, camel, pig, and equine was amplified using species-specific oligonucleotide primers, revealing amplicon sizes of 271, 212, 442, 208, and 439 bp, respectively. Through PCR, Table 3 shows that 88.9% of samples were positive for derivatives from cattle (Figure 1), 63.9% were positive for derivatives from chickens (Figure 2), 19.4% were positive for derivatives from camels (Figure 3), 8.3% were positive for derivatives from pigs (Figure 4), and 13.9% were positive for derivatives from horses (Figure 5). However, the Egyptian Food Codex forbids the inclusion of poultry and other meat species in products labeled as 100% beef. Bovine derivatives were detected in all samples, except 33.3% in each hawawshi and hot dog sample. Otherwise, chicken derivatives were detected in 83.3% of minced beef, 66.7% of each sausage, burger, hawawshi, and shawarma, and 33.3% of hot dog. On the other hand, 16.7% of all minced beef, sausage, hawawshi, hot dog, and shawarma samples, as well as 33.3% of burger samples, contained camel derivatives. Furthermore, 33.3% of minced beef samples and 16.7% of burger samples had camel derivatives that

Table 3: Total adulteration (%) and the detected species in the examined beef products (n=36).

Detected species	Total rate		Minced beef		Burger		Sausage		Hawawshi		Shawarma		Hot dog	
	No.	%	No	%	No	%	No	%	No.	%	No.	%	No.	%
Beef	32	89.9	6	100	6	100	6	100	6	100	4	66.7	4	66.7
Chicken	23	63.9	5	83.3	4	66.7	4	66.7	4	66.7	4	66.7	2	33.3
Camel	7	19.4	1	16.7	2	33.3	1	16.7	1	16.7	1	16.7	1	16.7
Pig	3	8.3	2	33.3	1	16.7	0	0	0	0	0	0	0	0
Equine	5	13.9	1	16.7	2	33.3	2	33.3	0	0	0	0	0	0

were absent in other samples. Additionally, only 33.3% of each sausage burger sample and 16.7% of the minced beef sample contained equine derivatives, while the hawawshi, hot dog, and shawarma samples did not contain any.

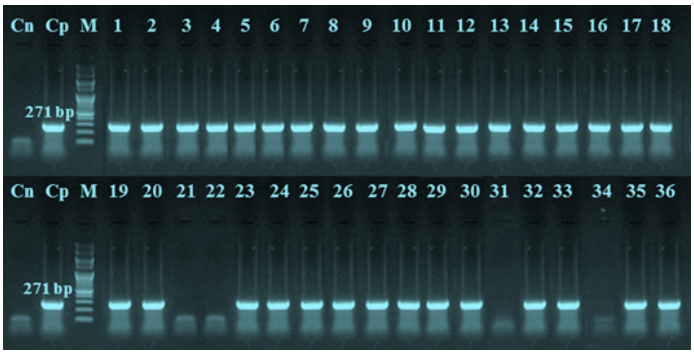

Figure 1: Electrophoretic gel for detection of bovine derivatives in beef products at 271 bp. Lanes 1-6: minced beef; Lanes 7–12: burger; Lanes 13–18: sausage; Lanes 19–24: hawawshi; Lanes 25–30: shawarma; and Lanes 31–36: hotdog. CN: control negative; CP: control positive. M: marker (100 bp).

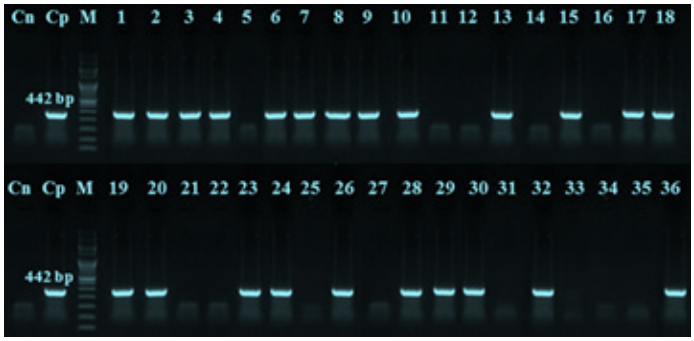

Figure 2: Electrophoretic gel figure for detection of chicken derivatives in beef products at 442 bp. Lanes 1-6: minced beef; Lanes 7–12: burger; Lanes 13–18: sausage; Lanes 19–24: hawawshi; Lanes 25–30: shawarma; and Lanes 31–36: hotdog. CN: control negative; CP: control positive. M: marker (100 bp).

Prevalence of Bacterial Contamination and their Virulence Genes

The total aerobic count (CFU/g) in the inspected beef sample was shown in Table 4, and sausage ($5.25\times10^5 \pm 2.7$) samples had the highest mean value, followed by burger ($2.8\times10^5 \pm 1.3$), minced beef ($4.66\times10^4 \pm 2.1$), and hawawshi ($5.1\times10^3 \pm 2.5$), while hot dog ($2.5\times10^3 \pm 1.3$) and shawarma ($2.3\times10^3 \pm 2.05$) were the lowest with significant difference between examined samples.

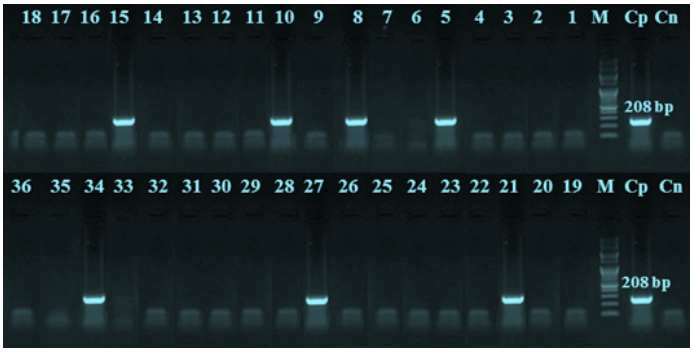

Figure 3: Electrophoretic gel for detection of camel derivatives in beef products at 208 bp. Lanes 1-6: minced beef; Lanes 7–12: burger; Lanes 13–18: sausage; Lanes 19–24: hawawshi; Lanes 25–30: shawarma; and Lanes 31–36: hotdog. CN: control negative; CP: control positive. M: marker (100 bp).

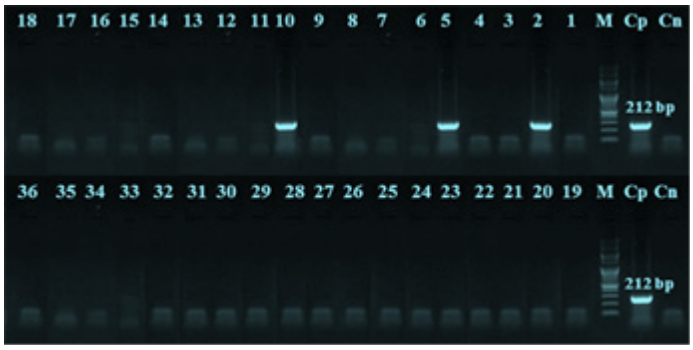

Figure 4: Electrophoretic gel figure for detection of pig derivatives in beef product at 212 bp. Lanes 1-6: minced beef; Lanes 7–12: burger; Lanes 13–18: sausage; Lanes 19–24: hawawshi; Lanes 25–30: shawarma; and Lanes 31–36: hotdog. CN: control negative; CP: control positive. M: marker (100 bp).

Table 5 reveals that 41.1% of the examined samples contained *P. aeruginosa*. Minced beef has the highest *P. aeruginosa* contamination rate (66.7%), while hot dog and shawarma have the lowest (20%). All examined *P. aeruginosa* isolates carried the *rpoB* gene, and PCR revealed that 70% and 40% of them were positive for the *lasB* and *exoS* virulence genes (Figure 6).

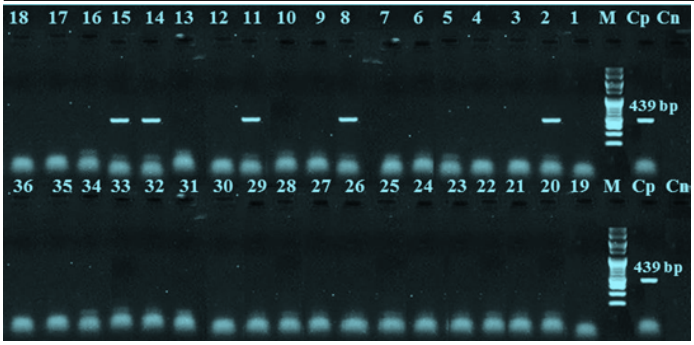

Figure 5: Electrophoretic gel for detection of equine derivatives in beef products at 439 bp. Lanes 1-6: minced beef; Lanes 7–12: burger; Lanes 13–18: sausage; Lanes 19–24: hawawshi; Lanes 25–30: shawarma; and Lanes 31–36: hotdog. CN: control negative; CP: control positive. M: marker (100 bp).

Table 4: Total aerobic count (CFU/g) in inspected beef products (n=90)

Product	Minimum	Maximum	Mean + SD
Minced beef	6.33×10^2	2.06×10^4	$4.66 \times 10^4 \pm 2.4^b$
Sausage	6.28×10^3	4.2×10^6	$5.25 \times 10^5 \pm 2.7^a$
Burger	1.50×10^3	1.81×10^6	$2.80 \times 10^5 \pm 1.3^a$
Hawawshi	0.66×10^2	1.73×10^4	$5.10 \times 10^3 \pm 2.5^c$
Hot dog	3.40×10^2	1.10×10^4	$2.50 \times 10^3 \pm 1.3^d$
Shawarma	1.20×10^2	4.10×10^4	$2.30 \times 10^3 \pm 2.05^d$

$p<0.001$, considered extremely significantly different. Mean values with the same letters are not significantly different.

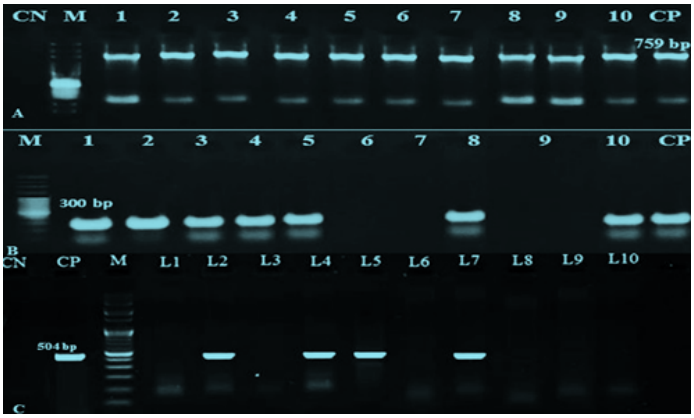

Figure 6: PCR electrophoresis of *P. aeruginosa rpoB* genes at 759 bp (**A**), *LasB* at 300 bp (**B**), and *exoS* at 504 bp (**C**). M: 100bp ladder. CP: positive control. CN: negative control

Table 5 shows that 30% of the inspected samples contain *E. coli*, with the highest isolation rate (46.7%) from minced beef, followed by 40% from each sausage, burger, and hawawshi, and 6.7% from hot dogs and shawarma. Molecular investigation of the *E. coli* isolates reveals that 50%, 80%, and 20% harbour *hylA*, *stx1*, and *stx2* virulence genes (Figure 7).

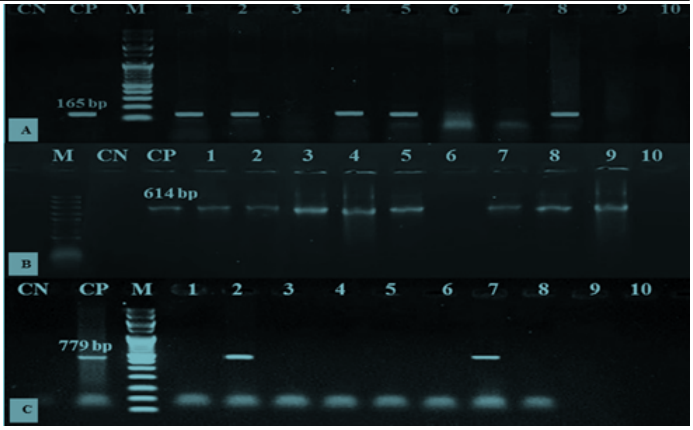

Figure 7: PCR electrophoresis of *E.coli hylA* genes at 165 bp (**A**), *stx1* at 614 bp (**B**), and *stx2* at 779 bp (**C**). M: 100bp ladder. CP: positive control. CN: negative control.

Table 5: Incidence of contaminated bacteria detected in the examined samples

Product	No.	P. aeruginosa		E-coli	
		No.	%	No.	%
Minced Beef	15	10	66.7	7	46.7
Sausage	15	6	40	6	40
Burger	15	8	53.3	6	40
Hawawshi	15	7	46.7	6	40
Hot dog	15	3	20	1	6.67
Shawarma	15	3	20	1	6.67
Total No.	90	37	41.1	27	30

The adulteration of livestock products has emerged as a significant concern for consumers worldwide. For this reason, the variety of meat from which the meat product is produced is a serious concern in terms of consumer protection and food control. However, it is seen as crucial to recognise the species of meat included in different meat products, particularly in Islamic nations where people only eat Halal meat (Özlü *et al*., 2023). The Egyptian Food Codex prohibits the inclusion of poultry and other species' flesh in products labeled as 100% beef (Nady *et al*., 2024). There were meat products in numerous markets throughout Egypt that did not conform to their labels, potentially causing economic losses for consumers and posing a risk to public health. Some native Egyptian plants have been linked to the adulteration of sausage, kofta, and hawawshi with low-quality, prohibited, and unknown meat sources (Hassanin *et al*., 2018). Therefore, in the current investigation, we used the PCR technique to search for meat from poultry, camels, horses, and pigs in these meat items. In this study, 63.9%, 19.4%, 8.3%, and 13.9% of inspected products have chicken, camel, pig, and equine derivatives, contrary to the data delivered on their label. The current research findings also showed that 88.9% of samples tested positive for bovine derivatives, while 11.1% showed no bovine derivatives, leading to their classification as beef, suggesting that

soybeans were the adulterant in these meat products.

For a variety of reasons, meat products may be cheating. One objective is to reduce production expenses. To cut costs, adulterators could mix non-meat substances or less expensive meats into the meat product. Another justification is to mislead customers. Adulterators might pose as sellers of more expensive meat varieties, including beef, when selling contaminated meat products (Setiadi *et al*., 2022). The current investigation attributed the high percentage of chicken derivatives to the use of chicken waste products such as blood vessels, cartilage, sinew, fat connective tissue, bloody effluvia, and even bone fragments mixed with meat and used as adulterants. Chicken meat is also less expensive than beef meat. Foodborne bacteria may contaminate these refuse products, making them less nutritious than meat. The occurrence of these microorganisms in final products due to insufficient heating temperature poses a potential health hazard to consumers (Hamouda and Abdelrahim, 2022).

PCR has become a valuable tool for identifying various animal species in meat products and is a potential replacement for current techniques (Kesmen *et al*., 2010). By enabling accurate, dependable, and prompt documentation of animal species in meat mixes, the PCR approach helps to avoid consumer fraud. Therefore, in contrast to other techniques, it makes it possible to identify animal meats that society does not normally consume more quickly, simply, or reliably (Hossain *et al*., 2023). The incidence of adulterated meat product samples varies depending on the country or area under investigation. Nevertheless, numerous studies have documented substantial rates of adulteration in meat product samples. For instance, an Egyptian investigation discovered that 6% of minced beef samples contained either pork or chicken (Abuelnaga *et al*., 2021). On the other hand, an Indian investigation discovered that nonmeat components such as starch and soy protein were present in 11% of samples of minced meat (Moirangthem *et al*., 2022). Moreover, the investigation by El-Sheikh *et al*. (2024) in Egypt documented the incidence of adulteration in 18% of the inspected beef samples, with 9% having dog meat, 5% having equine meat, and 4% having porcine meat. Moreover, Ha *et al*. (2017) identified three samples that verified pork out of 35 processed meat samples in Korea, while Keyvan *et al*. (2017) in Turkey proved that 13.5% of analysed products have chicken meat not stated on the label, and 2.7% have both chicken and equine meat. Additionally, the results of Hamouda and Abdelrahim (2022) recorded that 50% of each sausage and kofta samples and 25% of hawawshi samples were mixed with chicken meat, 33.3% of kofta testers were mixed with equine meats, and 8.33% of hawawshi testers were mixed with canine meat, and all analysed samples were negative for pork meat.

Studies of food fraud and adulteration are important for all types of products because these incidents can lead to public health threats and pose drastic potential impacts on the economies of the companies and/or countries involved (Moyer *et al*., 2017). Food authenticity is of primary importance for both consumers and food industries in all production stages, from the purchasing of raw materials to the distribution of finished products all over the world (Tibola *et al*., 2018). Regular enforcement of authenticity standards throughout the entire production chain helps prevent the occurrence of food fraud and adulteration events. However, international discussions continue to focus on the suitability of tools for assessing supply chain vulnerability to food fraud and adulteration, with the ultimate determination of the most effective mechanisms for global trade and supply chains (FAO, 2017). Adulterated meat products may expose consumers to various hazards. One potential issue involves misleading consumers about the true contents of meat products blended with less expensive meats like poultry or pork. Furthermore, falsified meat products can contain hazardous bacteria or other pollutants (Han *et al*., 2020). In the current study, the TAC in examined beef products has a significant difference between minced beef and other samples; in comparison, no significant variances are detected for sausage and burger, hot dog and shawarma. In addition, the TAC ranges from $5.25\times10^5 \pm 2.7$ CFU/g in sausage testers to $2.3\times10^3 \pm 2.05$ CFU/g in shawarma. The TAC outcomes achieved in the current investigation were lower than those informed by Abuzaid *et al*. (2020), Abuelnaga *et al*. (2021) in Egypt, and Erdem *et al*. (2014) in Istanbul.

It is noteworthy that the findings indicate that samples of adulterated beef products are more susceptible to bacterial contamination than those of unadulterated beef products. Furthermore, the sample's bacterial count can be influenced by the type of adulterant used, which is affected by a variety of factors. Initially, adulterants have the potential to modify the nutritious contents of meat, which in turn influences the proliferation of microbes. Second, the moisture content of each variety of meat significantly influences bacterial proliferation. Finally, adulterants may have an indirect impact on the bacterial count by altering meat storage conditions (Momtaz *et al*., 2023). As a result, bacterial counts in samples contaminated with horse and porcine meat are higher than in samples contaminated with chicken and camel meat.

Emerging food bacterium *P. aeruginosa* spreads widely in its environment and often functions as an opportunistic human pathogen. It is critical to the deterioration of a variety of foods (Rezaloo *et al*., 2022). Therefore, it is critical to determine its incidence and other epidemiological characteristics. This particular bacterium causes illnesses due to

a variety of virulence factors. *P. aeruginosa* virulence factors are exoenzymes (*exoS*) and elastase (*lasB*) genes responsible for adhesions and attachments, inflammatory reactions, and ultimately attack of the host cell (Jurado-Mart´ın *et al*., 2021). The current research discovered *P. aeruginosa* in 41.1% of the examined samples, which harbours one or more virulence genes (*lasB* and *exoS*). Additionally, minced beef had a higher occurrence rate (66.7%).

Results obtained by Elbayoumi *et al*. (2022) showed that the occurrence of *P. aeruginosa* was 4% in minced beef, burgers, and sausage, respectively. Furthermore, Benie *et al*. (2017) identified P. aeruginosa at a rate of 53.04% in beef samples collected from West Africa, detecting the exoS and lasB virulence genes in 96.70% of the isolates. Rezaloo *et al*. (2022) reported that *P. aeruginosa* contaminated 7.83% of beef testers, with *exoS* (75.86%) and *lasB* (51.72%) being the most identified virulence factors. The importation of inferior meat or its prolonged storage in hazardous conditions before customs clearance could be the cause of the high contamination level in the specimens under examination. *P. aeruginosa* in these samples may have originated from inadequate application of the heat chain, improper cooking time, and pollution in samples of beef products following manufacture. Furthermore, according to Sofy *et al*. (2017), *P. aeruginosa* exhibits strong environmental adaptation, low water activity (72%–97%), and ambient temperatures ranging from 4 to 42 °C.

The high distribution of virulence factors, especially *exoS* and *lasB*, were another important characteristic of *P. aeruginosa* occurrence in the samples under examination. The primary function of these genes is to facilitate the adhesion and incursion of microbes into host cells. Thus, consuming food with virulent *P. aeruginosa* strains may result in serious food-borne illness (Rezaloo *et al*., 2022). The present study found that PCR revealed *lasB* and *exoS* virulence factors in 70% and 40% of *P. aeruginosa*, respectively. The high distribution of the *lasB* gene indicates that these protease enzymes may play a major role in *P. aeruginosa's* pathogenesis by cleaving collagen and elastin. The *exoS* helps bacteria spread and kills tissue. It can also damage the links between epithelial cells; make more IL-8, and lower the innate immune response, immunoglobulins, and complement components (Rezaloo *et al*., 2022). Because of the wide range of virulence factors, it appears that *P. aeruginosa* isolates from meat product samples in our review can induce serious gastrointestinal illnesses.

Enterobacteriacae, particularly *E. coli*, are major food-poisoning pathogens as well as indicators of potential faecal contamination (Synge, 2000). Researchers have identified *E. coli* as severe foodborne bacteria and have linked it to several foodborne outbreaks. This bacterium encompasses a diverse array of strains, including lethal commensal strains and highly pathogenic strains that induce varying levels of infection in both humans and animals (Kaper *et al*., 2004). The main mechanism that makes *E. coli* harmful is its ability to make Shiga toxins (*stx1* and *stx2*), which are needed for the organism to stick to the intestinal epithelium. Haemolysins (*hly*) are what allow the bacterium to attach to the intestinal mucosa and lyse erythrocytes (Shehab Eldin *et al*., 2020). In the current investigation, the incidence of *E. coli* was 30%, and the molecular investigation revealed that 50%, 80%, and 20% of isolated *E. coli* contain *hylA*, *stx1*, and *stx2* virulence genes that are considered major public health hazards. Minced beef has the highest contamination rate with *E. coli* (46.7%), while hot dog and shawarma have the lowest (6.7%). Hassanin *et al*. (2014) in Egypt and Rahman *et al*. (2017) in Bangladesh observed similar results. As well as the outcomes achieved by Abuelnaga *et al*. (2021) in Egypt, 29.3% of examined beef samples had *E. coli*, which occurred in 45%, 10%, and 30% of minced beef, burger, and sausage products. Ragab *et al*. (2016) also found *E. coli* in 50% of the inspected minced beef and 30% of beef burgers in Egypt. Furthermore, the findings obtained by El-Sheikh *et al*. (2024) indicated that 13% of the inspected minced beef had *E. coli,* and the occurrence percentage was higher in adulterated samples compared to normal ones. Molecular recognition of *E. coli* virulence factors shown by Shehab Eldin *et al*. (2020) had success rates of 27.2% for *hylA*, 45.4% for *stx1*, and 63.6% for *stx2* genes.

This is a cause for concern, as *E. coli* can result in food poisoning, which can manifest in a variety of signs, such as diarrhoea, vomiting, and pains. *E. coli* food poisoning can occasionally be extremely serious and even fatal. There are numerous reasons why adulterated meat product samples may be more susceptible to *E. coli* contamination. Adulterated meat could potentially originate from unhealthy animals or those slaughtered in unhygienic conditions. Another explanation suggests that handling or processing of adulterated meat may increase the risk of bacterial contamination (Sharif *et al*., 2018). It is crucial to recognise that not all *E. coli* isolates are detrimental. In reality, the majority of strains are benign and essential for preserving intestinal health and a healthy digestive system. According to Martinson and Walk (2020), these strains help break down meals to produce vital vitamins and nutrients that our bodies need. Nevertheless, certain genotypes and serovars have the potential to produce toxins that can result in food poisoning (Rey *et al*., 2003).

The primary way to reduce food adulteration is to develop industry standards and certifications, as well as identify incidents. Innovations in testing methods, promotion of inspection services, expansion of law enforcement activity, and introduction of new legislative provisions will all help reduce food fraud and adulteration. These findings emphasise the need to use correct food handling and

preparation techniques to reduce bacterial contamination in meat products. By addressing factors such as moisture content and temperature control, we can considerably reduce the risk of microbial proliferation and improve food safety. As a result, consumers must be aware of the risks associated with eating adulterated meat products. By purchasing from reputable suppliers, verifying the complete cooking of meat products, and maintaining separation between meat and other foods during handling and cooking, consumers can mitigate these risks. We faced numerous challenges during the preparation of this study, primarily due to the absence of guidelines and legislation that govern food adulteration, and the difficulty in detecting it using a single analytical method. Therefore, the study only focused on specific products and did not simultaneously address various food adulterations and their detection techniques.

CONCLUSION AND RECOMMENDATIONS

The current study's results reveal that some foodborne bacteria, like *E. coli* and *P. aeruginosa*, have contaminated significant samples under investigation. These bacteria possess one or more virulence genes, thereby increasing the risk of foodborne disease and potentially facilitating its transmission. Additionally, the study revealed evidence of adulteration of multiple meat species, a finding that contradicts the information on the label. Therefore, it is imperative to evaluate the microbial quality of livestock products to ensure their safety and quality. It is strongly recommended that the PCR method devised in this study be employed as a screening technique for the recognition of adulteration and the abuse of labelling requirements in meat products. We require additional research to create innovative detection methods, identify adulteration in various products, and encompass a broad spectrum.

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NOVELTY STATEMENT

As a food safety concern in Aswan City, Egypt, the recent investigation attempts to identify the bacterial load and recognise some foodborne bacteria and their virulence genes in various beef products to avoid health and safety-related symptoms. Additionally, it looked into the use of PCR as a sensitive and specific technique to find the adulteration of beef products offered in Aswan City with other species in various markets and restaurants.

AUTHOR'S CONTRIBUTIONS

Nady Elbarbary and Eman Qenawy conducted conceptualization, data curation, and methodology. Eman Qenawy conducted a formal analysis. Nady Elbarbary, Mohamed Abou-ellail and Fatma Abdel-Motaal: supervision, investigation, and validation. Nady Elbarbary, Mohamed Abou-ellail and Mohammed Alshaharni wrote the original draft, revised, and edited the paper. The authors contributed equally and accepted the absolute manuscript.

Ethical Approval

The Scientific Research Committee and Bioethics Board of Aswan University, Faculty of Veterinary Medicine (3/2024), reviewed and accepted the procedures used for this research, which follow the Canadian Council on Animal Care guidelines (CCAC, 2005).

Conflict of Interest

The authors have declared no conflict of interest.

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