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Incidence of some foodborne pathogens in frozen beef marketed at Menofia governorate, Egypt

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ABSTRACT

The research was performed to detect the incidence of some foodborne pathogens in frozen beef meat. A total of one hundred random frozen meat specimens (each 50 g in weight) were collected from various supermarkets and butchers' shops at Menoufia governorate, Egypt and examined for the total colony, coliform and psychotropic colony count, *as well as Salmonella*, *E. coli* and *Staph aureus*. The average total colony count, coliform count and psychotropic colony count (expressed as a $\log_{10}$) in frozen meat samples was 4.51±0.96, 3.11±0.95 and 4.27±0.75 CFU/g, respectively. The incidence and serotyping of *E. coli* strains isolated from frozen meat was Enterohaemorrhagic *E. coli* 33.3%, Enteropathogenic *E. coli* 49.4%, Enterotoxogenic *E. coli* 8.3%, Enteroinvasive *E. coli* .3%.Serotyping and frequency of *Salmonella* isolated from the investigated frozen meat samples were *S. Typhimurium* (4%) and *S.* Enteritidis (6%). *Staph aureus* was isolated from 21 out of 50 samples, represented 42%,so freezing of beef is considered a good method for reduction the incidence of food borne disease than fresh beef.

1. INTRODUCTION

Food borne illnesses are considered by the World Health Organization (WHO) as diseases either infectious or toxic made by causative agents in ingested food. The reports in 2005 recorded 1.8 million people died from diseases causing diarrhea and high proportion of which was attributed to contamination of food and drinking water (WHO 2007). The majority of pathogens that of bacterial origin in meat products as well as beef meat cause of food borne contamination involve *E. coli*, *Salmonellae*, and coagulase-positive *S. aureus* (Hamed et al., 2015).

Salmonellosis is considered as major because of their survival of Salmonella at exact reduced temperature. They might appear in variant strains as S. typhi, serotypes S. enteritidis, S. typhimurium (Hazaa et al., 2019; Shaltout et al., 2022). Freezing is a high heat transfer utilized for meat products maintenance and it causes minimal loss of quality throughout long-term storage. Freezing is also one of the quickest, easiest, most versatile, and most fit technique for preserving food. Properly frozen food maintains its original color, texture, nutrients, and flavor than foods maintained by other procedures (Julie, 2013).

Bacterial infection of meat can be determined by utilizing aerobic plate count, Staphylococci. and total Escherichia coli. This provides a good idea of the hygienic quality of meat (Hamed et al., 2015).

This study aimed to evaluate the total colony count, total coliform count, total *psychotropic* count, isolation and identification of *E. coli*, Salmonella and *Staph. aureus* from frozen beef.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of one hundred random frozen beef specimens (each of 50g in weight) from various supermarkets and butchers' shops at El-Menofia governorate were examined. All samples have been put in a separate sterile plastic bag and stored in an ice box, subsequently sent to the laboratory under aseptic conditions without undu delay and examined as quickly as possible. The gathered specimens have been exposed to bacteriological methods to assess their quality.

2.2. Bacteriological Examination:

2.2.1. Preparation of samples (ISO 6887-2, 2017a.)

To 25 g of the examined frozen sample, two hundred twenty-five milliliters of sterile peptone solution (0.1%) has been added and extensively mixed utilizing a sterile blender for 1 – 1.5 min., from which ten-times serial dilutions have been prepared.

2.2.2. Aerobic Plate Count (APC) (ISO 4833-1, 2013)

One milliliter from each of the earlier prepared sterile dilutions has been transmitted into 2 sterile Petri-dishes to which approximately fifteen milliliters of sterile tempered and melted standard plate count agar at forty-five degrees Celsius has been added. Then thorough mixing, the inoculated and controlled plates have been permitted to solidify prior to be incubated in an inverted position (thirty-seven degrees Celsius for twenty-four hours). The APC per gram has been determined on plates containing thirty to three hundred colonies as well as each count has been documented individually.

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2.2.3. Determination of total coliform count (ISO 4832, 2006):
Violet Red Bile Agar media has been utilized to enumerate the total coliform(cfu) using the pour plate method.

2.2.4. Total psychrotrophic Count (FDA 2001)
Psychrotrophic bacteria were enumerated on Plate Count Agar at 7 ^{0}C (Oxoid, UK)

2.2.5. Isolation and identification of E. coli (ISO 16649-1, 2018)
A loop of homogenized samples overnight culture has been streaked onto Eosin Methylene Blue agar in duplicate and incubated at thirty-seven degrees Celsius for eighteen to twenty-four hours.
Pre-enrichment of *E. c*oli (ICMSF, 1996).
Morphological examination of *E. c*oli (Cruickshank et al*.,* 1975)
Biochemical identification of *E. c*oli (Kreig and Holt, 1984)

2.2.5.1 Serodiagnosis of E. coli
The isolate has been recognized serologically regarding to Kok et al. (1996).

2.2.6. Isolation of Salmonellae (ISO 6579-1, 2017b)
A loopful of Rappaport-Vassiliadis broth has been spread onto xylose lysine deoxycholate (XLD) agar & incubated at thirty-seven degrees Celsius for 18–24 hours. The purified isolates have been recognized morphologically, biochemically, and serologically (Kauffman, 1974).

2.2.7. Isolation and identification of S. aureus: ISO 6888-2021
Baird-Parker agar has been utilized for the isolation of *S. aureus*. Morphological examination of *S. aureus* (Cruickshank et al*.,* 1975)
Biochemical identification of *S. aureus*
- Catalase activity test according to (MacFaddin, 1976)
- Mannitol test according to (Bailey and Scott, 1978)
- Coagulase test according to (APHA, 1984)

2.3. Molecular characterization of E. coli strains and salmonellae isolates (Table 1)

2.5. Statistical Analysis
The outcome data statistically evaluated by utilization of the Analysis of variance (ANOVA) test regarding psychotropic and coliform counts Feldman et al. (2003).

Table 1 PCR primers and conditions for *E. c*oli and Salmonella gene amplification

	Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
E. coli genes	*stx1* (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya (2008)
	Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
	Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	Dhanashree and Mallya (2008)
	Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		
	eaeA(F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Mazaheri et al. (2014)
	eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
Salmonella genes	*stn*(F)	5′ CTTTGGTCGTAAAATAAGGCG ′3	260	Makino et al. (1999)
	stn (R)	5′ TGCCCAAAGCAGAGAGATTC ′3		
	hilA (R)	5′ CTGTCGCCTTAATCGCATGT ′3	497	Guo et al. (2000)
	hilA (F)	5′ CTGCCGCAGTGTTAAGGATA ′3		
	fimH (R)	5′ AAG CTT TTA ATC ATA ATC GAC TC ′	1008	Menghistu (2010)
	fimH (F)	5′ GGA TCC ATG AAA ATA TAC TC ′3		

3. RESULTS

From the outcome documented in Table 1, it's clear that the mean, minimum and maximum values of total colony count of examined frozen beef samples expressed as a log cfu/g were 4.15±0.096, 3.30, 5.03., the result obtained in table (2) also detected that the mean value of coliform count was 3.11±.095,in addition to that, the data showed in table (2) detected that the mean value of total psychotropic colony count of frozen beef samples expressed as a log cfu/g was as follows: 4.27±.075.

Table 2 Total colony, coliform, and psychotropic count of examined frozen beef samples expressed as (log cfu/g).

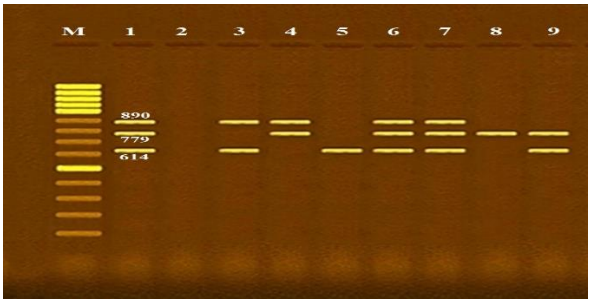
Item			P value
Total colony count	Min.	3.3	0.000
	Max.	5.03	
	Mean± Standard error	4.15±0.096^b	
Total coliform count	Min	2.3	0.000
	Max.	3.94	
	Mean± Standard error	3.11±.095^b	
Total psychotropic count	Min.	3.7	0.017
	Max.	4.95	
	Mean± Standard error	4.27±.075^b	

From the outcome documented in table (3), it's obvious that *E. c*oli serovars have been determined from frozen beef samples, three isolate with percentage of 25% from strain (O_{26}:H_{11}) strain (O_{55}:H_7),2(16.6%) (O_{103})2(16.6%) and strain (O_{127}:H6) (8.3%), (O_{111}:H_2) 2(16.6%), (O_{113}:H_4)1 (8.3%) and one strain O_{124}) (8.3%), the incidence and serotyping of *E. coli* strains isolated from frozen meat was Enterohaemorrhagic *E. coli* 33.3%, Enteropathogenic *E. coli* 49.4%, Enterotoxogenic *E. coli* 8.3%, Enteroinvasive *E. coli* .3%.

Table 3 Frequency and serotyping of *E. coli* species isolated from examined frozen beef samples.

E-coli serovars	Types of isolates	Isolates	
		No.	%
O_{26}:H_{11}	EHEC	3	25%
O_{55}:H_7	EPEC	2	16.6%
O_{127}:H_6	ETEC	1	8.3%
O_{103}	EPEC	2	16.6%
O_{111}:H_2	EPEC	2	16.6%
O_{113}:H_4	EHEC	1	8.3%
O_{124}	EIEC	1	8.3%
Total		12	

EPEC = Enteropathogenic *E. coli* ETEC = Enterotoxigenic *E. coli*
EHEC= Enterohaemorrhagic *E. coli* EIEC = Enteroinvasive *E. Coli*



Photograph 1 Agarose gel electrophoresis of multiplex Polymerase chain reaction of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) virulence genes for description of Enteropathogenic *E. coli.* Lane M: 100 bp ladder as molecular size deoxyribonucleic acid indicator. Lane 1: Control (+) *E. coli* for eaeA, stx2, and stx1 genes. Lane 2: Control (-). Lane 3 (*E. coli* O26): (+) strain for eaeA and stx1 genes. Lane 4 (*E. coli* O55): (+) strain for eaeA and stx2 genes. Lanes 5 (*E. coli* O127): (+) strains for stx1 gene. Lanes 6, 7 (*E. coli* O103 and O111): (+) strains for eaeA, stx2 and stx1genes. Lane 8 (*E. coli* O113): (+) strain for stx2 gene. Lane 9 (*E. coli* O124): (+) strain for stx1 and stx2 genes.

The result conducted in table (6) showed that frequency of virulent genes of Shiga toxin-producing *E. c*oli isolated from studied frozen meat stx_1 present in all strains except O $_{55}$ and O_{113},stx_2 gene present in all strains except in O $_{26}$ and O $_{127}$

and eae in all strains except in O_{127}, O_{124} and O_{113}.The result showed in Table (4) was clarified that Occurrence of virulent genes of Shiga toxin-producing *E. coli* isolated from examined frozen beef. The result conducted in table (5) showed that Frequency and serotyping of *Salmonella* isolated from examined frozen beef in which the *S. Enteritidis* were represented by 2% from one sample and from groupD_1, *S. Typhimurium* was with percentage of 2% from one samlpe from group B,*S. Kentucky* was represented by 2% from one sample from group B and *S. Infanti* was from group C_1 with zero % ,also this table (5) detected that the antigenic structures of these types of isolated *salmonella* which included O and H antigen. The results attained in Table (6) have been clarified that occurance of virulent genes of *Salmonella* strains seperated from the examined samples of frozen beef included *S. Typhimurium*, *S. Enteritidis,* with *S. Kentucky*, *S. Infantis*, in which *Stn* genes present in all strains, hilA gene present in all strains except in *S. Kentucky,* while *FimH* was not found in those strains.

Table 4 Occurrence of virulent genes of Shiga toxin-producing *E. coli* isolated from examined frozen beef samples

E. coli Serovars	*stx1*	*stx2*	*eae*
O_{26}:H_{11}	+	-	+
O_{55}:H_7	-	+	+
O_{127}:H_6	+	-	-
O_{103}	+	+	+
O_{111}:H_2	+	+	+
O_{113}:H_4	-	+	
O_{124}	+	+	

Table 5 Frequency and serotyping of *Salmonella* isolated from examined frozen beef (n=50)

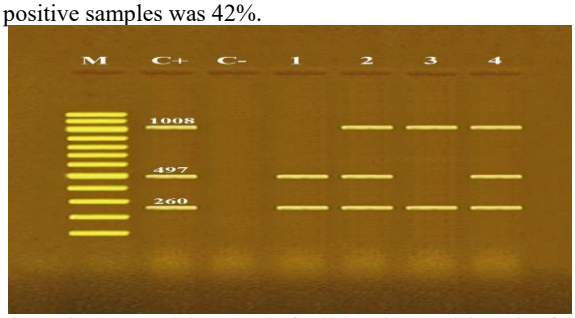
Salmonella Strains	Frozen meat No	%	Group	Antigenic *O*	structure *H*
S. Enteritidis	1	2	D1	1,9,12	g,m : -
S. Typhimurium	1	2	B	1,4,5,12	i : 1,2
S. Kentucky	1	2	B	1,4,5,12	i: Z6
S. Infantis	-	-	C1	6,7	r:1,2
Total	3	6			

Table 6 Occurrence of virulent genes of *Salmonella* strains isolated from examined frozen beef.

Salmonella strains	*stn*	*hilA*	*FimH*
S. Enteritidis	+	+	+
S. Typhimurium	+	+	+
S. Kentucky	+	-	+
S. Infantis	+	+	-

stn: Enterotoxin gene *hilA*: hyper-invasive locu *fimH*: fimbrial gene

The result conducted in photograph (2) was clarified that Agarose gel electrophoresis of multiplex Polymerase chain reaction of *stn*(260 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulent genes distinguation of *Salmonella s*trains in which Lane M:100 bp ladder as molecular size deoxyribonucleic acid indicator. Lane C+: Control positive *S.* for *fimH, hilA,* and *stn* genes.Lane C-: Control negative.Lane 1: (*S. infantis*): (+) strain for *hilA* and *stn* genes.Lanes 2, Lane 4: (*S. enteritidis*) and (*S.* Typhimurium): was positive strains for *stn*, *hilA* and *fimH* genes , Lane 3: (*S. Kentuchy*) was positive strain for *stn* and *fimH* genes. The outcomes investigated in table (7) showed that the frequency of *Staphylococcus i*n examined frozen beef in which the rate of positive samples was 42%.



Photograph 2: Agarose gel electrophoresis of multiplex Polymerase chain reaction of *stn* (260 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulent genes distinguation of *Salmonella* strains. Lane M: 100 bp ladder as molecular size deoxyribonucleic acid indicator. Lane C+: Control (+) *S.* for *fimH, hilA,* and *stn* genes. Lane C-: Control (-). Lane 1: (*S. infantis*): (+) strain for *hilA* and *stn* genes. Lanes 2 and Lane 4: (*S. enteritidis*) and (*S.* Typhimurium): (+) strains for *stn*, *hilA* and *fimH* genes. Lane 3: (*S. Kentuchy*): (+) strain for *stn* and *fimH* genes.

Table 7 Incidence of *S. aureus* in examined frozen beef samples (n=50)

Bacterial Isolates	Frozen beef No. of positive sample	Percentage (%)
S. aureus	21	42%

4. DISCUSSION

The frequent way to expand the shelf life of meat is via cooling. As the temperature decreases enzymatic activity and growth of bacteria (Johanna, 2005). Freezing is one of the majority crucial and extensively utilized maintenance approaches for prolonging the shelf life of meat, thus permitting the customers and processors to sustain meat safety and quality (Doulgeraki et al. 2012). Contaminated meat, especially frozen beef may pose a public health risk (Hamed et al., 2015). Foodborne illnesses, either toxic or infectious, due to causative agents in ingested food (WHO 2007).

Aerobic plate count, total psychrotrophic counts, and total coliform counts can determine bacterial contamination, hygiene practices during meat manufacturing and storage, and poor-quality frozen meat products (Zweifel et al., 2005; Hamed et al., 2015). Aerobic plate count is widely recognized as a standard for assessing microbial contamination of carcasses & serves as a valuable marker of the hygienic conditions within an abattoir (Cohen et al., 2007).

The present outcomes were less in comparison with those documented by Refai et al. (1991), who observed that the mean aerobic bacterial count of investigated frozen meat specimens was $2.7x10^6$ (cfu/g). The aerobic bacterial count could reflect the quality of food sanitation during manufacturing, shipping and storage, and also provides an index of food freshness (Jyh-wei and Yin-hung, 2000).

Coliform bacteria are enteric bacteria, naturally found in the human intestines. They are regarded as markers of contamination of food by fecal contaminants from handlers, contaminated water, and dirty equipment (Pelczar et al., 2005).

Bacteria of Psychrotrophic can rise in refrigerated conditions and temperature might influence different microbial evaluation variables involving total bacterial counts and maximum growth rate (Mataragas et al., 2006).

Abd El-Hady (2014) recorded that psychotropic count in exported frozen beef chunk and ribs was 1 x 10^3 to 1.8x 10^5 with mean value $2.78 \times 10^4 \pm 5.24 \times 10^3$ colony-forming unit per gram, 1.7 × 10 3 to 7.9 × 10^5 with a mean value 3.87 x× $10^4 \pm 4.35 \times 10^3$ cfu/g, respectively. With the frequency of twenty-four (96%), respectively. Psychrotrophic bacteria proliferate at temperatures 7°C or below, with an optimal growth range of twenty to thirty degrees Celsius. Psychotropic organisms are transmitted to frozen meat through storage, transportation, handling, and from un-sanitized tools (Venugopal et al., 1984). *E. c*oli provides dependable indicators of potential fecal contamination (Synge 2000).

Temperature might impact the spoilage possible of bacteria, & various strains of identical species don't regularly exhibit identical growth rates.

At present study, these outcomes go against those of Abou Aly et al. (2007) and Abd El-Aziz-Wafaa (2015), who reported greater coliform count. The *E. C*oli isolation from meat specimens demonstrates the fecal contamination and revelled that other pathogens of fecal origin could exist. The greater prevalence of *E. Coli* in the examined samples due to mishandling through processing, in addition to distribution, and/or the utilization of contaminated water throughout slaughtering & evisceration. (Aycicek et al., 2004; Gwida et al., 2014).

The frequency of *S*almonellae in frozen meat may be originate through various infected touch of butchers and food handlers, fecal hand contamination, contaminted clothing, by presence of rodent & other non-hygienic practices in the slaughterhouse area. The risk of progression for *Salmonellosis* is the main cause of survival of *Salmonella* at exact reduce temperatures too.

Nearly the same outcomes have been attained by Edris et al. (2011) where they isolate *S. t*yphi two percent and S. typhimurium (4%), and Shaltout et al. (2016) where they isolated *S. t*yphi (4%), and greater outcomes have been documented for S. typhimurium (8%)

Non-typhoidal salmonellosis is one of the greatest frequent foodborne illnesses, which occurs mainly cause of consumption of contaminated animal products involving imported frozen cattle and organs (Wheeler et al.,2014). *Salmonella spp*. could survive long-term frozen storage (-20 ºC), like in frozen beef trimming (Bosilevac et al., 2007).

Staphyloccocus. aureus is the second foodborne pathogen leading to food poisoning in the world following Salmonella (Atanassova et al., 2001).

Staph aureus is a frequent pathogen that leads to food poisoning outbreaks of high economic significance during the world (Alarcón et al., 2006). It is commonly distributed in animals, nature, and humans.

Frozen meat is frequently above significantly contaminated, caused by the incidence of spoilage microorganisms that are responsible for objectionable pathogens or changes that can result in food intoxication or infection (Tauxe et al., 2002). *Staph aureus* is present in hair, membrane, palms, throat and fingernails so the human and are considered the main reservoir for *Staphylococcus aureus* (Plaatjies et al.,2004). Accordingly, all samples investigated have been accepted according to EOS (2005), in which the maximum limit for psychotropic bacterial count in imported frozen meat must not be more than 10^6 cfu/g. Higher outcomes have been attained by Ibrahim et al., (2011)

Exposing frozen meat to refreezing and rethawing in street vendors and market shops must be prevented as it yields to an abundant supply of bacteria as it is, a perfect medium for multiplication and microbial growth (Abd El-Aziz, 2000). Multiplex-PCR assay might be a useful technique in epidemiological research & surveillance by relating separates from various sources to an often origin (Rabie et al., 2012). Utilization of polymerase chain reaction, 4 separate strains contained Stn gene, which is a distinctive gene for *Salmonella spp.* (Jamshidi et al., 2009). Also, *hila* strains, which are specific for the revealing of salmonella, have been distinguished in all the strains separated at which was in line with wih those reported by Chagas et al. (2013)

These factors, in turn, are closely correlated with the evolution of spoilage bacteria. The poor food handling and abuse of temperature control could encourage the evolution of microorganisms, which cause spoilage of food (Gour et al., 2014).

5. CONCLUSIONS

Freezing of beef meat is considered a good method to preserve meat, but contamination of the meat before freezing or after thawing, inefficient freezing, improper handling, and cooking of frozen meat lead to the growth and proliferation of food borne microorganisms and subsequently food borne illness, which is a public health hazard.

CONFLICT OF INTEREST

The authors announce that they have no Conflict of interest.

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