

Incidence of Some Foodborne Pathogens in Retailed Beef Luncheon and Kofta at El-Gharbia Governorate, Egypt

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Abstract

This study was conducted to evaluate the microbial aspect of some meat products by performing *S. aureus* count, detection of *S. aureus* enterotoxins as well as isolation and identification of some food poisoning bacteria. Therefore, fifty random samples of beef luncheon and kofta (25 of each) were equally collected from different localities in Gharbia governorate, then bacteriologically examined, where values of *S. aureus* count were $7.61 \times 10^2 \pm 0.54 \times 10^2$ in beef luncheon, $2.82 \times 10^3 \pm 0.39 \times 10^3$ (cfu/g) in kofta samples. Meanwhile, in beef luncheon enterotoxin B and enterotoxin (A&D) were detected with a percentage of 4% of each, while in kofta samples enterotoxin A, enterotoxin D, and enterotoxin (A&C) were detected with a percentage of 8%, 4%, and 4%, respectively. Moreover, *Salmonellae*, *E. coli*, *Klebsiella pneumoniae*, and *Listeria* were detected at 16%, 20%, 20%, and 20% in the examined beef luncheon. while, in kofta samples 24 %, 36%, 28% and 16%, respectively. Meanwhile, *E. coli* identified as O26 : H11, O44 : H18, O78, O91 : H21, O103 : H2, O111 : H4, O127 : H6, O146 : H21 and O159 with various percentages. *Salmonella* spp. identified as *S. Enteritidis*, *S. Typhimurium*, *S. Labadi*, *S. Infantis*, and *S. Molade*. On the other side, *Listeria* species serologically identified as *L. monocytogenes*, *L. ivanovii*, *L. innocuai*, and *L. welshimeri*. Moreover, isolated serotypes of *K. pneumoniae* as K1 and K2 were 16%, and 4% in luncheon samples. While 16% and 12% were in kofta samples. Furthermore, high virulent *K. pneumoniae* (HVKP) and classic *K. pneumoniae* (CKP) were 12% and 8% in luncheon, while were 20% and 8% in kofta samples, respectively. Achieved results in the current study proved that most of the examined meat products were contaminated with *E. coli*, *Salmonella* spp., *S. aureus*, *Listeria* spp. and *K. pneumoniae*, this considered objectionable, as they render the product of inferior quality and unfit for consumption

KEYWORDS

Luncheon, Kofta, APC, *Salmonellae*, *E. coli*, *S. aureus*, *K. pneumoniae*, *Listeria*.

INTRODUCTION

Bacteria such as *S. aureus*, *Salmonella*, *L. monocytogenes*, and *E. coli* are the major zoonotic bacterial pathogens that associated with the consumption of the contaminated animal products, and are the causative agents of foodborne illness and death in the world. Most of these microbes have zoonotic importance, as they can significantly impact both public health and economic sectors (Abebe *et al.*, 2020). Because of the high levels of moisture, nitrogenous substances, minerals, a few fermentable carbohydrates like glycogen, and perfect pH that promotes the growth of most bacteria, meat products can serve as an effective culture medium for the growth of various organisms (Alahakoon *et al.*, 2015).

Coliform microorganisms are related to the digestive systems of human beings and animals. Presence outside the intestines can be a contaminant demonstrated with fecal discharges (Park *et al.*, 1999). Fecal contamination of food can occur at the time of slaughter or during processing (Dsani *et al.*, 2020). Pneumonia, encephalitis, infections of the urinary system, and diarrhea all have been linked to pathogenic *E. coli* (Elbayoumi *et al.*, 2018b).

Coliform bacteria (*E. coli* and *Klebsiella* spp.) are of great im-

portance in food microbiology. They are vital biosanitary indicators that emphasize hygiene in the processing and handling of food products (Filimon *et al.*, 2010). *K. pneumoniae* is an important opportunistic pathogen that spreads through contaminated food materials and is often considered an agent of foodborne illness. The pathogen can be found in frozen foods and fresh meat that causes a variety of infectious diseases in humans, including septicemia, liver abscesses, diarrhea, and pneumonia (Guo *et al.*, 2017). It is the primary reason for the hospitalization of immunocompromised patients and individuals with severe diseases (Russo and Marr, 2019).

Staphylococcus aureus was in contaminated foods causing human illness; linked to food poisoning. Furthermore, *S. aureus* can develop and convey virulence in a wide range of foods, including meat and meat products. Heat-stable enterotoxins of *S. aureus* strains can cause staphylococcal food poisoning, a common cause of gastroenteritis worldwide (Shaltout *et al.*, 2019).

Salmonella is an important foodborne zoonotic pathogen with relevance to global public health (Gomes *et al.*, 2022). *Salmonella* serovars continue to pose a threat to the public's health even if they don't always result in fatal illness, it is mainly located within the intestinal tract leading to gastroenteritis (Mohammed-

and Cormican, 2016). Diarrhea, cramping in the abdomen, and fever within 8 to 72 hours are the most common characteristics of salmonellosis (Elbayoumi et al., 2018a).

Listeriosis is an important bacterial food-borne zoonotic disease caused by *Listeria monocytogenes*. The pathogenic strains of *Listeria* can cause severe illness in men by ingestion of contaminated food products, especially meat (Deka et al., 2022).

Serious symptoms of invasive listeriosis, such as sepsis, encephalitis, and meningitis, are common. which affects mainly pregnant women, immunocompromised people, and newborns (Cherifi et al., 2020). Because of the severity of the symptoms and the high case fatality rate, *L. monocytogenes* research and control are critical for food safety worldwide (Thomas et al., 2015). As the contamination of meat products such as luncheon and kofta with different food-borne pathogens constitute dangerous problems for consumers. So, this study was done to evaluate the pathogenic microorganisms and bacteriological quality of these products.

MATERIALS AND METHODS

Collection of samples

Fifty random samples of meat products were equally collected from different localities in Gharbia governorate, Egypt from February to August 2022. Samples were luncheon and kofta (25 of each). Samples were kept in separate sterile plastic bags and preserved in an ice box then transferred to the laboratory quickly for bacteriological examination.

Preparation of samples (ISO, 2008)

In aseptic conditions, 25 grams of each sample were collected and transferred to a sterile homogenizer flask containing 225 ml of sterile peptone water (0.1%). homogenized for 3 minutes at 14000 rpm then allowed for 5 minutes at room temperature. One ml of the homogenate was added to a tube containing 9 ml of sterile peptone water (0.1%) tenfold serial dilutions were prepared for bacteriological examination.

Bacteriological examination

S. aureus was detected according to Mac Faddin (2000) and counted according to FDA (2001). Enterotoxin was detected based on the method described by Shingaki et al. (1981). Sam-

ples were examined for *E. coli* according to ISO (2013) and serologically based on Kok et al. (1996). Detection of *Salmonellae* was done according to ISO (2013) and serologically to Kauffman (1974). Detection of *Listeria* spp. based on the methods of Mac Faddin (2000) and serologically according to Pagotto et al. (2001). *Klebsiella pneumoniae* was detected as described in ISO (2004), and serologically based on Edmondson and Cooke (1979).

Statistical Analysis

Using the SPSS tool, the collected results were statistically evaluated (Statistical Package for Social Sciences, version IBM 23). Statistical analysis was made using one way analysis of variance (ANOVA) for determinations of the minimum, maximum and means of the different organisms isolated from the meat samples products according to Feldman et al., (2003).

RESULTS

In Table 1, the incidence of *S. aureus* in beef luncheon and kofta samples were 40% and 52% with mean values of $7.61 \times 10^2 \pm 0.54 \times 10^2$ and $2.82 \times 10^3 \pm 0.39 \times 10^3$ Cfug, respectively. Also, enterotoxin B and enterotoxin A and D with a percentage of 4% in beef luncheon, while enterotoxin A (8%), enterotoxin D (4%), and enterotoxin A and C (4%) in kofta samples, respectively (Table 2).

Moreover, *E. coli* was detected with a percentage of 20 % and 36% in beef luncheon and kofta samples as follows O26: H11(4%), O91: H21 (8%), O111: H4(4%) and O146: H21(4%) in beef luncheon. While in kofta O26 : H11(12%), O44 : H18 (4%), O78(4%), O103 : H2(4%), O127 : H6(8%) and O159 (4%),(table 3).Furthermore, *Salmonella* was detected with a percentage of 16 % and 24% in beef luncheon and kofta samples, respectively as followed by *S. Infantis* (8%) in luncheon and (4%) in kofta, also, *S. Enteritidis* (4%) in luncheon and (8%) in kofta, *S. Typhimurium* (4%of each), *S. Labadi* and *S. Molade* 4% in kofta samples only (Table 4).

Listeria spp. were 20% and 16% in luncheon and kofta samples, respectively. In beef luncheon, *L. ivanovii* with a percentage of 12%, *L. monocytogenes* and *L. innocua* (4% of each). Meanwhile, in kofta samples, *L. monocytogenes* with a percentage of 8%, *L. ivanovii* and *L. welshimeri* (4% of each) (Table 5).

K. pneumoniae with a percentage of 20% and 28% in beef luncheon and kofta, respectively as K1 and K2 percent were

Table 1. Count of *S. aureus* in the examined samples of meat products (n = 25).

	No. of affected samples		Min	Max	Mean±S.E*
	No.	%			
Beef luncheon	10	40	< 10 ²	2.0×10 ³	7.61×10 ² ±0.54×10 ²
Kofta	13	52	< 10 ²	7.0×10 ³	2.82×10 ³ ±0.39×10 ³

S.E* = Standard error

Table 2. Incidence of *S. aureus* enterotoxins isolated from samples of meat products (n=25).

Enterotoxins	Meat luncheon		Kofta	
	No	%	No	%
A	-	-	2	8
B	1	4	-	-
D	-	-	1	4
A+C	-	-	1	4
A+D	1	4	-	-
Total	2	4	4	8

16% & 4% in beef luncheon samples. While 16% & 12% were in kofta samples, respectively. High virulent *K. pneumoniae* (HVKP) with a percentage of 12% and 20% in luncheon and kofta. Furthermore, classic *K. pneumoniae* (CKP) with a percentage (of 8% of each) (Table 6).

DISCUSSION

Accomplishing food safety is a worldwide health objective and food-borne manifestations that have attracted major at-

tention in global health. Hence, the determination of microbial pathogens in food is the key to the identification and prevention of problems related to well-being and safety (Gokulakrishnan and Vergis, 2015).

Concerning luncheon samples, *S. aureus* count were nearly similar to results obtained by Abuelnaga et al. (2021) ($5.20 \times 10^2 \pm 0.41 \times 10^2$) and Saad et al. (2023) ($8.2 \times 10^2 \pm 3.6 \times 10^2$) Cfu/g, lower level reported by Hassanien et al. (2018) (0.1×10^2) Cfu/g and Shaltout et al. (2022) ($6.3 \times 10^2 \pm 1.6 \times 10^1$) Cfu/g, and higher value obtained by Tarabees et al. (2016) (3.09×10^5 cfu/g). On the other side, a lower incidence of *S. aureus* by Abuelnaga et

Table 3. Incidence of *E. coli* serotyping of samples of meat products (n=25).

Products	Meat luncheon		Kofta		Strain Characteristics
	No.	%	No.	%	
<i>E. coli</i> strains					
O17: H18	-	-	-	-	EPEC
O26: H11	1	4	3	12	EHEC
O44: H18	-	-	1	4	EAEC
O78	-	-	1	4	ETEC
O91: H21	2	8	-	-	EHEC
O103: H2	-	-	1	4	EHEC
O111: H4	1	4	-	-	EHEC
O121: H7	-	-	-	-	EPEC
O127: H6	-	-	2	8	ETEC
O146: H21	1	4	-	-	EPEC
O159	-	-	1	4	EIEC
Total	5	20	9	36	

Table 4. Incidence of *Salmonellae* and serotyping isolated from samples of meat products (n=25).

Products	Beef luncheon		Kofta		Antigenic structure	
	No.	%	No.	%	O	H
Serotypes						
<i>S. Enteritidis</i>	1	4	2	8	1,9,12	g,m: -
<i>S. Kentucky</i>	-	-	-	-	8,20	i: Z6
<i>S. Infantis</i>	2	8	1	4	6,7,14	r: 1,5
<i>S. Labadi</i>	-	-	1	4	8,20	d: Z6
<i>S. Molade</i>	-	-	1	4	8,20	Z10: Z6
<i>S. Papuana</i>	-	-	-	-	6,7	r: e,n,Z15
<i>S. Typhimurium</i>	1	4	1	4	1,4,5,12	i: 1,2
<i>S. Wingrove</i>	-	-	-	-	6,8	c: 1,2
Total	4	16	6	24	-	-

Table 5. Incidence of *Listeria* species and serotyping isolated from samples of meat products (n=25).

Products	Beef luncheon		Kofta	
	No.	%	No.	%
<i>Listeriae</i>				
<i>L. monocytogenes</i>	1	4	2	8
<i>L. ivanovii</i>	3	12	1	4
<i>L. innocua</i>	1	4	-	-
<i>L. grayi</i>	-	-	-	-
<i>L. welshimeri</i>	-	-	1	4
Total	5	20	4	16

Table 6. Incidence of *K. pneumoniae* and serotyping of samples of meat products (n=25).

Products	Positive samples		Serotypes							
	No.	%	K1		K2		HVKP		CKP	
			No.	%	No.	%	No.	%	No.	%
Luncheon	5	20	4	16	1	4	3	12	2	8
Kofta	7	28	4	16	3	12	5	20	2	8

al. (2021) 30% and Saad et al. (2023) 20% in luncheon samples. The presence of *S. aureus* in heat-treated food is a pointer to largely poor personal hygiene, improper storage, and an unhygienic environment (Achi and Madubuike, 2007).

Regarding kofta samples, *S. aureus* count was nearly similar to the result obtained by Hassan et al. (2018) ($3.72 \times 10^3 \pm 0.51 \times 10^3$) and Hassanin et al., (2018) ($3.10 \times 10^3 \pm 0.74 \times 10^3$), lower result obtained by Abd El Satter (2016) ($4.3 \times 10^2 \pm 2.06 \times 10^2$) (cfu/g), Abuelnaga et al. (2021) ($4.4 \times 10^2 \pm 9.5 \times 10$) and Shaltout et al. (2022) ($8.7 \times 10^2 \pm 1.5 \times 10^2$). Lower incidence of *S. aureus* was recorded by Abuelnaga et al. (2021) (20%), Hassanin et al. (2018) (46.67%), and Shaltout et al. (2022) (40%) in kofta samples. The high *S. aureus* count could be due to the neglected hygienic practices of the workers and the technique used for evisceration (Cohen et al., 2007). Moreover, the existence of *S. aureus* in meat products is considered a trustworthy index of improper handling during processing (Nadim, 2016).

S. aureus strains that produce sea are known to be the most frequent in food poisoning outbreaks. The sea is an extremely potent gastrointestinal toxin, with a low infective dose of 100 ng sufficient to cause toxicity (Balaban and Rasooly, 2000).

In luncheon samples, a high rate of *E. coli* was obtained by Mosbah (2017) (24%); Abuelnaga et al. (2021) (55%) and Shaltout et al. (2022) (30 %). Lower results were reported by Saad et al. (2018) (12%) Similar results were recorded by Tarabees et al. (2015) (20%).

Meanwhile, in kofta samples high rate of *E. coli* obtained by Hassan et al. (2018) (46.67%) and Shaltout et al. (2022) (40%), lower results reported by Younes et al. (2019) (12%) and Abuelnaga et al. (2021) (25%).

The presence of *E. coli* in food is considered an indicator of faults during preparation, handling, storage, or service (Shaltout et al., 2016). Fecal contamination of carcass and contamination of raw food is common source of *E. coli* infection (Roberts, 1990).

The isolated serotypes agree with Hassan et al. (2018) who isolated O26:H11 (6.67%) & O111: H4 (EHEC) (6.67 %) and Abuelnaga (2021) O26, O126, O111, O158, O146 from luncheon samples. While Hassan et al. (2018) O26:H11 (13.33%), Saad et al. (2018) O127: H6 (ETEC) (4%), and Abuelnaga (2021) O26 from kofta agree with the obtained results.

Regarding the detection of *Salmonella* in the examined beef luncheon, a lower rate of *Salmonella* was reported by Saad et al. (2018) 8% and Abuelnaga et al. (2021) 15% and Elbayoumi et al. (2021) 7.5%, a similar result was recorded by Essa et al. (2009) (16.7%). While others did not detect *Salmonella* (Sharaf-and Sabra- 2012; Saad et al. (2018); Armany et al. 2021; Saad et al. 2023). Comparing the obtained findings for detection of *Salmonella* in the examined kofta samples, high results were obtained by Hassan et al. (2018) (26.67%) and Elbayoumi et al. (2021) (27.5%), lower results were obtained by Saad et al. (2018) (16%) and Abuelnaga et al., (2021) (15%) and Elbayoumi et al. (2021) (27.5%). While Younes et al. (2019) did not detect *Salmonella*.

The transmission of salmonellosis occurs when feed and water are contaminated by *Salmonella*-contaminated stools or fecal-oral routes making feces important reservoirs of *Salmonella* serovars (Atlaw, 2022). As well as poor hygiene conditions, the temperature of storage, equipment, and personal hygiene (Stevens et al., 2006). More than 2500 *Salmonella* serotypes are recognized, of which, *S. Enteritidis*, *S. Typhimurium*, and *S. Kentucky* are identified as the most frequent causative agents causing disease burden on consumers (Bugarel, 2017).

The isolated serotypes of *Salmonella* agree with Eldesouky et al. (2016) isolated *S. Enteritidis* (47%), *S. Typhimurium* (23.52%), and Elbayoumi et al. (2021) *S. Enteritidis* 2.5% and *S. Typhimurium* 5% from luncheon samples. While Hassan et al. (2018) isolated *S. Enteritidis* 6.67 % (1), *S. Typhimurium* 13.33 % (2), and Elbayoumi et al. (2021) isolated *S. Enteritidis* 12.5% (5), *S. Infantis* 7.5% (3)

Listeria causes contamination of various processed meat in any stage of processing and storage (Zanette, 2015). High incidence of *Listeria* spp. obtained by Mahmoud et al. (2019) 40% from luncheon. While Eldaly et al. (2013) could not isolate *Liste-*

ria spp. from luncheon but in examined kofta samples high incidence of *Listeria* spp. obtained by Ahmed (2001) (22.5%), Eldaly et al. (2013) (35%), and Mahmoud et al. (2019) (70%).

Concerning *L. monocytogenes* in luncheon samples, lower results were obtained by Abdel-Aziz and Hassanien (2022) (3.1%), while Armany et al. (2021) failed to find *L. monocytogenes* and higher results were reported by Saad et al. (2001) (7.5%) and Mahmoud et al. (2019) (10%) in luncheon samples.

The capability of *L. monocytogenes* to reproduce at the refrigeration zone of temperature a risk of processed cooked meat like luncheon (Schuchat et al., 1992). Therefore, the presence of *L. monocytogenes* reflects bad hygienic measures (Marinšek and Grebenc, 2002). While in examined kofta samples lower results of *L. monocytogenes* were obtained by Eldaly et al. (2013) (5%) and higher results were obtained by Mahmoud et al. (2019) (20%) in kofta samples. Contamination of kofta by *L. monocytogenes* may occur during animal slaughtering, transportation, workers' hands, and equipment used such as mincing machines, knives, and packaging tools.

Recently, the number of foodborne illness outbreaks caused by *K. pneumoniae* increased. However, characteristics of *K. pneumoniae* isolated from foods now, are not detected (Zhang et al., 2018).

Lower result (12.6%) for *K. pneumoniae* in beef luncheon was reported by Abdel-Rhman (2020), while (HVKP) and (CKP) (26 and 18 isolates, respectively) and Meshaal et al. (2021) detected *K. pneumoniae* in 4 of 12 beef luncheon samples. Moreover, Chepkemei et al. (2022) isolated *Klebsiella* spp. (7%) from raw meat. A relatively high prevalence of *Klebsiella* spp. may be attributable to the employment of conventional food preparation techniques, improper temperatures, and improper hygiene (Barro et al., 2006).

CONCLUSION

The contamination of meat products with such serious pathogens not only renders these meals of inferior quality and unfit for human consumption but is also considered an indication of fecal contamination. It demonstrated that there is a high bacterial load besides a relatively high rate of pathogens of public health importance and this may be due to mishandling and the negligence of hygienic aspects either at production levels or product selling.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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