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Bacteriological quality of ready to eat meals served at governmental hospital in Egypt.

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ABSTRACT

The consumption of ready-to-eat (RTE) meat and chicken products in modern times represents high health threat as the opportunistic and commensal microorganisms may cause severe problems. Ninety random samples of cooked meat, grilled kofta and fried chicken (30 of each) as well as, 90 swabs of worker's hands, table surfaces and knives (30 of each) were collected from central restaurant of a governmental hospital, Cairo Governorate to evaluate their bacteriological quality. Aerobic plate count (APC), *Enterobacteriaceae* count, *coliform* count, *S. aureus* count were determined. Moreover, detection of Enteropathogenic *E. coli*, *S. aureus*, *Salmonella spp.* and *K. pneumoniae* was performed. The revealed results indicated significant differences between cooked meat, grilled kofta and fried chicken at (P<0.05). The mean values of APC, *Enterobacteriaceae*, *coliform* and *S. aureus* counts were 6.09×10^4, 5.45×10^3, 2.57×10^3 and 6.12×10^3 (cfu/g) in cooked meat, 1.81×10^5, 1.81×10^4, 6.05×10^3 and 9.44×10^3 for grilled kofta, and 3.24×10^5, 2.97×10^4, 8.83×10^3, 1.75×10^4 for fried chicken, respectively. The most accepted product was cooked meat while the lowest accepted one was fried chicken. Furthermore, the incidence of enterotoxins secreted by *S. aureus* was 3.3% and 6.7% (type A) for grilled kofta and fried chicken, respectively, while type C was only present in fried chicken (3.3%). Type D enterotoxin was recovered in cooked meat, grilled kofta and fried chicken with equal percent (3.3%). Types (A+B) *S, aureus* enterotoxins were found in grilled kofta (3.3%), however types A and C were detected in cooked meat and fried chicken (3.3% of each). The results revealed that fried chicken samples were most contaminated with *E. coli*, *Salmonella* and *K. pneumonia*. Moreover, *K. pneumonia* were isolated at 13.3%, 20% and 30%, respectively. High virulent and classic *K. pneumonia* also were isolated. The Incidence of pathogenic bacteria *S. aureus*, *E. coli*, *Salmonellae* and *K. pneumonia* in the swabs of worker hands were 30%, 0%, 0% and 10%, respectively and 10%, 20%, 10%, 20% form table surfaces and 20%, 10%, 0% and 10% from knives.

1. INTRODUCTION

Although, ready to eat (RTE) meat meals are favoured due to excessive biological value, agreeable taste as well as easily serving (Mahros et al., 2021), but also regarded as high-risk food and may additionally lead to foodborne illness (Shao-qin et al., 2012). So, presence of *Salmonella* species in RTE products makes it risky (Muth, 2009).

Food poisoning due to *Staphylococcus* spp. characterized by means of fast onset, nausea, and violent vomiting (Argudin et al., 2010). Another very vital organism involved in foodborne disease is *E. coli* that regarded as appropriate indicator of faecal contamination (Synge, 2000). Some strains of *E. coli* produce toxin (STEC), which is a group of pathogenic *E. coli* producing one or extra Shiga toxins (Monaghan et al., 2011).

Furthermore, the present study was planned out to determine APC, *coliform*, *Enterobacteriaceae* and *S. aureus* also counts isolation and identification of *E. coli*, salmonella, and *S. aureus* in RTE cooked meat, grilled kofta and fried chicken meals.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 90 samples of ready to eat meals represented by cooked meat, grilled kofta and fried chicken (30 of each) and 90 swabs of worker's hands, table surfaces and knives (30 of each) have been collected from central restaurant of a governmental hospital. Each sample was kept in a separate sterile plastic bag and preserved in an ice container then transferred to the laboratory underneath complete aseptic conditions without undue delay and examined as rapidly as possible. The collected samples had been subjected to the bacteriological examination to determine the sanitary conditions under which they have been prepared and served.

2.2. Bacteriological examination:

Preparation of samples (ISO 4833-1, 2013):

Accurately, 25 grams of each sample were homogenized aseptically with 225 ml of 0.1% sterile peptone water in a stomacher (Colworth, 400) for 1.5 minutes from which tenfold serial dilution were prepared.

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Preparation of swabs:
Swabs were prepared by sterile cotton screw capped plastic tubes which are ready for used for worker's hand, table surface and knives.

2.2.1. Aerobic Plate Count was conducted according to ISO 4833-1 (2013)

2.2.2. Enterobacteriaceae Count was recorded according to ISO 4833-1 (2013)

2.2.3. Total coliform count was performed according to ISO 4832 (2006)

2.2.4. Screening for Enteropathogenic Escherichia coli was adopted according to ISO 16649-2, (2001)
Biochemical identification (Kreig and Holt, 1984):

Serological identification of E. coli:
The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

2.2.5. Screening for Salmonellae
Pre-enrichment broth:
From the original dilution, one ml was inoculated into sterile peptone water and incubated at 37 ^{o}C for 18 hours.
Enrichment broth was recorded according to Harvey and Price (1981)
Selective Plating:
Identification of Salmonellae was performed according to ISO, (2002).

Biochemical identification:
Serological identification of Salmonellae:
Serological identification of *Salmonellae* was performed according to Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

2.2.6. Identification of K. pneumoniae:
String test for detection of mucoviscosity (Shon et al., 2013):
Serological identification of capsular antigen by Quelling test "Neufeld reaction" (Edmondson and Cooke, 1979).

2.2.7. Identification of S. aureus count (FDA, 2001):
Identification of Staphylococci species was recorded according to FDA, (2001):

2.3. Statistical analysis:
The obtained results were statistically evaluated by application of ANOVA test according to Feldman et al. (2003).

3. RESULTS

It is evident from the results recorded in table (1) that the mean values of APC, *Enterobacteriaceae* counts, *Coliform* count and *S. aureus* count of the examined cooked meat samples 6.09 x10^4, 5.45x10^3, 2.57 x10^3 and 6.12 x10^3 (cfu/g, respectively and 1.81x10^5, 1.86 x10^4, 6.05 x10^3 and 9.44 x10^3 for grilled kofta while, the mean values of APC, *Enterobacteriaceae* counts, *Coliform* count and *S. aureus* count of the examined fried chicken samples were 3.24x10^5, 2.97x10^4, 8.83x10^3 and 1.75x10^3 (cfu/g), respectively.

According to Centre for food safety, (2014) the accepted samples based on their APC data presented in (table 2) were 76.7%, 70% and 56.7% for cooked meat, grilled kofta and fried chicken, respectively. Also, the accepted samples based on their *Enterobacteriaceae* counts were 73.3%, 63.3% and 46.7% for cooked meat, grilled kofta and in fried chicken. Moreover, the accepted samples based on their coliform count were 70%, 56.7% and 43.3% in cooked meat, grilled kofta and in fried chicken, while the accepted samples based on *S. aureus* count were 83.3%, 80%, 73.3% for cooked meat, grilled kofta and fried chicken, respectively.
Enterotoxins secreted by *S. aureus* isolated from cooked meat samples were (6.7%), 10% from grilled kofta samples and 16.7% of fried chicken samples (table 3). Also, data in table (4) revealed that 12 isolates of *E. coli* were identified from the examined samples with different percentages as follow O26:H11 (EHEC) (3.3%), O86 (EPEC) (3.3%) and O146:H21(EPEC) (3.3%) for cooked meat, O26:H11(EHEC) (3.3%), O119:H6 (EPEC) (3.3%) and O128:H2 ETEC (6.7%) for grilled kofta and O55:H7 (EPEC) (3.3%), O78 (ETEC) (6.7%), O91:H21 (EHEC) (3.3%), and O159 (EIEC) (3.3%) for fried chicken, respectively.

Table 1 Mean values of certain bacterial group (cfu/g) in RTE meat served at central restaurant of a governmental hospital (n=30).

Cooked foods	APC (cfu/g)	Enterobacteriaceae (cfu/g)	Coliforms (cfu/g)	*S.aureus* (cfu/g)
Cooked meat	90.6×10^4± 0.77x10^{4c}	5.45×10^3± 0.69×10^{3c}	2.57x10^3± 0.41x10^{3c}	6.12x10^3± 0.96x10^{3c}
Grilled kofta	1.81x10^5± 0.28×10^{5b}	1.81x10^4± 0.28×10^{4b}	6.05x10^3± 0.72×10^{3b}	9.44x10^3± 1.51x10^{3b}
Fried chicken	3.24x10^5± 0.56x10^{5a}	2.97×10^4± 0.46x10^{4a}	8.83x10^3± 1.16x10^{3a}	1.75x10^4± 0.34x10^{3a}

*Mean values with different superscripts in the same column are significantly different at (P<0.05).

Table 2 Fitness of the RTE meat served at central restaurant of a governmental hospital based on their contamination with certain bacterial groups cfu/g (n=30).

	Cooked foods		Accepted samples		Unaccepted samples	
		acceptable range*	No.	%	No.	%
APC (cfu/g)	Cooked meat	<10^5	23	76.7	7	23.3
	Grilled kofta	<10^5	21	70	9	30
	Fried chicken	<10^5	17	56.7	13	43.3
Enterobacteriaceae (cfu/g)	Cooked meat	<10^4	22	73.3	8	26.7
	Grilled kofta	<10^4	19	63.3	11	36.7
	Fried chicken	<10^4	14	46.7	16	53.3
Coliform count (cfu/g)	Cooked meat	<10^2	23	70	9	30
	Grilled kofta	<10^2	17	56.7	13	33.3
	Fried chicken	<10^2	13	43.3	17	56.7
S.aureus (cfu/g)	Cooked meat	<10^4	25	83.3	5	16.7
	Grilled kofta	<10^4	24	80	6	20
	Fried chicken	<10^4	22	73.3	8	26.7

*Centre for Food Safety (2014)

Data presented in table (5) revealed that 11 isolates of *Salmonellae* were identified from the examined samples as follow *S. Enteritidis* (3.3%) for grilled kofta and fried chicken, *S. Infantis* (3.3%) from fried chicken, *S. Kentucky* (6.67%) from fried chicken, *S. Montevideo* (3.3%) from cooked meat, *S. Shangani* (3.3%) from fried chicken, *S. Tsevie* (3.3) from grilled kofta and *S. Typhimurium* (3.3%) and (6.67%) for cooked meat and grilled kofta, respectively.

Table (6) indicated that *K. pneumonia* isolated from cooked meat, grilled kofta and fried chicken as K1 percent was 10%, 10% and 16.7%, respectively. While K2 percent was 3.3%, 10% and 13.3% for cooked meat, grilled kofta and fried chicken, respectively. High virulent *K. pneumonia* (HVKP) 0%, 13.3%, 20% for cooked meat, grilled kofta and fried chicken, respectively. Furthermore, classic *K. pneumonia* (CKP) was isolated from cooked meat, grilled kofta and fried chicken 13.3%. 6.7% and 10%, respectively. The Incidence of pathogenic bacteria *S. aureus*, *E. coli*, *Salmonellae* and *K. pneumonia* in the swabs taken from worker's hands were 30%, 0%, 0% and 10%, respectively and 10%, 20%, 10%, 20% form table surfaces and 20%, 10%, 0% and 10% from knives (table 7).

Table 3 Occurrence of enterotoxins secreted by S. aureus isolated from cooked food served at central restaurant of a governmental hospital (n=30).

Enterotoxins	Cooked meat		Grilled kofta		Fried chicken	
	No.	%	No.	%	No.	%
A	-	-	1	3.3	2	6.7
C	-	-	-	-	1	3.3
D	1	3.3	1	3.3	1	3.3
A+B	-	-	1	3.3	-	-
A+C	1	3.3	-	-	1	3.3
Total	2	6.7	3	10	5	16.7

Table 4 Incidence of Enteropathogenic E. coli detected in RTE meat served at central restaurant of a governmental hospital (n=30).

Product	Cooked meat		Grilled kofta		Fried chicken		Strain characteristics
E.coli Strains	No.	%	No.	%	No.	%	
O26 :H11	1	3.3	1	3.3	-	-	EHEC
O55 :H7	-	-	-	-	1	3.3	EPEC
O78	-	-	-	-	2	6.7	ETEC
O86	1	3.3	-	-	-	-	EPEC
O91:H21	-	-	-	-	1	3.3	EHEC
O119 :H6	-	-	1	3.3	-	-	EPEC
O128 :H2	-		2	6.7	-	-	ETEC
O146 : H21	1	3.3	-	-	-	-	EPEC
O159	-	-	-	-	1	3.3	EIEC

ETEC= Enteropathogenic *E. coli* EIEC = Enterotoxigenic *E. coli*
EHEC= Enterohaemorrhagic *E. coli* ETEC= Enterotoxigenic *E. coli*

Table 5 Incidence and serotyping of Salmonella organisms isolated from cooked food served at central restaurant of a governmental hospital (n=30).

Products	Cooked meat		Grilled Kofta		Fried chicken		Group	Antigenic structure	
Salmonella Strains	No.	%	No.	%	No.	%		O	H
S. Enteritidis	-	-	1	3.3	1	3.3	D1	1,9,12	g,m : 1,7
S.Infantis	-	-	-	-	1	3.3	C1	6,7	*R:1,5*
S.Kentucky	-	-	-	-	2	6.7	C3	8,20	I:z6
S. Montivideo	1	3.3	-	-	-	-	C1	6,7	g,m,s:1,2,7
S. Shangani	-	-	-	-	1	3.3	E1	3,10	D: 1,5
S.Tsevie	-	-	1	3.3	-	-	B	1,4,12	I:e,n,z15
S.Typhimurium	1	3.3	2	6.67	-	-	B	1,4,5,12	i: 1,2

Table 6 Incidence and serotyping and pathogenicity of K. pneumonia contaminating the cooked food served at central restaurant of a governmental hospital.

Meat meals	Sero diagnosis of *K. pneumonia*		String test	
	K1	K2	HVKP	CKP
	No. (%)	No. (%)	No. (%)	No. (%)
Cooked meat (30)	3 (10)	1 (3.3)	0 (0)	4 (13.3)
Grilled kofta (30)	3 (10)	3 (10)	6 (13.3)	2 (6.7)
Fried chicken (30)	5(16.7)	4(13.3)	6 (20)	3 (10)
Total (90)	11(12.2)	8 (8.9)	10 (11.1)	9 (10)

HVKP=High virulent *K. pneumoniae* CKP =Classic *K. pneumoniae*

Table 7 Incidence of pathogenic bacteria in the swabs taken from worker's hands, table surfaces and knives at central restaurant of a governmental hospital (n=30).

Swabs	Worker hands		Table surfaces		Knives	
Pathogens	No.	%	No.	%	No.	%
S.aureus	5	30	2	10	3	20
E. coli	1	0	4	20	2	10
S. almonellae	0	0	3	10	2	0
K. pneumonia	2	10	6	20	5	10

4. DISCUSSION

Food security has turn out to be as a vital global affair with public health implications and worldwide trade (Zeighami et al., 2020).

The results of APC revealed significance difference ($P<0.05$) between the examined samples cooked meat, grilled kofta and fried chicken. The results were lower than that obtained by Edris et al. (2020a) who reported that the mean values of APC in grilled and fried chicken meals were 9.97 $x10^3$ and 6.02$x10^3$ (cfu/g), while similar results were mentioned by Shaltout et al. (2015) who found that values of APC of grilled kofta was 8.51×10^5 cfu/g. The APC is very important indicator for sanitary condition of RTE meals. EEC (2005) The acceptability of the RTE meat based on APC revealed that cooked meat was more acceptable than grilled kofta and fried chicken, respectively. This may attribute to differences in heat treatment of the examined samples. Also, high incidence of aerobic plate counts indicated that the cooking process was inappropriate, or post cooking contamination had occurred (Khater et al., 2013).

The mean values of total Enterobacteriaceae counts/g in the examined samples of RTE meat showed significant difference ($P<0.05$) between the examined samples of cooked meat, grilled kofta and fried chicken. The current results were higher than recorded by Edris et al. (2020b), who reported that the mean values of Enterobacteriaceae of kofta was 9.14×10^3 cfu/g, unlike results were recorded by Shaltout et al. (2013) as they isolated Enterobacteriaceae from street vended kofta samples was(1.5×10^7cfu/g). Also, the acceptability of the cooked foods based on Enterobacteriaceae revealed that cooked meat was more acceptable than grilled kofta and fried chicken, respectively. So, the data in table (1) revealed that the mean values of total coliform counts cfu/g showed significance difference ($P<0.05$) between the examined samples. The current results were lower than that reported by (Saad et al., 2011) who found that the mean values of coliform were 5.17×10^2 cfu/g. Also, similar results obtained by Abd El-Aal (2015) ($6.40\times10^3\pm 1.23\times10^3$) for fried chicken.

Staphylococci count in examined samples were lower than those mentioned by and Abd El-Aal (2015) ($2.10\times10^3\pm 0.32\times10^3$) for fried chicken meat. also, Edris et al. (2020b) isolated *S. aureus* 1.26×10^3 cfu/g and 7.58×10^2 for grilled and fried chicken. EFSA (2016) stated that toxins of bacterial origin are considered as the third most significant reason of foodborne outbreaks worldwide. Moreover, the isolated staphylococcal enterotoxin from kofta and other food products as recorded by Osama et al. (2021).

The Incidence of Enteropathogenic *E. coli* detected in cooked foods was recorded in table (4) *E. coli* is one of the common foodborne bacterial pathogens that secrete toxins (Ma et al., 2019). Contamination of RET chicken with Enteropathogenic *E. coli* during storage usually causes safety and economic losses (Huang et al., 2020).

Incidence of Salmonellae serotypes detected in cooked foods in table (5) showed that the most common serotypes that isolated were*S. Enteritidis, S. infantis, S. kentuckey, S. Montevideo, S.Shangani, S.Tsevie and S. typhimurium.* Similar serotypes isolated by Edris et al. (2020a) form

ready to eat meat and chicken meals. Salmonella spp. were previously isolated from ready to eat meat meals by Soliman et al. (2002).

Incidence and serotyping of *K. pneumoniae* in table (6). indicated that fried chicken is more contaminated with such organism.

Moreover, results were interfered with those recorded by Abd El-Aal (2015) who isolated *K. pneumonia* from cooked meat (6.67%). and agree with those reported by Ali (2011) (60%).

Meanwhile, table (6) cleared that the incidence of food poisoning bacteria for the swabs taken from meat handlers, table surfaces and knives was 5(30%) for worker's hands contaminated with *S.aureus* and 2 (10%) of them were contaminated with *K. pneumonia*, while 4 (20%) of table surfaces had *E.coli* , 3(10%) has *Salmonellae*, 2 (10%) has *S. aureus* and 6 (20%) have *K. pneumoniae* and knives have 2(10%) E. coli, 3(20%) *S. aureus* and 5 (10%) *K. pneumoniae.*

5. CONCLUSION

The obtained results indicated that fried chicken samples were more contaminated *with S. aureus, E. coli, coliform, Enterobacteriaceae,* as compared with those of grilled kofta and cooked meat.

The presence of these microorganisms in large number is not only renders these meals of inferior quality and unfit for human consumption, but also considered as an indication for the faecal contamination. To improve the quality of cooked meals to be safe for human consumption, the following recommendations should be adopted. Good Raw materials stored under proper conditions should be considered. High quality meat and additives should be used. Food handlers should have the ability to handle food hygienically.

Worker's hands should be thoroughly washed and sanitized. Hands should be thoroughly washed and sanitized before work and after visiting the toilet.

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