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Hygienic profile of some ready to eat meat product sandwiches sold in Benha city, Qalubiya Governorate, Egypt

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ABSTRACT

Ready-to-eat (RTE) meat product sandwiches have become one of the most important sources of nutrition, especially with changing consumer's dietary and social habits. Unfortunately, it may be loaded with many microorganisms especially accidental post-cooking cross-contamination. Therefore, this study aimed to investigate the bacteriological quality of 120 ready to eat meat product sandwiches (beef kofta, bovine liver, fish fillet, and chicken nuggets), 30 of each, which were collected randomly from restaurants and street vendors located in Benha city, Qalubiya Governorate, Egypt. Results revealed that kofta samples were the most contaminated samples where mean values of aerobic plate count (APC) and coliforms were $9.6x10^6$, and $1.9x10^2$ CFU/g, respectively; followed by chicken nuggets, fish fillet, and bovine liver samples. Furthermore, *E. coli* and salmonellae of different serological types were detected in 5.8% and 2.5% of examined samples, respectively. Obtained results indicated that RTE meat-based sandwiches may pose a risk to consumer's health in the absence of food safety knowledge and hygiene application; strict hygienic measures and authority inspection is strongly recommended.

1. INTRODUCTION

Roadside foods are ready to eat foods that prepared and sold by vendors in streets and similar public places. They supply a source of Ready-To-Eat (RTE), high quality and nutritional value meals with reasonable price, while giving a good income for the vendors (Swanepoel et al., 1998). They feed a lot of consumers daily with a wide variety of RTE foods. Dependence on such foods is more interesting in its convenience than in its safety and hygiene (Mensah et al., 2002).

Street vendor foods raise concerns with respect to their potential for serious food poisoning outbreaks due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants, and improper food handling practices based on unrespect of good manufacturing practices and good hygienic practices (Estrada et al., 2004).

Ready to eat meat products are highly demanded due to their biological value, reasonable price, and agreeable taste; also, they represent rapid easily prepared meals and solve the problem of shortage in fresh meat of high price which is not available for many families with limited income (Samapundo et al., 2015). Meat products are considered as a major source of most reported foodborne outbreaks and spoilage bacteria and/or foodborne pathogens (Gracey et al., 1999), as they may be exposed to contamination with several types of microorganisms from different sources during the chain of production, processing, and cooking (Gundogan et al., 2005); thus, when they used as street vended food they may posing potential risks to public health.

Salmonella and *E. coli* are commonly implicated food commodity in outbreaks (accounted for 8% of outbreaks) causes illness ranging from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis (Johnson et al., 2006; Jackson et al., 2013).

In Egypt, street-vended meat products may pose a health hazard because of the preparation, handling, serving in bad hygienic conditions as using poor quality raw materials, poor personal hygiene, and post-cooking holding for long times encourages heavy bacterial loads in foods with pathogenic microorganism; such contamination may render the product to be of an inferior quality or unfit for human consumption (El-Ziqaty et al., 2016); with the shortage of the available data concerning the incidence of foodborne infections related to fast foods, lacking of information about the quality and safety of street-vendor foods, and where effective

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food safety controls by concerned regulatory agencies are yet to be realized, evaluation of roadside food bacterial hazards and their indicators would help provide specifications for setting microbial guideline values (Hazaa, 2015).

Therefore, this study aimed to investigate the hygienic measures of some RTE meat products sandwiches with special reference to presence of *E. coli* and Salmonellae contamination.

2. MATERIAL AND METHODS

2.1. Collection of samples:

Hundred-twenty random samples of RTE meat products sandwiches represented by (beef kofta, bovine liver, fish fillet, and chicken nuggets), 30 of each, were collected from restaurants and street vendors in Benha city, Qalubiya governorate, Egypt. Samples were transferred to the laboratory in ice box within 1hr and examined for its bacteriological quality.

2.2. Bacteriological examination:

- Preparation of sample according to APHA (2013).
- Determination of Aerobic plate count (APC) according to (ISO 4833-1, 2013).
- Determination of coliform count (ISO 4832, 2006).
- Enumeration of E. coli was performed according to (ISO 16649-2, 2001): 1 ml from the previously prepared serial dilution was cultured in TBX agar by pour-plate technique and incubated at 44 ºC for 24 hours. Suspected colonies were counted and isolated for more identification.

2.3. Serological identification was performed according to (Kok et al., 1996).

Detection of salmonellae was performed according to (ISO 6579, 2017)

Prepared sample was incubated in buffered peptone water broth at 37 ± 1°C for 18 ± 2 hours, then transferred to Rappaport Vassilidis broth (RV broth) and incubated at 43°C/24hr. One ml of enriched sample was plated on selective XLD agar and Brilliant Green agar, and incubated at 37°C/24hrs, plates were examined for suspected Salmonella colonies which then isolated for confirmation.

Suspected purified salmonella colony was cultured on three biochemical media represented by TSI agar, Urea agar, and L-Lysine decarboxylation media and incubated at 37°C/24hrs.

Serological identification

Pure culture was classified using slide agglutination tests with commercial polyvalent somatic (O-) and flagellar (H-) antisera.

2.4. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

The results of APC were summarized in table (1) and declared that kofta samples were the most contaminated samples with mean value of $9.6x10^6$ CFU/g, followed by chicken nuggets, fish fillet, and bovine liver samples. The statistical results revealed a significant increase in kofta samples when compared with other samples when $P \leq 0.05$; while, bovine liver samples showed a significant decrease of APC when compared with other samples when $P \leq 0.05$.

Regarding to the importance of fecal contamination inspection, coliform bacteria results as shown in table (2), kofta samples recorded the most contamination with incidence and mean value of 50%, and $1.9x10^2$ CFU/g, respectively, followed by chicken nuggets, fish fillet, and bovine liver samples. The statistical results revealed significant differences between all examined samples when $P \leq 0.05$.

For further identification of some entero-pathogenic bacteria, incidence of *E. coli* detection and serotyping results as illustrated in tables (3), *E. coli* could be detected in 3(10%), 1(3.33%), 1(3.33%), and 2(6.66%) of examined beef kofta, bovine liver, fish fillet, and chicken nuggets samples, respectively; serological identification of isolated *E. coli* strains revealed detection of $O_{128}:H_2$, and $O_{26}:H_{11}$ in kofta samples, O_{78} and $O_1:H_7$ in chicken nuggets samples, $O_{91}:H_{21}$ in fish fillet samples, and $O_{119}:H_6$ could be isolated from bovine liver samples.

Table 1 Statistical analytical results of Aerobic Plate Count (CFU/g) in the examined sandwiches samples (n=30).

Products	Count (CFU/g)		
	Min.	Max.	Mean± S.E.
Beef Kofta	$3.20x10^5$	$1.7x10^7$	$9.6x10^6 \pm 1.0x10^{6a}$
Bovine liver	$0.92x10^5$	$0.32x10^7$	$1.2x10^6 \pm 0.26x10^{6c}$
Fish fillet	$0.90x10^5$	$1.1x10^7$	$3.6x10^6 \pm 0.85x10^{6b}$
Chicken nuggets	$8.20x10^5$	$1.2x10^7$	$5.8x10^6 \pm 0.09x10^{6b}$

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$).

Table 2 Statistical analytical results of Coliform counts (CFU/g) in the examined sandwiches samples (n=30).

Products	+ ve samples		Count (CFU/g)		
	No.	%*	Min.	Max.	Mean± S.E.
Beef kofta	15	50	9.5x10	$3.5x10^2$	$1.9x10^2 \pm 0.19x10^{2a}$
Bovine liver	12	40	1.5x10	$2.0x10^2$	$1.1x10^2 \pm 0.15x10^{2c}$
Fish fillet	9	30	6.2x10	$2.4x10^2$	$1.3x10^2 \pm 0.14x10^{2bc}$
Chicken nuggets	9	30	9.3x10	$2.8x10^2$	$1.6x10^2 \pm 0.14x10^{2ab}$
Total	45	37.5**			

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$). * Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

Furthermore, Salmonellae, as summarized in table (4), could be detected in 2(6.66%) kofta samples, and 1(3.33%) liver sample, while failed to be detected in

nuggets and fillet samples; in addition, serological identification of isolated strains revealed detection of *S. Typhimurium, S. Infantis* in kofta samples, and *S. Enteritidis* in liver sample.

Table 3 Prevalence and serotyping of E. coli in examined sandwiches samples (n=30).

Products	+ve Samples		*E. coli* serotypes	Strain characterization
	No.	%*		
Beef kofta	3	10	O_{128}:H_2	ETEC
			O_{26}:H_{11} (2 isolates)	EHEC
Bovine liver	1	3.33	O_{119}:H_6	EPEC
Fish fillet	1	3.33	O_{91}:H_{21}	EHEC
Chicken nuggets	2	6.66	O_{78}	EPEC
			O_1:H_7	EPEC
Total	7	5.8**		

* Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

4. DISCUSSION

Nowadays, due to modern lifestyle changes, most people do not have time to cook and prepare homemade meals considered as one of the main reasons for increasing fast food demand (Dipeolu et al., 2007). Despite the economic and nutritional benefits of fast foods, the consumption of fast foods have a potential risk of foodborne infections as it may be contaminated from different sources; and in the fact of that the early preparation of large quantities of meat products and hold for hours without heat control enhances the growth of microorganisms which contaminated such products during processing, handling, and serving (Dawson, 1992; Tambekar et al., 2008). So, APC is considered as the hygienic image of the meat product as reported by Jay (1997) who reported that, although the APC of any food product is not precisely indicative for its safety for consumption, but it is considered as supreme importance in judging the hygienic conditions under which it has been produced, handled and stored.

Aerobic plate count results in table (1) were somewhat agreed with those reported by Ibrahim et al. (2014) ($4.78x10^5$ CFU/g-chicken nuggets); Magawata and Ahmed (2014) ($3.5x10^6$ CFU/g-RTE fish fillet), and Ateya (2018) (8.48×10^6 and 2.8×10^6 CFU/g for kofta and liver samples, respectively). However, they were higher than results recorded by El-Kewaiey (2012) ($8.2x10^4$ CFU/g for nuggets samples); Hassanien et al. (2014) ($5.02x10^3$ CFU/g-fried RTE fish), and Hassan (2016) (1.3×10^5 and 7.8×10^5 CFU/g for kofta and liver, respectively).

The presence of coliform bacteria in meat products have an epidemiological interest as some of them are pathogenic, and may result in serious infections and foodborne diseases; so, the total coliforms count may be used as a fecal contamination indicator in foods due to inadequate heat treatment and/or post-processing cross-contamination of meat products (Mousa et al., 2001). Results of coliform bacteria in table (2) were in agreement with El-Kewaiey (2012) ($2.4x10^2$ CFU/g for nuggets samples); Zaki (2012) ($7.8x10^2$ for kofta samples); Hassanien et al. (2014) ($3.2x10^2$ CFU/g for fried RTE fish samples); El-Ziqaty (2016) (6.8×10^2 CFU/g for liver samples); however it was higher than those recorded by Abdu-Elaziz (2018) (2.3x10 CFU/g for liver sandwiches); Hussein et al. (2018) (3.9x10 CFU/g for kofta samples); while disagreed with Tavakoli and Riazipour (2008) who failed to detect coliform bacteria in RTE fish samples; on the other hand, recorded results were lower than that recorded by Eid et al. (2014) ($2.1x10^3$ CFU/g for examined nuggets samples); Mashak et al. (2014) ($1.5x10^4$ CFU/g for RTE chicken meat product sandwiches); Shaltout et al. (2017a) (2.5×10^3 (60%) and 9.0×10^4 (80%) for kofta and liver sandwiches, respectively), and Oje et al. (2018) ($1.2x10^4$ CFU/g for fried fish samples collected from Ado-Lagos).

Variations may be attributed to the processing defect and/or post-processing contamination from workers, utensils and contact surfaces which indicate inadequate hygiene. The presence of high APC and coliform counts in RTE food indicates deplorable poor hygiene and sanitary practices employed in the processing and packaging of this food product.

As a member of coliforms, *Escherichia coli* (*E.* coli) is used as an indicator for fecal contamination and poor sanitation during processing; its presence in RTE foods indicates that the food has been prepared under poor hygienic conditions (Eisel et al., 1997; Khater *et al*., 2013).

Table 4 Prevalence and serotyping of Salmonellae in examined sandwiches samples (n=30).

Products	+ve Samples		Salmonella serotyping	Group	Antigenic structure	
	No.	%*			O	H
Beef kofta	2	6.66	*S. Typhimurium*	C1	6,7,14	r : 1,5
			S. Infantis	B	1,4,5,12	i : 1,2
Bovine liver	1	3.33	*S. Enteritidis*	D1	1,9,12	g,m : -
Fish fillet	Free	---	Free	---	---	---
Chicken nuggets	Free	---	Free	---	---	---
Total	3	2.5**				

* Percentage in relation to total number of each sample (30). ** Percentage in relation to total number of samples (120).

E. coli detection results in table (3) were nearly similar to those recorded by Hazaa (2015) (15% in beef kofta samples); El-Shafeei (2017) (4% from liver samples), and Ibrahim et al. (2018) (16.6% for examined nuggets samples), while lower than that recorded by Abdel Fattah (2014) (40 and 33.3% of examined kofta and chicken nuggets samples); Hassanien et al. (2014) (5.8% of examined RTE fish samples), and Meshref (2018) (24 and 32% of examined liver and kofta samples, respectively), on the other hand, El-Kewaiey (2012) failed to detect *E. coli* in examined nuggets samples.

Serogroups of O_{26}, O_{91}, O_{128}, and O_{78} of non-O_{157} pathogenic *E. coli* were recorded to cause HUS, bloody diarrhea, and other gastrointestinal illnesses, entero-hemorrhagic *E. coli* entered the intestines attaching to the intestinal mucosa and produces toxins causing the intestinal wall porous, allowing further toxin to enter the bloodstream and induced the other systemic clinical manifestations (Ursula *et al.* (2012); Son *et al.* (2014)).

Moreover, the obtained Salmonellae results in table (4) were in agree with those recorded by Abd El-Ghany (2010) (failed to detect salmonella in fried RTE fish samples); El-Kewaiey (2012) (failed to detect salmonella in examined nuggets samples); Meshref (2018) (4 and 8% of examined liver and kofta samples, respectively); while lower than those recorded by Abdel Fattah (2014) (detected salmonella in 13.3% of examined nuggets samples); Shaltout et al. (2017b) (32, and 60% of examined kofta and liver, respectively), on the other hand, obtained results disagreed with Abdu-Elaziz (2018), and Elshazly (2018) (failed to detect salmonella in examined liver, and kofta samples, respectively).

Salmonella Typhimurium and *S. Enteritidis* were recorded to come among the top fifteen salmonella serovars isolated from foodborne outbreaks in Japan between 2010-2014; the symptoms of salmonella foodborne infection are gastroenteritis with mild fever, diarrhea, abdominal pain, nausea and vomiting. Additionally, in immune compromised adults can cause a systemic infection with additional complications (Haimovich and Venkatesan (2006); IDSC (2014)).

The variation in the results between different authors may be due to the differences in hygienic measures during manufacturing practices, handling, and the effectiveness of thermal treatment applied during preparation. The presence of such pathogenic bacteria in RTE foods is considered as an indicator of faults during preparation, handling, storage or service which may come through using of contaminated raw materials, food handlers and the surrounding environment.

The recent results proved that the examined kofta sandwich samples were the most contaminated samples, followed by chicken nuggets, fish fillet, and liver samples which may be due to the fact that longer time of heat treatment in case of frying has a more lethal effect on the bacterial contaminations.

Generally, the high incidences of bacterial detection in such RTE examined meat products may be due to contamination of raw materials used for production, in addition, spices, equipment, dressings, knives, and other additives are considered as source of contamination of RTE products during preparation (Klein and Luwers, 1994).

5. CONCULOSIONS

From the obtained results; the study concludes that RTE beef kofta sandwich samples were the most contaminated samples, followed by chicken nuggets, fish fillet, and bovine liver sandwich samples, respectively. Generally, it indicated that the examined RTE products were prepared under low hygienic measures of food preparation and handling; roadside restaurants and street vendors still considered as a potential source of a health hazard and recommended authorities for more restrict hygienic measures applications over ready to eat food outlets.

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