



Listeria spp. and Enterobacteriaceae Group in Sandwiches of Meat and Meat Products

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the Egyptian street-vended sandwiches of meat and meat products for the presence of *Listeria monocytogenes* and Enterobacteriaceae group. To evaluate the microbiological quality of street-vended sandwiches of meat and meat products sold in Cairo-Egypt.

Place and Duration of Study: Department of Food Microbiology, National Research Center, Cairo, at during the period of January 2011 to September 2012.

Methology: Seventy sandwiches of meat and meat products including ten samples each of burger, hawawshi, kofta, liver, luncheon, sausages and shawerma sandwiches were randomly collected from the street-vendors and food shops in Great-Cairo governorate. Samples were investigated for their loads of Enterobacteriaceae counts as well as the presence of *L. monocytogenes*. Enterobacteriaceae counts was done using the conventional International method (FDA), however isolation and identification of *L. monocytogenes* was carried out using three different microbiological examination methods including classic selective conventional media, chromogenic media, as well as rapid identification method "food-system kits". 168 isolates of *Listeria spp* were identified

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following the biochemical identification tests (Bergey's Manual) and confirmed using Hi *Listeria* identification kits and latex test kits.

Results: Enterobacteriaceae group were detected in 51% of the examined samples with an average counts of 37×10^2 cfu/g. *Listeria spp.* were detected in range between 24% and 36%, depended on the method used, with numbers ranged from 16×10^2 to 23×10^2 cfu/g. All samples that were contaminated with *Listeria spp.* harboured *L. monocytogenes*. *Listeria spp.* was not detected in all the examined Hawawshi sandwiches with an exception of one positive sample detected using the chromogenic media. The obtained results indicated that 37 out 68 (54%) isolates, picked up from classic selective conventional media, and 62 out of 100 (62%) isolates from chromogenic medium were confirmed as *L. monocytogenes* indicating that chromogenic medium may be the superior for isolation of the pathogen from ready-to-eat sandwiches of meat and products.

Conclusion: The obtained results indicated that these foods presented a source of infection to the consumer. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving should be taken. The chromogenic media was the most efficient for the isolation of the pathogen during this course of study.

Keywords: Meat; meat products; sandwiches; Enterobacteriaceae; *Listeria monocytogenes*.

1. INTRODUCTION

Ready-to-eat foods are commonly sold by street vendors at where street-food vending is common, there has been little information regarding the incidence of street-food related diseases. This has raised many concerns because the conditions under which street vendors operate are usually unsuitable for the preparation and selling of food [1-3]. In most cases running water is not available at vending sites and hand and dishwashing are usually done in one or more buckets or pans of water, sometimes without soap. Waste water and garbage are discarded in the streets providing food and harborage for insects and rodents. Foods are usually not effectively protected from dust and flies which may harbor food borne pathogens also safe food storage temperatures are difficult to maintain [1,2]. Thus, there are potential health risks associated with initial contamination of raw foods with pathogenic bacteria as well as subsequent contamination by vendors during preparation and through post-cooking handling and cross contamination [1]. According to Scott and Gravani [4], temporary food service, such as mobile unit may operate on a more regular basis but unlike modern food service establishments operate under less than optimum conditions. The Enterobacteriaceae are a large family of gram-negative bacilli that are normal inhabitants of the gastrointestinal tract of humans and other animals [5]. These organisms are a common cause of community-acquired and health-care-acquired infections. Although this family includes more than 70 genera, the health-care-associated Enterobacteriaceae most commonly reported to CDC's National Healthcare Safety Network (NHSN) surveillance system are *Escherichia coli*, *Klebsiella* species and *Enterobacter* species [6]. *Listeria monocytogenes* bacterium is of particular importance as it can cause a disease called listeriosis. This infection is responsible for an estimated 2500 serious illnesses and 500 deaths each year in the United States. The illness can be serious for elderly people, newborns, pregnant women and those with weakened immune systems. During the last decade *L. monocytogenes* has been recognized as an agent of food borne illness. The pathogen has been associated with foods such as raw milk, cheeses, ice cream, raw vegetables, raw and cooked poultry, raw meats and raw and smoked fish [7,8]. Meat and meat products such as raw meat, ground meat and liver as well as meat products such as Sausage, Kofta, Burger and Luncheon sandwiches have been

incriminated human listeriosis in many countries specially the bacterium can grow and multiplies readily at refrigerator temperature [9,10]. The chromogenic media have been developed to provide convenient management and identification of *Listeria* spp including *L. monocytogenes*. This has been done according to enzymes expressed by the pathogen and production of acids by the fermentation of sugars. The medium is easy to prepare and interpret, thus the presumptive identification of *L. monocytogenes* is possible after 24 h, compared with 3-4 days using Oxford and other conventional agar [11]. The aim of this work was to assess to what extent sandwiches of meat and meat product, sold in streets, are contaminated by Enterobacteriaceae group and can expose the consumer for catching listeriosis. Comparison between efficiency of three different microbiological examination methods for isolation of *Listeria* spp, including classic conventional selective media, chromogenic media, as well as rapid food-system kits was another goal.

2. MATERIALS AND METHODS

2.1 Samples

Seventy sandwiches of meat and meat products were randomly collected from the street-vendors and food shops in Great-Cairo governorate, during the period of January 2011 to September 2012, including ten samples each of burger, hawawshi, kofta, liver, luncheon, sausages and shawerma sandwiches. All samples were collected aseptically, placed in sterile containers, stored at 4°C, transferred to the laboratory and examined the same day of collection.

2.2 The Microbiological Analysis

Samples were investigated for their loads of Enterobacteriaceae group as well as the presence of *L. monocytogenes* using three different microbiological examination methods including classic selective media, chromogenic media, as well as rapid identification method "food-system kits". Samples were well homogenized, twenty-five gram of homogenate was mixed and diluted with buffered peptone water (Himedia, Mumbai), to make the sufficient ten-fold dilutions. Enumeration of Enterobacteriaceae family was carried out by spreading 0.1 ml of dilution onto the surface of violet red bile glucose agar medium as recommended by [12,13]. Plated were incubated at 37°C for 24 h. *Listeria monocytogenes* was detected by mixing 25 g of the sample with 225 ml *Listeria* selective enrichment supplement (M890A, Himedia, Mumbai) as recommended by Louvett et al. [14]. Samples were incubated at 30°C for 7 day. A plate of Oxford agar base, represented as conventional method, (M1145, Himedia, Mumbai) supplemented with *Listeria* supplement was daily streaked from each sample and incubated at 35°C for 48h as recommended by Curtis et al. [15]. Suspected colonies were picked up and propagated for further specific morphological, biochemical tests as recommended by FDA. [13]. In the same time, using the same dilutions, Hi-chromogenic media (HiChrome *Listeria* Agar Base (M1417, Himedia, Mumbai) was inoculated in parallel for isolation of the pathogen, to compare its efficiency for isolation with the conventional selective medium. Two colonies from each duplicate plates of the highest dilution showing growth for each sample were randomly picked and purified on Nutrient Agar plates. For more accuracy and comparison, miniaturized biochemical food-system, kits (micro titer plates) for detection of pathogenic germs, was delivered from Liofilchem. Via Scozia-Zona Ind. Le-64026 Roseto D.A. (TE) Italy were also used to compare their results with those obtained by the Conventional selective and the chromogenic media. According to instruction supplemented by the company, 25 g of sample was homogenized in buffered peptone water

(225 ml) and incubated at 36°C for 24 hrs. Aliquot of 0.2 ml of the sample was dispensed into the vial of the physiological solution supplied in the kit and 0.2 ml (4 drops) of the sample suspension was transferred into each well of the system, and incubated at 36°C for 18 – 24 hrs. *Listeria spp.* was detected in their specified wells according to the instructions provided by the company and attached with the kits.

2.3 Identification of the Isolates

Suspected isolates were subjected to microscopic examination as well as their chemical and biochemical confirmation tests [13,16]. Nevertheless, the rapid detection methods (Hi kits and Latex test kits) were used for confirming the identification of these isolated.

3. RESULTS AND DISCUSSION

Seventy sandwiches of street-vended meat and meat products including ten samples each of burger, hawawshi, kofta, liver, luncheon, sausages and shawerma were investigated for their loads of Enterobacteriaceae counts as well as the presence of *L. monocytogenes* using three different microbiological examination methods including classic selective media, chromogenic media. The obtained results are shown in Table 1.

As seen from Table 1, Enterobacteriaceae group bacteria were detected in 50% of susage, 60% of liver, kofta and burger, 20% hawawshi, 70% luncheon and 40% of shawerma sandwiches with counts ranged from 25×10^2 to 50×10^2 cfu/g, with an average of 37×10^2 cfu/g. Our obtained results are in harmony with those obtained in Latin America [17]. Also, in Brazil, Bezerra et al. [18] reported that the Brazilian hamburgers were categorized as unsuitable for human consumption in 31.4% of samples which were positive for coliform. Our obtained results as well as those obtained in Brazil and Latin America confirm that coliform bacterial group is still considered good indicators for assessing general hygienic status of food contact surfaces [19].

The incidence of *Listeria spp.*, using both the conventional classic selective as well as chromogenic media, emphasize that the contamination percentage ranged from 10% to 40% according to the type of used medium and the type of the examined sandwiches (Table 1). Using the classic selective medium *Listeria spp.* was detected in counts ranged from 10×10^2 to 30×10^2 cfu/g with an average of 16×10^2 cfu/g in 10% of sausages, 30% of liver, kofta and burger, 40% of luncheon, and 30% of shawerma respectively, however, the bacterium was not detected in any sandwiches of hawawshi. When chromogenic medium was used for detection of the bacterium, at least one more positive sample was detected in all the examined food types indicating that chromogenic medium was more efficient rather than the conventional selective medium. More positive samples were detected in range from 10% to 50% of the examined samples when the food system kits were used to detected *Listeria spp.* In this regard, similar results were recorded by Greenwood et al. [11]; Reissbrodt [20] and Odumeru et al. [21].

Table 1. Incidence of Enterobacteriaceae and *Listeria spp.* in the examined foods using classic and chromogenic method

Kind of meat samples	No of samples	<i>Listeria spp.</i>				Enterobacteriaceae	
		classic and chromogenic methods		Food System		Positive samples	Average count cfu/ml (mean)
		Positive samples	Average count cfu/ml (mean)	Positive samples	%		
Sausage sandwiches	10	1(10%)* 2 (20%)**	10x10 ^{2*} 17x10 ^{2**}	3	30%	5 (50%)	35x10 ²
Liver sandwiches	10	3(30%)* 4 (40%)**	19x10 ^{2*} 24x10 ^{2**}	5	50%	6 (60%)	50x10 ²
Kofta sandwiches	10	3(30%)* 4 (40%)**	30x10 ^{2*} 37x10 ^{2**}	5	50%	6 (60%)	46x10 ²
Burger sandwiches	10	3(30%)* 5 (50%)**	14x10 ^{2*} 20x10 ^{2**}	6	60%	6 (60%)	31x10 ²
Hawawshi sandwiches	10	ND* 1(10%)**	0* 13x10 ^{2**}	3	30%	2 (20%)	25x10 ²
Luncheon sandwiches	10	4(40%)* 5 (50%)**	20x10 ^{2*} 29x10 ^{2**}	6	60%	7 (70%)	45x10 ²
Shawerma sandwiches	10	3(30%)* 4 (40%)**	16x10 ^{2*} 21x10 ^{2**}	5	50%	4 (40%)	30x10 ²

(*): classic method, (**): chromogenic method, (Food system): food-system kits (micro titer plates) for detection of pathogenic germs, (ND): Notdetected.

These results may reveal that the food system kits were the superior followed by the chromogenic media and lasted by the traditional classic media for isolation of *Listeria spp.* in the examined food type (Table 1). It is known that the food system kits only allow the detection of the bacteria, depend on their biochemical reactions; however the chromogenic media detect the bacterial colonies allowing isolation of the detected strains in addition to their low cost and processing time. These criteria may give preference for using the chromogenic media for detection of *L. monocytogenes*. Generally, the high percentage of contamination with *L. monocytogenes* observed in this study was similar to that reported by Greenwood et al. [11].

Sixty-eight isolates were picked up from the selective agar media(*Listeria* Oxford base medium) as well as 100 isolates were picked up from the HiChrome *Listeria* agar, of the positive examined samples, for identification/confirmation as *L. monocytogenes*. Sources and numbers of these isolates are presented in Table 2.

Table 2. Identification of *Listeria spp.* isolated from meat product sandwiches using classic and chromogenic methods

Type/no. of samples	No of samples (Positive samples)	<i>Listeria spp.</i> isolates	<i>Listeria monocytogenes</i> strain
Sausage sandwiches	10(1)*	4*	2*
	10(2)**	8**	5**
Liver sandwiches	10(3)*	12*	5*
	10(4)**	16**	9**
Kofta sandwiches	10(3)*	12*	7*
	10(4)**	16**	10**
Burger sandwiches	10(3)*	12*	5*
	10(5)**	20**	11**
Hawawshi sandwiches	10(0)*	0*	0*
	10(1)**	4**	2**
Luncheon sandwiches	10(4)*	16*	11*
	10(5)**	20**	14**
Shawerma sandwiches	10(3)*	12*	7*
	10(4)**	16**	11**
Total	70	68*	37*
		100**	62**

These isolates were identified according to their morphology, physiological and biochemical characteristics [13,16]. Additional rapid identification kits (Hi *Listeria* identification kit and Hi *Listeria* latex test kit) were used for confirmation of the isolates, as *L. monocytogenes*. The obtained results indicated that 37 out 68 (54%) and 62 out of 100 (62%) isolates were confirmed as *L. monocytogenes* emphasize again, that chromogenic medium may be the superior for isolation of the pathogen from ready-to-eat sandwiches of meat and products.

According to the Egyptian guideline standards numbers 1114, 1688, 1972 and 3492 issued in 2005 as well as number 1473 issued in 2007, specified for meat products, the examined products should be free from any pathogenic organisms.

According to these microbiological specifications, most of the examined meat products (76%) were found to be acceptable, due to contamination with *L. monocytogenes*, however only 24 % of samples were found unacceptable by these standards Table 3.

Table 3. Comply of the examined sandwiches to the Egyptian Standards specified for *Listeria monocytogenes*

Meat product	Total samples	Accepted samples (%)	Rejected samples (%)
Sausage sandwiches (10)	10	9 (90%)	1 (10%)
Liver sandwiches (10)	10	7 (70%)	3 (30%)
Kofta sandwiches (10)	10	7 (70%)	3 (30%)
Burger sandwiches (10)	10	7 (70%)	3 (30%)
Hawawshi sandwiches (10)	10	10 (100%)	0 (0%)
Luncheon sandwiches (10)	10	6 (60%)	4 (40%)
Shawerma sandwiches (10)	10	7 (70%)	3 (30%)
Total	70	53(76%)	17 (24%)

Over all the obtained results of this study indicated that meat products, as ready-to-eat foods sold in different location of Great-Cairo Governorate, showed high degree of contamination and can poses a serious challenge to the public health authorities and hazardous food for the consumer.

Improper handling of food is responsible for most cases of foodborne diseases and intoxication, including improper use of preparation and storage temperatures, cross contamination and poor personal hygiene [22]. Also, when food handlers do not practice proper personal hygiene or correct food preparation, they may become vehicles for microorganisms, through their hands, mouth, and skin among others [23,24]. Moreover, foods sold near polluted environments are exposed to contamination by such pathogenic microorganisms. Some of these factors and may be another resulted in this large extent of contamination with *L. monocytogenes*.

4. CONCLUSION

In conclusion, results obtained in this study indicated that consuming of meat and meat products sandwiches, as fast meal and ready-to-eat foods, sold in many locations of Great-Cairo city, may possess a serious challenge to the public health authorities and hazardous food for the consumer. The obtained results will help to clarify the epidemiology of diseases in the country. It may also help/enforce the dissection makers to take the proper methods/actions to control such outbreaks that may arise from the consumption of these foods. It may also be useful in the development of any microbiological guidelines specified for those vendors. Meanwhile, the non-governmental organizations together with the governmental health agencies encourage taking simple measures to educate those vendors on topics of food safety and hygienic practices. On the other hand, careful handling/preparing, washing, cleaning, and above all personal hygiene awareness would help to minimize such contamination and subsequently prevent consumers from food borne illness.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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