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Incidence of some epidemiologically relevant food-borne pathogens in street-vended sandwiches

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ABSTRACT

Ready-to-eat foods are popular consumed in the world including Egypt. It could be easily contaminated with various Food-borne pathogens and thus could be a main source of food-borne illness. Meat and meat products are considered as an excellent source for supporting growth of such pathogens. The present study was intended to monitor the microbiological quality of twenty sandwiches of ready-to-eat liver (kibda) and minced meat (hawawshi) sold in Egypt. Food samples were analyzed for the presence of coliform bacteria, *Escherichia coli* O157:H7, *Salmonella* spp, *Listeria monocytogenes*, *Bacillus cereus*, as well as the presence of coagulase-positive *Staphylococcus aureus* and their ability to produce various types of enterotoxins. Coliform bacteria were detected in 40% and 20%, *E. coli* O157:H7 in 20% and 10%, *Salmonella* spp. in 30% and 20%, *L. monocytogenes* in 30% and 10%, while *B. cereus* was detected in 60% and 40% of the liver and minced meat sandwiches respectively. Although the coagulase-positive *S. aureus* was detected in 40% of liver and 20% of meat sandwiches but none of these isolates was able to produce any type of staphylococcal enterotoxins. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving of these foods should be undertaken.

Keywords: Ready-to-eat foods, *Escherichia coli* O157:H7, *Salmonella* spp, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*.

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INTRODUCTION

Ready-to-eat foods are popular consumed all over the world including Egypt. These foods are well appreciated by consumers because of their taste, low cost, nutrient value and ready availability for immediate consumption [1]. In Egypt, Ready-to-eat foods are commonly sold in restaurants however a great part of these foods are vended in streets by street vendor where foods are not effectively protected from dust and flies. Moreover, the safe food storage temperatures are difficult to maintain[2,3]. Consequently, these foods could be easily contaminated with various food-borne pathogens and thus could be a main source of food-borne illness. Meat and meat products including liver are nutritious meals for the consumer since it consider as an excellent source of high quality animal protein, vitamins, and certain minerals [4]. In the same time, they are considered an ideal culture medium for growth of many organisms, including food-borne pathogens, which can contaminate foods during the preparation process by the food handlers [5-8]

Thus, there is potential health risks associated with consumption of these foods. The most common food-borne pathogens of major concern, to public health, in ready-to-eat foods are coliform bacteria as general indicator for fecal pollution, and in particular *Escherichia coli* O157:H7, *Salmonella spp*, *Listeria monocytogenes* and *Bacillus cereus* as well as the presence of coagulase-positive *Staphylococcus aureus* and their ability to produce various types of their enterotoxins.[9-11]

Consequently, questions have been raised about the safety and microbiological quality of these sandwiches since such prepared foods are considered to be susceptible to post-preparation contamination by pathogenic bacteria.[12,13] In Egypt, there has been little information regarding the incidence of street-food related diseases.

This study was done to evaluate the microbiological quality of the ready-to-eat minced-eat (hawawshi) and liver (kibda) sandwiches sold in Qalubiya, Egypt. The presence of coliform bacteria, *Escherichia coli* O157:H7, *Salmonella spp*, *Listeria monocytogenes*, *Bacillus cereus* as well as the presence of coagulase-positive *Staphylococcus aureus* and their ability to produce various types of their enterotoxins was investigated.

MATERIALS AND METHODS

Food Samples:

A total of twenty samples of ready- to- eat liver and hawawshi sandwiches were randomly collected from street-vendors and food shops in Qalubiya, Egypt including ten samples each. The samples were collected under aseptic conditions, placed in plastic bags, stored in cool box and transferred to the laboratory. All samples were examined the same day of collection.

Sample preparation:

Twenty-five gm. of each sample was mixed, homogenized in sterile mixer and diluted with 225 ml buffered peptone water .Ten-fold dilutions of homogenates were prepared and subjected to all the microbiological analysis.[14]

Microbiological analysis:

Liver and hawawshi samples were analyzed for the presence of coliform group using violet red bile agar medium, plates were incubated for 24 hrs at 35°C, *Escherichia coli* O157:H7 by spreading 0.1 ml of the sufficient dilution onto plates of sorbitol MacConkey agar medium which were incubated at 35°C for 24 hrs.

Listeria monocytogenes was analyzed by mixing 25 g of each sample with 225ml *Listeria* selective enrichment medium, in 500 ml flasks, and incubated at 30°C for 7 days. Serial dilutions were made and plated onto Oxford agar base supplemented with *Listeria* supplement and incubated at 35°C for 48 hrs.

Salmonella spp. were detected by mixing 25 g of each sample with 225 ml of sterile buffer peptone water and incubated at 35°C for 24 hrs. One ml mixture was transferred to 10 ml selenite cystein broth and

incubated at 35° c for 72 hrs. Then, serial dilutions were done, plates onto Salmonella & Shigella agar and incubated at 35° c for 24 hrs. *Bacillus cereus* by the surface plating technique onto the *Bacillus cereus* agar medium, supplemented with polymyxin B and egg yolk.

***Staphylococcus aureus* was detected** using Baird-parker media (Oxoid) supplemented with egg yolk and potassium tellurite. Plates were incubated at 37°c for 48 hrs. Typical colonies were identified by morphology, catalase and coagulase production [14]. Randomly, two confirmed isolates, isolated from each positive food sample, were serologically tested for their ability to produce enterotoxin A, B, C and D by the Reversed Passive Latex Agglutination technique, as recommended by [15,16] using Oxoid SET-RPLA (a kit for the detection of staphylococci enterotoxins A, B, C and D manufactured by Denka Seiken Ltd, Japan for Oxoid Ltd).

Identification of isolates:

Three to five suspected isolates of each organism, isolated from each positive sample, were subjected for identification using the microscopic examination as well as their chemical and biochemical confirmation tests [14, 17].

RESULTS AND DISCUSSION

The microbiological quality of ready to-eat sandwiches of liver and minced meat depend greatly on the initial quality of the used materials and the other ingredients. Other factors may include the efficacy of cooking process and proper sanitary practices for personnel and cooking/processing utensils [18]. Even though their ingredients reach a temperature that is ideal to ensure that the food is cooked thoroughly, cross-contamination during preparation of these sandwiches could be traced back to the use of uncooked green vegetables and unhygienic handling [19]. The results of the microbiological analysis of the minced meat (hawawshi) and liver sandwiches are presented in tables (1). The presence of Coliform group in the investigated sandwiches is an indicator of the fecal pollution. As seen from table 1, four samples of liver sandwiches, representing 40% of total samples, and two samples of hawawshi sandwiches representing 20% of the total examined samples, were fecally contaminated with counts ranged from (two to four orders of magnitude) 24×10^2 to 40×10^4 cfu/g. It is worthy to note that the higher counts were detected in liver sandwiches however the lowest were detected in the minced meat. This may due to the nutritious constituents of the liver that may enhance/support the presence of the microorganism.

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis associated with consumption of hamburgers from a fast food chain restaurant [20]. In this work, this organism was detected in 20% and 10% of liver and minced meat sandwiches respectively (Table 1). A maximum of two orders of magnitude were detected in the positive examined samples. Although not all the fecal polluted food samples, only 3 of 5, were contaminated by such organism, but this may support the importance of consideration the presence of coliform group as an important alarm for the presence of such pathogen that has been associated with meat outbreaks [21] particularly with the consumption of undercooked ground beef [22]. The presence of this pathogen in liver sandwiches can be attributed to improper handling and processing, use of contaminated raw materials or the use of dirty processing utensils like knife and trays [23].

Salmonella spp. are found in the intestinal tract of wild and domesticated animals and humans. Some serotypes of *Salmonella*, such as *S. typhi* and *S. paratyphi* are only found in humans [24]. Although, the Ready-to-eat foods should be free of *Salmonella* spp., but the present study showed that this pathogen was detected in 30% and 20% of examined liver and minced-meat (hawawshi) sandwiches respectively, Kegode *et al.*, 2008 [25], studied the occurrence of *Salmonella* in 456 fresh raw meats (turkey, chicken, pork, beef) raw meats sold in retail grocery stores in North Dakota in the Midwestern United States; and found that *Salmonella* spp were found in only 13 samples representing 2.9% of the total examined samples. On the contrary, of our obtained results, *Salmonella* spp. failed to be isolated in different kind of foods by other researchers [26,27] and in meat products [28-30]. The presence of this high percentage of this pathogen in this study may indicated that the hygienic conditions of the processed sandwiches were very poor and that these sandwiches examined in this study are of great public health concern. Probably, this high incidence may indicate that the examined samples were occasionally being contaminated with *Salmonella*.

Table1. Incidence of some food-borne pathogens in the examined minced meat and liver sandwiches.

Source of isolates	Region	Coliform group	<i>Escherichia coli</i> O157:H7	<i>Salmonella</i> <i>typhimurium</i>	<i>Listeria</i> <i>monocytogenes</i>	<i>Staphylococcus</i> <i>aureus</i>	<i>Bacillus</i> <i>cereus</i>
Liver Sandwich	Toukh	Nil	Nil	Nil	Nil	45x10 ¹	8x10 ¹
Liver Sandwich	Toukh	40x10 ⁴	Nil	4x10 ²	Nil	23x10 ¹	Nil
Liver Sandwich	KafrShukr	Nil	Nil	Nil	1x10 ³	Nil	72x10 ¹
Liver Sandwich	KafrShukr	Nil	Nil	Nil	Nil	4x10 ¹	3x10 ²
Liver Sandwich	Benha	24x10 ²	4x10 ²	45x10 ¹	Nil	Nil	Nil
Liver Sandwich	Benha	Nil	Nil	Nil	37x10 ¹	Nil	Nil
Liver Sandwich	Benha	32x10 ²	9x10 ²	Nil	1x10 ³	Nil	Nil
Liver Sandwich	Shoubra	Nil	Nil	Nil	Nil	Nil	9x10 ²
Liver Sandwich	Shoubra	5x10 ³	Nil	2x10 ²	Nil	3x10 ²	45x10 ¹
Liver Sandwich	Shoubra	Nil	Nil	Nil	Nil	Nil	5x10 ¹
Hawawshi Sandwich	Toukh	Nil	Nil	Nil	Nil	2x10 ²	Nil
Hawawshi Sandwich	Toukh	Nil	Nil	Nil	Nil	37x10 ¹	Nil
Hawawshi Sandwich	KafrShukr	Nil	Nil	Nil	Nil	Nil	Nil
Hawawshi Sandwich	KafrShukr	Nil	Nil	Nil	Nil	Nil	5x10 ¹
Hawawshi Sandwich	Benha	Nil	Nil	Nil	Nil	Nil	Nil
Hawawshi Sandwich	Benha	35x10 ²	7x10 ²	27x10 ¹	Nil	Nil	Nil
Hawawshi Sandwich	Benha	24x10 ²	Nil	12x10 ¹	44x10 ¹	Nil	Nil
Hawawshi Sandwich	Shoubra	Nil	Nil	Nil	Nil	Nil	4x10 ¹
Hawawshi Sandwich	Shoubra	Nil	Nil	Nil	Nil	Nil	12x10 ¹
Hawawshi Sandwich	Shoubra	Nil	Nil	Nil	Nil	Nil	3x10 ²

Listeria monocytogenes is commonly found in soil and water. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin, such as meats, meat and dairy products [31,32]. Thus, *L. monocytogenes* may be present in the environment of many food-processing and retail food facilities, and its complete elimination is extremely difficult. In this work, *Listeria monocytogenes* was isolated from 30 and 10% of the liver and meat samples respectively. The current percentage of contamination with *L. monocytogenes* in ready-to-eat foods, observed in this study, overlap the percentages obtained by other researchers[33,34]. Generally, Ready-to-eat foods are vulnerable to recontamination with *L. monocytogenes* during handling, processing or packaging at the retail level, or in the domestic streets environment. Other factor support the presence/persistence of this organism could be its ability to grow at low temperatures during any subsequent period of storage during preparation of the final food product.

Staphylococcus aureus plays a great role in bacterial contamination of fast foods/ready-to-eat-foods, since food handlers/workers may contaminate this type of foods during preparation and /or processing. In this work, this pathogen was isolated from 40% and 20% of liver and minced meat sandwiches respectively (Table 1). El-Sherbeeney *et al.*, [36] analyzed 114 street-vended ready-to-eat Egyptian food samples, their study revealed that that this pathogen was present in 80% of these samples. Our obtained results are less than those obtained by these investigators. In the same time, our results agreed with those obtained by Tambekar *et al.* [37] who found that 41% of 114 street-vended ready-to-eat Egyptian food samples, were contaminated with *S. aureus*. The presence of this pathogen in our examined samples could be consider as another indicator of less personal hygiene from food processors/vendors since such organism is widely distributed in nature and on the surface of human skin, eyes and nasal secretions as well as in respiratory tracts[37-39]. Also, it can arise from unclean clothes and surrounding poor unhygienic environment which my assist for cross-contamination by such pathogen. Fortunately, none of the tested twelve coagulase positive *Staphylococcus aureus* strains was able to produce any types of enterotoxin A, B, C or D.

Bacillus cereus was detected in 60% of examined RTE liver sandwiches and 40% of examined hawawshi sandwiches. The presence of this bacterium in meat has been widely reported from different parts of the world[40, 41]. For example Mosupye and Von Holy [42], reported (22%) incidence of *B. cereus* in a related study in South Africa. Ismail [19] reported higher incidences (48%) in beef and Lamb ready to eat foods. More, higher incidence (72%) was recorded by El-Sherbeeney *et al.*, [36] in sandwiches of liver. This high percentage, obtained in this study, may support its ubiquitous distribution in the environment especially in streets environment.

In conclusion, the results of this study indicate that hygienic conditions of some processed RTE meat sandwiches were very poor and may constitute a considerable hazard to human health. Using of high quality raw materials, efficient heat treatment, adequate cleaning and sanitization of utensils may assist reducing this cross contamination. The policy makers should address legislations for street-vendors to assure their personal hygiene and sanitation.

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