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Microbial Status of Shawerma Sandwiches in Kafr El-Sheikh Governorate

Nehad I.E. Salem[1], Adel M. El Gamel[2], Amal M.A. Khalifa[2], Hala A.M. Abd-El-Hady[2], Mayada A.M. Abou Zeid[2]

[1]Department of food hygiene, [2]Department of bacteriology, Regional Kafr Sheikh Animal Health Research Institute

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Correspondence to:
Nehad Ibrahem Salem, nournabil18@yahoo.com

Abstract

This study was carried out to analyze the microbial status of shawerma sandwiches in Kafr-Elsheikh Governorate. Fifty random samples were collected from different localities (25 chicken shawerma and 25 meat shawerma sandwiches) and analyzed for the presence of microorganisms using appropriate selective media. Inoculations were done by using the spread plate technique. The mean values for aerobic plate (APC), coliforms, yeast and mould in examined chicken samples were 2.8x106 ±1.0x106, 9.6 x102 ±3.0 x102 and 1.9 x102 ±3.9 x101 cfu/g, respectively. While in examined meat samples the mean values were 2.7x107 ±1.5x107, 5.0 x102 ±2.1 x102 and 2.2 x102 ±6.7 x101 cfu/g, respectively. While mould count was negligible. Staphylococci, coliforms, clostridia spp. and Bacillus cereus organisms were isolated from 16 (64%), 19 (76%), 24 (96%) and 10 (40%) in examined chicken samples respectively, while these organisms were isolated from 13 (52%), 13 (52%), 22 (88%) and 3(12%) in examined meat samples respectively. Moreover staphylococci spp. was identified in examined chicken samples into Staph. aureus (26%), S. epidermidis (6%), S. saprophyticus (4%), S. intermidis (2%), S. capitus (0%) and micrococcus spp. (6%) respectively, while these results in examined meat samples were (14%), (4%), (0%), (0%), (2%) and (2%), respectively. Coliforms organisms in examined chicken samples were identified into Klebsiella. ozaenae ,K. pneumoniae ,Citrobacter. freundii ,C. diversus ,Enterobacter. coloacae ,E. erogenens and E. aglomerance as 5%, 10%, 10%, 5%, 10%, 15% and 5%.While these results in meat samples were 0%, 10%, 4%, 0%, 7%, 11% and 0%. Also clostridia spp. in chicken samples were identified into C. sporogenes, C. tertium, C. perfringenes, C. butyricum, C. putrefaciens, C. sordelli and C. bifermentans by 5%, 2%, 14%, 4%, 4%, 2% and 1%. While these results in meat samples were 7%, 3%, 9%, 3%, 0%, 0% and 2%. The obtained results cleared that the chicken samples harbourd 9 (28%) strains of C. perfringens with Lecithinase activity, while meat samples have 7 (15%) strains, Toxigenic strains from these pervious strains in chicken samples were identified into Type (A) , Type (B) , Type (C) and Type (D) by 3(33%) , 1(11%) ,0(0%) and 1(11%), such results in meat samples were 1(14%),0 (0%),1(14%) and 0 (0%). Moreover, heat resistance strains of clostridia were identified. The results were statistically evaluated and the possible sources of contamination as well as the public health importance of isolated bacteria and the hygienic measures which should be followed were discussed.

1. INTRODUCTION

Different terms can be used to describe Ready-to-Eat foods these include convenient, ready, and instant and fast foods. such RTE food includes; pastries, meat pie, sausage rolls, burger, doughnut, shawerma, salads or coleslaw, milk and milk products RTE food can be described as the status of food being ready for immediate consumption at the point of scale, it could be raw or cooked, and can be consumed without further treatment **(**Tsang, 2002).There is an increase in the consumption of RTE fast food because of a change in social patterns characterized by increased mobility, large numbers of itinerary workers and less family centered activities. Thus, good manufacturing practices, good sanitation or sanitary measure and proper food handling have been transferred from individuals/families to the food vendor who rarely enforces such practice (Musa and Okande, 2002).

Street vended food can contribute to food security of those involved in its production, particularly, suppliers of raw produce, food processors, vendors and consumers (Opeolu et al., 2010). Microbiological quality problems of RTE food (Shawerma) depends greatly on the following factors: low initial quality of raw meat and other ingredients, inefficient cooking process, improper sanitary practices for personnel, and for cooking/processing utensils (Kayaardi et al., 2006). One or several of these factors may lead to potential health hazard for humans (Evans et al., 1999) and (Harakeh et al., 2005). The main sources of pathogenic bacteria in food are contaminated raw food, food handlers, dust, water, utensils & insects (Ray, 1996). (RTE) food has been implicated in cases of food poisoning or gastroenteritis in human beings (Eley, 1996). Hot foods have been the source of outbreaks of Staphylococcus aureus, Clostridium perfringens & Salmonella enteritidis food poisoning (Hatakka, 1998).

RTE foods must be examined at regular intervals in order to assess their microbiological quality as it reflects its sanitary condition during its production and distribution (Hubbert et al., 1996). Poultry are known to be a reservoir of large number of bacteria which are may be pathogenic to human being. Typically, these occur in low sanitation levels and may pose a threat to the consumer if the product is not handled in a safe manner. Therefore, the production, transportation and sale of meat products must be performed with the almost care and preferably be subjected to hazard analysis critical control point (HACCP) evaluation, to prevent the presentation of any undue hazard (Madden, 1994).

Therefore, this work was planned to evaluate the bacteriological status of RTE food (Shawerma Sandwiches) sold in Kafr Elsheikh Governorate.

2. MATERIAL AND METHODS

1-**Collection of Samples**: A total of 50 random RTE samples of chicken and meat Shawerma (25 of each) were collected from different localities in Kafr El-Sheikh governorate, weight of each sample was 100g and aseptically transferred, without delay, in an insulated ice box to the laboratory and then subjected to the following examinations:

2-Bacteriological Examination

2-1-preparetion of samples: were done as described in (APHA, 2001): Twenty-five grams of the examined samples were homogenized with 225 ml of sterile buffered peptone water (0.1%) to give a dilution of (10^{-1}). One ml of the clear homogenate was mixed with 9ml of buffered peptone water (0.1%) and then decimal serial dilutions were prepared.

2-2- Aerobic Plate Count (APC) (cfu/gm): It was carried out according to (FAO, 1992).

2-3- Count of *coliforms* (MPN/gm): was carried out according to (FDA, 2002).

2-4- Isolation of *Staph. aureus*: according to (FDA, 2002).

2-5- Isolation and identification of *coliforms* according to (ISO, 2005)

2-6- Isolation of *bacillus Cereus*: The technique used was recommended by (ISO, 2004a).

2-7- Isolation and identification of *clostridia spp.* according to (ISO, 2004b).

2-8- Lecithinase activity of *C. perfringens* was recovered on Egg yolk agar media (Nagler's reaction) according to (Murray et al., 2003).

2-9- Demonstration of *C. perfringens* toxins was adopted by application of dermonecrotic reaction in Guinea pig (Sterne and Batty, 1975).

3-Mycological examination:

Total mold and yeast count: It was carried out according to (Bailary and Scott, 1998):

3. RESULTS AND DISCUSSION

Table (1): Statistical analytical results of bacteriological evaluation of examined RTE chicken and meat shawerma samples (25 of each).

Samples type	APC meant ±SE	Coliform counts meant ±SE	Yeast counts Mean ±SE	Mold counts mean ±SE
Chicken	$2.8x10^6 \pm 1.0x10^6$	$9.6\ x10^2 \pm 3.0\ x10^2$	$1.9\ x10^2 \pm 3.9\ x10^1$	<10
Meat	$2.7\ x10^7 \pm 1.5\ x10^7$	$5.0\ x10^2 \pm 2.1x10^2$	$2.2x10^2 \pm 6.7x10^1$	<10

Table (2): Percentage of isolated organisms of *Staph. aureus*, coliforms, clostridia and bacillus cereus in examined RTE chicken and meat shawerma samples (25 of each).

Isolated. Organism	Chicken No. of +VE isolates	%	Meat No. of +VE isolates	%
Staph.aureus	16	64	13	52
Coliforms	19	76	13	52
Clostridia	24	96	22	88
Bacillius cereus	10	40	3	12

Table: (3) Percentage of *Staphylococcus* spp. isolated from RTE Samples (n=25).

Identified organism	% of meat isolates	% of chicken isolates
Staph. aureus	**14**	**26**
Staph. epidermidi	**4**	**6**
Staph. saprophytics	**0**	**4**
Staph. intermedius	**0**	**2**
Staph. capitis	**2**	**0**
Micrococcus Spp	**2**	**6**

Table (4): Identification of coliforms bacteria isolated from RTE samples.

Identified bacteria	% of meat isolates	% of chicken isolates
Klebsiella ozaenae	0	5
Klebsiella pneumoniae	10	10
Citrobacter freundii	4	10
Citrobacter diversus	0	5
Enterobacter cloacae	7	10
Enterobacter aerogenes	11	15
Enterobacter agglomerans	0	5

Table (5): Identification of clostiridia *Spp* isolated from RTE samples.

Identified bact.	% of meat isolates	% of chicken isolates
C. sporogenes	7	5
C. tertium	3	2
C. perfringes	9	14
C. butyricum	3	4
C. putrefaciens	0	4
C. sordelli	0	2
C. bifermentans	2	1

Table (6) Lecithinase activity of *C. perfringenes* recovered from the examined samples of chicken and meat shawerma.

-ve isolates of lecithinase		+ve isolates of lecithinase		Products
%	No.	%	No.	
15	5	28	9	Chicken
8	2	15	7	Meat

Table (7) Typing of lecithinase +ve strains of C.perfringenes by dermonecrotic reaction in Guniea pig.

Type of toxin produced	A		B		C		D	
	No.	%	No.	%	No.	%	No.	%
Chicken	3	33	1	11	0	0	1	11
Meat	1	14	0	0	1	14	0	0

Table (8): Heat resistance of *C. Perfringens* type A isolated from the examined samples of chicken and meat shawerma.

Toxigenic strains / Products	Resistance time				
	60 min	90 min	120 min	150 min	180 min
	No.	No.	No.	No.	No.
Chicken shawerma	3	2	2	2	1
Meat shawerma	1	1	1	0	0

With the increasing pace of globalization and tourism, the safety of street food has become one of the major concerns of public health, and a focus for governments and scientists to raise public awareness. The primary goal of food service programs is to protect the consumers from any food contamination or at least to reduce the effect of any health hazard (Montney and Gould, 1988)

Generally, microbiological examination is a sensitive measure collectively verifying the quality of raw material the perfection of processing, as well as the proper storage.

The results illustrated in Table (1) showed that the mean values of aerobic plate count in examined samples of chicken and meat shawarma were $2.8x10^6$ $\pm1.0x10^6$ and $2.7\ x10^7$ $\pm1.5\ x10^7$ cfu/mg, respectively. These results were higher than that recorded by Elfaki and Elhakim (2011); Eman and Sherifa (2012); Odu and Akan (2012); Abdalhamid et al., (2013) with mean values of $5.1x10^4$, $1.2x10^5$, $1.0x10^6$ and $8.4\ x10^5$ cfu/g. respectively.

The total bacterial count is considered as an index of sanitary and quality of food (Forsythe and Hayes, 1998). These higher results may by attribute to the Processing plants, contamination of Products. Heavy bacterial loads enter the processing operations with the product and these bacteria can be disseminated throughout the plant during processing, so diseases can also resulted when these products are not properly cooked and post-processing contaminated.

Also results in table (1) revealed that the mean values of total coliform counts in examined chicken and meat shawarma samples were $9.6\ x10^2$ $\pm3.0\ x10^2$ and $5\ x10^2$ $\pm2.1\ x10^2$ cfu/g. respectively, these results were nearly similar to Farooq et al., (2013) with mean of $8.1x10^2$.cfu/g, and higher than that recorded by Elfaki and Elhakim, (2011) with mean value of 1.7 x10 cfu/g and lower than that recorded by Odu and Akan, (2012); Abdalhamid et al., (2013) with mean values of $9.4\ x10^4$. and $4.3x10^4$cfu/g. respectively. The presence of coliforms in examined samples may constitute a public health hazard (Robinson, 1990).

Coliforms are mainly found in water, soil and feacal matter as they are widely distributed in water, soil and vegetation (Rompre et al., 2002) They are among the most common bacteria that cause disease. The presence of these organisms in ready-to-eat food (shawerma) depicts a deplorable state of poor hygiene and sanitary practices employed in the processing and packaging of this food product (Jay, 2005).

Results in table (1) revealed that the mean values of yeast counts in examined chicken and meat samples were $1.9\ x10^2$ $\pm3.9\ x10^1$ and $2.2x10^2$ $\pm6.7x10^1$ cfu/g, while the molds counts in both examined samples were negligible. These results of yeast count were lower that recorded by Eman and Sherifa, (2012) With mean values of 5.2x105 cfu/g . and nearly similar to that recorded by Elfaki and Elhakim, (2011) Mould and yeast contamination usually occurred due to handling, deboning, processing, packing and washing with polluted water and may due to dust, flies, air, workers, equipments and fluctuation of temperature during transportation and storage (Refaie, 1991; Farghaly, 1998).

In table (2) the results showed that the number of isolates of *Staph.aureus*, *Coliforms*, *Clostridia.spp* and *Bacillius.cereus* in examined chicken samples were 16 (64%), 19 (76%), 24 (96%) and 10 (40%), while these numbers of isolates in examined meat samples were 13 (52%), 13 (52%), 22 (88%) and 3 (12%), respectively and in table (3) by identification of Staphylococci we found that in chicken samples the percentages of *S. aureus*, *S. epidermdi*, *S. saprophyticus*, *S. intermedius*, *S.capitus* and micrococcus were 26, 6, 4, 2, 0 and 6 while these percentages in shawerma meat samples were 14, 4, 0, 0, 2 and 2, respectively .In most countries, the most common food borne illness is Staphylococcus *spp.* food intoxication and Enterotoxigenic spp have been isolated from foods implicated in illnesses (Adesiyun, 1995), *S.* aureus strains can be pathogenic and relatively non-pathogenic. They produce disease when the bacteria contaminate food they produce some enzymes which are implicated with Staphylococcus invasiveness and many extracellular

substances, some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal (Prescott et al., 2005). Though the bacteria can be killed during extensive cooking but the toxin may not be destroyed. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individual to the toxins. (Talaro and Talaro, 1996).Their presence in food indicates poor personal hygiene and poor manufacturing practices of the food vendor (Musa and Okande, 2002). They also can withstand high sodium chloride concentration (Jawetz et al., 2008).

Results in table (4) showed the identification of coliforms bacteria where the percentages of *Klebsiella.ozaenae, Klebsiella. pneumoniae, Citrobacter. freundii, Citrobacer .diversus, Enterobacter.cloacae,* and *Enterobacter. agglomerans* in examined shawerma chicken samples were 5, 10, 10, 5, 10, 15 and 5 while these results in examined meat samples were 0, 10, 4, 0, 7, 11 and 0.respectively Similar organisms were isolated by Abdalla et al., (2009) and Okonko et al., (2009).

Coliforms are mainly found in water, soil and feacal matter (Rompre et al., 2002). They belong to the family Enterobacteriaceae; they are short gram-negative rods. Those do not form spores, (Jawetz et al., 2008). They are among the most common bacteria that cause disease, the presence of these organisms in ready-to- eat food (shawerma) depicts a deplorable state of poor hygiene and sanitary practices employed in the processing and packaging of this food product (Jay, 2005),

Results in table (5) showed the identification of clostridsia organisms where the percentages of *C. sporogenes, C. tertium, C. perfringes, C. butyricum ,C. putrefaciens C. bifermentans* and *C. sordelli* in chicken shawerma samples were 5, 2, 4, 14, 4, 2 and 1 while in shawerma meat samples were 7, 3, 3, 9, 0, 0 and 2 respectively.

Table (6) showed that the positive strains of *C. perfringes* for lecithinase activity recovered from chicken shawerma samples were 9 (28%) while that recovered from meat shawarma samples were 7(15%), While results in Table (7) showed the typing of Lecithinase positive strains of *C. perfringes* by dermonecrotic reaction in Guinea pig in examined chicken samples were the *C.perfringes* type "A″ represent 3 strains (33%) , each of type "B" and "D" represent only 1 strain (11%) and type "C" not recognized. While in examined meat samples *C.perfringes* type "A″ and "C" represent only 1 strain (14) and type "B" and "D" were not recognized.

Table (8) revealed the heat resistant Toxigenic strains of *C. perfringes* type (A) isolates from chicken shawerma samples after 60 min, 90 min, 120 min, 150 min and 180 min were 3, 2, 2, 2 and 1 strain respectively, while that isolated from meat shawerma samples after 60 min, 90 min, 120 min, 150 min and 180 min were 1, 1, 1, 0 and 0 strain, respectively.

C. perfringens is one of the most common causes of food borne illness in the United States. CDC estimated that it causes nearly 1 million cases of food borne illness each year. People infected with *C. perfringens* develop diarrhea and abdominal cramps within 6 to 24 hours without vomition (typically 8 to 12 hours). The illness usually begins suddenly and lasts for less than 24 hours. People infected with *C. perfringens* usually do not have fever or vomiting. The illness is not passed from one person to another. Although *C. perfringens* may live normally in the human intestine, illness is caused by eating food contaminated with large numbers of *C. perfringens* bacteria that produce enough toxins in the intestines to cause illness.

C. perfringens spores can survive high temperatures. During slowly cooling and holding of food at temperatures from 54°F–140°F (12°C–60°C), the spores germinate and then the bacteria grow. The bacteria grow very rapidly between 109°F – 117°F (43°C – 47°C). If the food is served without enough reheating to kill the bacteria, live bacteria may be eaten. The bacteria produce a toxin inside the intestine that causes illness. (Grass et al., 2010)

From 1998 to 2008, 1229 food borne outbreaks caused by *Bacillus cereus, Clostridium perfringens, and Staphylococcus aureus* were reported in the United States; 39% were reported with a confirmed etiology. Vomiting was commonly reported in *B. cereus* (median, 75% of cases) and *Staph. aureus* outbreaks (median, 87%), but rarely in *C. perfringens* outbreaks (median, 9%) (Bennett et al., 2008). *Bacillus cereus* can give rise to two distinct forms of food borne disease, the emetic and the diarrhoeal syndromes. The emetic syndrome is believed to be associated with an emetic toxin pre-formed in food. Cooked rice is the most common vehicle, and the symptoms are similar to those of *Staphylococcus aureus* intoxication. The diarrhoeal type is caused by an enterotoxin and the symptoms generally parallel those of the *Clostridium perfringens* food poisoning. The heat resistance of *B. cereus* spores and the non-fastidious nature of the organism facilitate its survival and/or growth in wide variety of foods. (Shinaqawa, 1990).

4. Conclusion and Recommendation

This study showed that despite of the preparation of shawerma by heating, there were still some pathogenic microorganisms observed on the samples

examined. This is as a result of the fact that some of the observed microorganisms can survive at high temperatures. Also, handling, storage and processing steps are major factors for the cross contamination of the major materials used for the preparation of Shawerma. In addition to personal hygiene and processing practice of the food vendors which determine the safety in the consumption of ready-to-eat-food (Shawerma) so we recommended the following items:

-Using of high quality raw material and efficient heat treatment.

-Adequate cleaning & sanitization of utensils day-by-day observance of proper personal and food handling of cooked food

-Consumption the contamination must be reduced by implementing satisfactory manufacturing practices.

-Effectively training for plant workers in hygiene, safety and quality assurance.

-Application of strict hygienic measures during handling preparation and serving the products.

-There should be an establishment of sub-units of committees like National Association of Food and Drugs Administration that would be involved in the regular check up of sanitary conditions of fast food centers.

-Lastly, there should be awareness on the health implication of pathogens introduced during cross contamination.

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