



Incidence of some pathogens in beef burger sold in Cairo

Moustafa A.El-Shenawy*, Zeinab I. Sadek, Samy Mohamed Abdelhamid, Mohamed T. Fouad
 Department of Dairy Science, Division of Food Industries and Nutrition, National Research Centre,
 12311 Dokki, Giza, Egypt



Abstract

Thirty burger samples were randomly collected from food shops super marks in Great-Cairo governorate. Samples were microbiologically investigated for the presence of some pathogenic microorganisms including *Staphylococcus aureus*, *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica* and *Bacillus cereus*. *Staphylococcus aureus* and *Bacillus cereus* were detected in 60% of the examined samples with an average counts of 14×10^2 and 5.1×10^2 cfu/g. however, *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* were detected in 50, 50 and 40% of the examined samples, with an average counts of 12×10^2 , 4.8×10^2 and 5.1×10^2 cfu/g respectively. Eighty-five isolates of pathogenic bacteria including 20 isolates each of *Bacillus cereus* and *Staphylococcus aureus* as well as 15 isolates each of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* were purified and identified following the biochemical identification tests (Bergey's Manual). Only 56 out of the testes 85 isolates were confirmed as pathogenic species using Hi identification kits and latex test kits. The obtained results indicated that these foods may pose 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.

Keywords; Super-markets; Egyptian sausage; microbiological analysis; pathogens.

Introduction

Meat and meat products are among the most important protein sources in the daily diet of people living in developed countries. Beef burger is almost the most popular meat product consumed by millions of people from all over the world. The common processes (such as mincing, cooking, and salt addition) applied in the production of burgers enhance the formation of reactive oxygen species; therefore, the resultant product is highly vulnerable to oxidation [1, 2].

The increasing number and severity of food poisoning outbreaks worldwide has considerably increased public awareness about food [3], especially meat and meat products which are one of the most important sources of human infections with a variety

of foodborne pathogens [4,5]. However, meat and meat products continues to be an important food group in the diet for many consumers [6-8], *Staphylococcus aureus* is considered to be one of the most important foodborne diseases worldwide due to its ability to produce wide arrays of toxins [9,10]. Meat products like luncheon, burger and minced meat are considered important sources of pathogenic *Salmonella* spp. which caused sever gastroenteritis in human, especially products manufactured from raw and minced meat and not subjected for heat treatment [11]. *Yersinia enterocolitica* in meat and meat products is a special concern since those organisms are capable of growth at refrigerator temperatures [12]. *Yersinia enterocolitica* is by far the most frequent cause of yersiniosis worldwide. *Yersinia*

*Corresponding author e-mail: elshenawy_moustafa51@yahoo.com (Moustafa A.El-Shenawy).

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enterocolitica occurs in several biotypes and serotypes, which differs in pathogenicity to humans, geographical distribution and animal reservoirs [13,14].

B. cereus occurred in 98% of test minced meat, 60% of sausage, 48% of rice grains, 44% of Koshari or ice-cream and 36% of pasteurized milk samples. [15]. Shehu and Adesiyun [16] reported 39.5% of milk to be positive for *E. coli*. Enterotoxigenic *Escherichiacoli* has been involved in food-borne illness and recovered from various food types, processed or raw [17]. Microbial food safety is an increase in public health concern worldwide. It is estimated that approximately 76 million foodborne illness occurring in the united states every year [18,19]. Contaminated, raw or undercooked poultry and red meats are particularly important in foodborne diseases. Microorganisms in fast and traditional fast foods are responsible for many human diseases. *Salmonella* is common cause of foodborne illness, particularly in chicken and undercooked eggs [20, 21], whereas *Listeria* spp. is common from chilled and frozen foods. Other foodborne microorganisms include *Camphylobacterspp*, *Staphylococcus* spp., *E. coli* and *Yersinia* spp., whose incidence was reported by Kaneko *et al.* [22] and Pelczaret *al.* [23, 24]. Consequently, this work has been done to evaluate the general bacteriological condition of the burger product sold in the Egyptian market. With a focus on the feasibility of the presence of some pathogenic microbes including *Staphylococcus aureus* counts, *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica* and *Bacillus cereus* in order to give a general idea of the health and safety condition of this food product.

Experimental

Three samples from 10 different food shops super markets in Great-Cairo governorate including (Bulak, Giza, Dokki, Ramsis, Bin El-Sarayat, El-Saeda, Ain-Shams, Attaba, Misr El-Gadida and Moasasa) were collected. The samples were handled in sterile plastic containers and transferred to the laboratory in ice-box within 2h of collection.

Samples 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 [25].

Microbiological Analysis:-

Ten-fold dilutions of the homogenates samples were inoculated onto plates of selective media. Enumeration of *Escherichia coli* O157: H7 was

carried out by spreading 0.1 ml of each dilution onto plates of sorbitol MacConkey agar medium and colourless colonies counts were done after 24 hrs incubation at 35° C incubation [26]. For detection of *Salmonella typhimurium*, (25g) of each sample was mixed with 225ml of sterile buffer peptone water and incubated at 35 °C for 24 hrs. One to ten ml of this mixture was transferred to selenite cystein broth and incubated at 35 °C for 72 hrs. Plates of *Salmonella & Shigella* ager were streaked from the last process and incubated at 35 °C for 24 hrs. Growth of *Salmonella typhimurium* is appears as colorless colonies with black centers [27].

For *Yersinia*, Each sample (25 g) was homogenized and mixed with 225ml *Yersinia* selective enrichment in 500ml flasks. Flasks were incubated at 30 °C for 48 hrs, and then spread onto plates of *Yersinia* selective agar medium. After 18-24 hrs incubation at 35 °C, suspected colonies of *Y. enterocolitica* which appear form dark red colonies resembling bulls' eye were picked up and further tested for specific identification. Enumeration of *S. aureus* in samples was carried out by spreading 0.1 ml of each dilution onto the surface of Baird Parker media supplemented with egg yolk and potassium tellurite solution. Plates were incubated at 37 °C for 48 hrs, typical colonies, which appear gray-black, shiny and convex with a narrow white margin surrounded by a clearing zone, were counted [28, 29]. Suspected colonies were picked up and propagated for further specific morphological and biochemical tests.

Bacillus cereus was determined by the surface plating technique using the *Bacillus cereus* agar medium, supplemented with polymyxin B and egg yolk. Plates were incubated at 37 °C for 48 hrs, a typical colony, which appears peacock blue-coloured and surrounded by precipitation zone were counted and tested for further specific identification [26].

Purification and Identification of the isolated strains:-

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 according to Bergy's Manual [30]. Additional kits used to help for accurate identification including Hi Staphylococcus identification kit, Hi *Staphylococcus* Latex test kit, Hi *E. coli* identification kit, Hi *E. coli* O157 Latex test kit, Hi *Salmonella* identification kit and Hi *Salmonella* Latex test kit. All results of identification tests done for these isolates were compared with a specific reference strains obtained from ATCC.

References strains

Strains of *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Yersinia enterocolitica* ATCC 27729 and *Salmonella typhimurium* ATCC 14028 were obtained from the Department of Dairy Science, National Research Centre, Cairo, Egypt. The abovementioned strains were served as reference indicators for comparison among the tested strains.

Results and discussion

Meat and meat products are considered as a major vehicle of most reported food poisoning outbreaks. Therefore, it is important to use the microbiological criteria as it gives guidance on the acceptability of burger samples and their manufacturing, handling and distribution processes. The incidence of different pathogens in the burger samples in present study were presented in table 1.

The incidence of *Escherichia coli* 0157: H7 in the burger samples was high in the present study, in this work, this organism was detected in 20% of total samples, where *Escherichia coli* 0157: H7 contaminated with counts ranged from 7×10^2 to 21×10^2 cfu/g. (Table 1). Our results agreed with those obtained by Saleh et al. [31] and El-Dosoky et al. [32] they found that incidence (12% and 15%) in burger samples was reported by respectively, whereas very low incidence (5.12%) was reported by El Shrek and Ali (2012) [33] in cooked meat products.

The incidence of *Salmonella typhimurium* in burger samples was 50% total samples, where *Escherichia coli* 0157: H7 contaminated with counts ranged from 2×10^2 to 11×10^2 cfu/g. El-Dosoky et al. [32] reported (10%) incidence of *Salmonella typhimurium* in beef burger. An incidence of 20% and 14% and 5.71% were reported by Edris [34], Mousa et al. [35] and Ibrahim et al. [36] respectively, which are far less than the incidence observed in the present study in the burger samples.

Staphylococcus aureus could cause food poisoning and if it grows in large numbers can leave toxins in the product, which may survive heating. It lives on the skins of humans and animals and can easily be transferred to food products. Table (1) showed that *Staphylococcus aureus* was detected in 60% of total samples of examined burger, where *Staphylococcus aureus* contaminated counts ranged from 2×10^2 to 20×10^2 cfu/g. counts of 8.3×10^2 , 2.8×10^2 and 1.05×10^2 to 2.3×10^2 cfu/gm were observed by Ali and Abd-El-Aziz [37], El-Mossalami et al. [38], and Min et al. [39] respectively, which were almost similar to the counts observed in the present study.

A rise in notified cases of food poisoning has occurred across the most of Europe and North America; in particular, in the incidence of microbial

food poisoning of animal origin [40]. *Bacillus cereus* was detected in 60% of examined burger. The presence of this bacterium in meat has been widely reported in different parts of the world [41]. For example Mosupye and Von Holy [42], reported (22%) incidence of *B. cereus* in a related study in South Africa, however Ismail [43] reported higher incidences (48%) in beef and Lamb ready to eat foods. Also table (1) indicated the incidence of *Yersinia enterocolitica* isolated from burger samples as 40%. Our results overlap those obtained by Mousa et al. [35], who found that the incidence of *Yersinia enterocolitica* isolated from meat product samples including beef burger, luncheon, pasterma and sausage were 46, 40, 54 and 34%, respectively. Most common sources for this microorganism could be in factories during preparation of the examined product.

Identification of the isolated pathogenic bacteria:

Table (2) summarizes the number of examined samples and the bacterial isolates detected, and their percentages. As shown in Table (2) all the selected 66 isolates of different pathogens bacteria were subjected to identification according to their morphological, physiological and biochemical characteristics as described in Bergy's manual of determinative Bacteriology [30].

Twenty typical isolate were picked up from Baird Parker media agar, 15 typical isolate were picked up from sorbitol MacConkey agar, 15 typical isolate were picked up from *Yersinia* selective agar medium, 15 typical isolate were picked up from *Salmonella* & *Shigella* agar and 20 typical isolate were picked up from *Bacillus cereus* agar medium. After Identification and confirmation, only 14 strains (out of 20) were found to belong to genus *Staphylococcus aureus*, representing 25% of the total strains (56), however the other 11 (out of 15), 9 (out of 15), 10 (out of 15) and 12 (out of 20) isolates were belonged to *Escherichia coli* 0157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium* and *Bacillus cereus*, representing 20, 16, 18 and 21% of the total strains respectively.

Final assessment of burger product according to Egyptian Standards (ES: 1668/2005)

According to the microbiological specifications of the Egyptian Standards (ES: 1668/2005), this product should be free of all examined pathogens. Consequently, most of burger samples representing 73% of total samples were not accepted to the ES due to one or more of criterion, however the remain 27% of the burger samples were accepted. The result demonstrates the fact that the unhygienic and poor sanitary conditions under which the meat and meat products are handled and processed are not acceptable from sanitary point of view. It has further evidence that the undesirable

level of contamination which might have acquired from the environment and agents and to obtain wholesome, safe and sound meat products, the principles Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) must be adopted.

Conclusion

The results of this study indicate that hygienic conditions of burger 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.

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Table 1: Incidence of different pathogens in burger samples from different sources

No.	Source	E. c.	Sal.	Sta.	B.c.	Yers.
1	Bulak	7 x10 ²	6 x10 ²	18x10 ²	3 x10 ²	Nil
		Nil	7 x10 ²	14x10 ²	5x10 ²	7 x10 ²
		Nil	Nil	Nil	Nil	Nil
2	Giza	9 x10 ²	5 x10 ²	Nil	Nil	Nil
		Nil	Nil	Nil	Nil	Nil
		Nil	Nil	16x10 ²	3x10 ²	5 x10 ²
3	Dokki	18 x10 ²	Nil	12 x10 ²	8 x10 ²	9 x10 ²
		Nil	3 x10 ²	Nil	7x10 ²	Nil
		Nil	Nil	Nil	Nil	Nil
4	Ramsis	11 x10 ²	2 x10 ²	15x10 ²	2 x10 ²	11 x10 ²
		12x10 ²	6x10 ²	17x10 ²	6x10 ²	Nil
		Nil	Nil	Nil	3x10 ²	Nil
5	Bin El-Sarayat	16 x10 ²	Nil	2 x10 ²	Nil	Nil
		Nil	Nil	Nil	Nil	Nil
		Nil	3 x10 ²	9x10 ²	7x10 ²	3 x10 ²
6	El-Saeda	Nil	Nil	Nil	Nil	Nil
		12 x10 ²	4 x10 ²	17x10 ²	5x10 ²	8 x10 ²
		Nil	Nil	11x10 ²	Nil	3 x10 ²
7	Ain-Shams	18x10 ²	7 x10 ²	23x10 ²	4x10 ²	Nil
		Nil	Nil	Nil	Nil	Nil
		Nil	Nil	Nil	Nil	Nil
8	Attaba	10 x10 ²	Nil	14x10 ²	6 x10 ²	10x10 ²
		16 x10 ²	6 x10 ²	8x10 ²	8x10 ²	6 x10 ²
		9 x10 ²	3 x10 ²	13x10 ²	4x10 ²	Nil
9	Misr El-Gadida	Nil	5 x10 ²	17x10 ²	Nil	3 x10 ²
		Nil	Nil	Nil	Nil	Nil
		13 x10 ²	Nil	Nil	4x10 ²	Nil
10	Moasasa	21x10 ²	4 x10 ²	12 x10 ²	2x10 ²	Nil
		11 x10 ²	6x10 ²	19x10 ²	7x10 ²	8 x10 ²
		13 x10 ²	5 x10 ²	16x10 ²	5x10 ²	4 x10 ²

Sta.=*Staphylococcus aureus*; E.C.= *Escherichia coli* 0157:H7; Yers.= *Yersinia enterocolitica*; Sal.= *Salmonella typhimurium*; B.c. = *Bacillus cereus*

Table 2. Number of positive samples and isolates form burger samples

	No.	Sta.	E.c.	Yers.	Sal.	B.c.
Sausages samples	30	18* (60%)	15* (50%)	12* (40%)	15 * (50%)	18* (60%)
Total isolates	85	20 (23%)	15(18%)	15 (18%)	15 (18%)	20(23%)
Total strains	56	14 (25%)	11 (20%)	9 (16%)	10 (18%)	12 (21%)

Sta.=*Staphylococcus aureus*; E.c.= *Escherichia coli* 0157:H7; Yers.= *Yersinia enterocolitica*; Sal. = *Salmonella typhimurium* ;B.c. = *Bacillus cereus*, () no. of isolates/ strains, *, positive samples