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Bacteriological Quality of Sausage Sold in Great Cairo Area

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Abstract

Twenty sausage samples were randomly collected from food shops super-markets 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***. *Bacillus cereus* were detected in 70% of the examined samples with an average counts of $4.2x10^2$ cfu/g. In the same time, *Staphylococcus aureus, Escherichia coli* 0157:H7, ***Salmonella typhimurium*** and *Yersinia enterocolitica* were detected in 40, 20, 20 and 30% of the examined samples, with an average counts of $20x10^2$, $25x10^2$,$15x10^2$ and $5.1x10^2$ cfu/g, respectively. Sixty six isolates of pathogenic bacteria including 22 isolates of *Bacillus cereus*, as well as 12 isolates each of ***Staphylococcus aureus*** and *Yersinia enterocolitica,* and 10 isolates each of *Escherichia coli* 0157:H7 and ***Salmonella typhimurium,*** were purified and identified following the biochemical identification tests. Only 47 (71%) out of the tested 66 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 handling should be taken.

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

1. Introduction

Fresh sausages are highly perishable and serve as substrates for several spoilage and pathogenic microorganisms due to their high water content and abundance of essential nutrients [1, 2]. Spoilage can be defined as any change in a food product that makes it unacceptable to the consumer from a sensory point of view. Microbial spoilage is by far the most common cause of spoilage and may manifest itself as visible growth (slime, colonies), as textural changes (degradation of polymers) or as off-flavors [3, 4]. In the case of meat and meat products, microbial spoilage leads to the development of off-flavors, oxidative rancidity, discoloration, gas production and, often, slime formation [5].

Traditionally, fermented sausages were considered healthy and safe foods. More recently, eating fermented sausages has been associated with health hazards caused by the high contents of saturated fats and Na Cl, presence of nitrite and degradation products such as nitrosamines, and use of smoking which can lead to toxic compounds such as polycyclic aromatic hydrocarbons in the products. Hazards can also be both of direct microbiological nature, the sausages potentially being contaminated with food pathogens, and of indirect microbiological nature by metabolic activity of microorganisms

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causing presence of biogenic amines and mycotoxins [6, 7].

Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well documented worldwide [8, 9]. Food-borne illness imposes a substantial economic and quality of life burden on society by way of acute morbidity and chronic sequel [10, 11]. Staphylococcal intoxication is a leading cause of food-borne intoxication and enterotoxigenic *Staphylococcus* strains have been isolated from foods implicated in illnesses [12-14].*Salmonella* spp. has been reported by the United States Department of Agriculture Food Safety and Inspection Service (FSIS) as one of the most common causes of food-borne illness associated with meat and poultry products. *Yersiniaenterocolitica* is a salt-tolerant, pschrotrophic rod that is widely distributed in nature, in aquatic and animal reservoir for human pathogenic strains [15]. The role of *Bacillus cereus* in outbreaks of food-borne illness is becoming well documented. In Egypt, Saleh*et al*. [16] examined 25 samples of each minced meat and sausage. He found that 96 and 60% of these samples were contaminated with *Bacillus cereus,*respectively.

Shehu and Adesiyun [17] reported 39.5% of milk to be positive for *Escherichia. coli*. Enterotoxigenic *Escherichiacoli* has been involved in food-borne illness and recovered from various food types, processed or raw [18]. Consequently, this work has been done to evaluate the general bacteriological condition of the sausage 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* 0157:H7, *Salmonella typhimurium, Yersinia enterocolitica* and *Bacillus cereu*s in order to give a general idea of the health and safety condition of this food product.

2. Experimental

Source and collection of samples:

Two samples from 10 different food shops (super markets) located 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 [19].

Microbiological Analysis:-

Ten-fold dilutions of the homogenates samples were inoculated onto plates of different 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 colorless colonies counts were done after 24 hrs of incubation at 35º C incubation [20]. For detection of *Salmonella typhimurium*, (25g) of each sample was mixed with 225ml of sterile buffer peptone water and incubated at 35 ºC for 24 hrs. One to ten ml of this mixture was transferred to selenite cystein broth and incubated at 35 ºC for 72 hrs. Plates of *Salmonella &Shigella* ager were streaked from the last process and incubated at 35 ºC for 24 hrs. Growth of *Salmonella typhimurium* is appears as colorless colonies with black centers [21].

For *Yersinia,* Each sample (25 g) was homogenized and mixed with 225ml *Yersinia* selective enrichment in 500ml flasks. Flasks were incubated at 30 ºC for 48 hrs, and then spread onto plates of *Yersinia* selective agar medium. After 18-24 hrs of incubation at 35 ºC, suspected colonies of *Y. enterocolitica* which appear form dark red colonies resembling bulls' eye were picked up and further tested for specificidentification. 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 ºC for 48 hrs, typical colonies, which appears gray-black, shiny and convex with a narrow white margin surrounded by a clearing zone, were counted [22, 23]. 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 ºC for 48 hrs, typical colonies, which appears peacock blue-colored and surrounded by precipitation zone were counted and tested for further specific identification [20].

Purification and Identification of the isolated strains:-

Three to five suspected isolates of each pathogen, 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 [24]. Additional kits to help for accurate identification including Hi Staphylococcus identification kit, Hi *Staphylococcus* Latex test kit, Hi *E. coli* identification kit, Hi *E. coli* 0157 Latex test kit, Hi

Salmonella identification kit and Hi *Salmonella* Latex test kit were used. 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 14028were obtained from the Department of Dairy Science, National Research Centre, Cairo, Egypt. These strains were served as reference indicators for comparison among the tested isolates.

3. Results and discussion

The microbiological quality of sausage depends greatly on the initial quality of the used materials and the other processing ingredients. The results of the microbiological analysis of the sausage are presented in table (1). As seen from table 1, four samples of sausage, representing 20% of total samples, were salmonella contaminated with counts ranged from $12x10^2$ to $18x10^2$cfu/g. The presence of this high percentage of this pathogen may indicate that the hygienic conditions of the processed product were very poor and that this examined product of great public health concern. [25].

Bacillus cereus was detected in 70% of examined sausage. The presence of this bacterium in meat has been widely reported in different parts of the world [26, 27]. For example Mosupye and Von Holy [28], reported (22%) incidence of *B. cereus* in a related study in South Africa, however Ismail in Egypt [29] reported higher incidences (48%) in beef and Lamb ready to eat foods.

More, higher incidence (72%) was recorded by El-Sherbeeny*et al*., [30] in sandwiches of liver. This high percentage, obtained in this study, may support its ubiquitous distribution in the environment especially in processing environment.

Escherichiacoli 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 [31]. In this work, this organism was detected in 20% (Table 1). The presence of this pathogen in sausage can be attributed to improper handling and processing, use of contaminated raw materials or the use of unclean processing utensils like knife and trays [32].

Staphylococcus aureus plays a great role in bacterial contamination of 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% of the examined samples (Table 1). Our results agreed with those obtained by Tambekar et al., [33] 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 [33-35]. Also, it can arise from unclean clothes and surrounding poor unhygienic environment which my assist for cross-contamination by such pathogen.

Yersinia enterocolitica were detected in 30% of examined sausage. Our results overlap those obtained by Mousa et al. [36], who found that the incidence of *Yersiniaenterocolitica* 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 [24].

Twelve typical isolate were picked up from Baird Parker media agar, 10 typical isolate were picked up from sorbitol MacConkey agar, 12 typical isolate were picked up from *Yersinia* selective agar medium, 10 typical isolate were picked up from *Salmonella &Shigella* ager and 22 typical isolate were picked up from *Bacillus cereus* agar medium. After Identification and confirmation, only 9 strains (out of 12) were found to belong to genus *Staphylococcus aureus*, representing 19% of the total strains (47), however the other 7(out of 10), 10(out of 12), 7(out of 10) and 14 (out of 22) isolates were belonged to *Escherichia coli* 0157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium* and *Bacillus cereu*s, representing 14.9, 21.3, 14.9 and 29.8% of the total strains, respectively.

Final assessment of sausages product according to Egyptian Standards (ES: 1972/2005)

According to the microbiological specifications of the Egyptian Standards (ES: 1972/2005), this product should be free of all examined pathogens. Consequently, most of sausage samples representing 70% of total samples were not accepted to the Egyptian Standards due to one or more of criterion, however they remain 30% of the sausage samples were accepted.

4. Conclusion

The results of this study indicate that hygienic conditions of sausage 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.Bacterial counts of sausage samples (cfu/g) from ten locations in Great-Cairo Governorate

No.	Source (place)	Sal.	B.c.	E. c.	Sta.	Yers.
1	Bulak	$12x10^2$	$8 x10^2$	$27x10^2$	$18x10^2$	$6 x10^2$
		Nil	$6 x10^2$	Nil	Nil	Nil
2	Giza	Nil	$5 x10^2$	Nil	$26x10^2$	Nil
		Nil	Nil	Nil	Nil	Nil
3	Dokki	Nil	$9x10^2$	$31 x10^2$	$22x10^2$	$5x10^2$
		Nil	Nil	Nil	Nil	Nil
4	Ramsis	$16x10^2$	$5x10^2$	Nil	$8x10^2$	$3 x10^2$
		Nil	$3 x10^2$	Nil	Nil	Nil
5	Bin El-Sarayat	Nil	Nil	Nil	Nil	Nil
		Nil	$2 x10^2$	$19x10^2$	$27x10^2$	Nil
6	El-Saeda	Nil	$3 x10^2$	Nil	Nil	Nil
		Nil	$3 x10^2$	Nil	Nil	Nil
7	Ain-Shams	Nil	$2 x10^2$	Nil	$26x10^2$	$6 x10^2$
		Nil	Nil	Nil	Nil	Nil
8	Attaba	$14x10^2$	$2 x10^2$	Nil	$16x10^2$	$7 x10^2$
		Nil	$4 x10^2$	Nil	Nil	Nil
9	Misr El-Gadida	Nil	Nil	Nil	Nil	Nil
		Nil	Nil	Nil	Nil	Nil
10	Moasasa	$18x10^2$	$3 x10^2$	$23x10^2$	$8x10^2$	$4 x10^2$
		Nil	$4 x10^2$	Nil	Nil	Nil

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

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

	No.	Sta.	E.c.	Yers.	Sal.	B.c.
Sausages samples	20	8* (40%)	4* (20%)	6* (30%)	4 * (20%)	14* (70%)
Total isolates	66	12 (18%)	10 (15%)	12 (18%)	10 (15%)	22(33%)
Total strains	47	9 (19%)	7 (14.9%)	10 (21.3%)	7 (14.9%)	14 (29.8%)

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