Alexandria Journal of Veterinary Sciences

www.alexjvs.com

AJVS. Vol. 51(2): 263-268. November 2016

DOI: **10.5455/ajvs.218707**

Bacteriological Quality of Fresh Sausage Sold in Alexandria Governorate

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Key words:
Fresh sausage, *Staphylococcus aureus*, Enteropathogenic *Escherichia coli*, *Salmonellae*, and *Yersinia enterocolitica*.

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Abstract

One hundred of fresh sausage samples were collected from different butchers at Alexandria governorate. The samples were analyzed for detection of some microbial groups (*Staphylococcus aureus*, Enteropathogenic *Escherichia coli*, *Salmonellae* and *Yersinia enterocolitica*). Results showed that the highest pathogen recovered was 85 *Staphylococcus aureus* isolates (65 isolates were coagulase positive and 20 isolates were coagulase negative) from all examined fresh sausage samples with an incidence of (85%). On the other hand, no *Salmonella* isolates were recovered from all fresh sausage samples examined among the enteric bacterial groups one isolate of *Proteus vulgaris* and 9 isolates of *Proteus mirabilis* with an incidence of 1% and 9% respectively were obtained. Four isolates of *Yersinia enterocolitica* with a percentage of (4%) were isolated. Moreover, 12 isolates of enteropathogenic *Escherichia coli* were recovered, with an incidence of (12%). Concerning serological identification of the recovered Enteropathogenic *Escherichia coli* , the results revealed that 3 strains of O1 , 2 strains each from O26 , O44 , O55 , 2 strains each from O125 , O127 and 2 isolates were untypable with an incidence of 25 , 16.66 , 8.33 , 8.33 , 16.66 , 8.33 and 16.66% respectively.

1. INTRODUCTION:

Sausage manufacturing began over two thousand years ago and the product played an important part in man's diet since then. The modern word "sausage" is derived from the Latin word "*salsus*," which means "salted" (Rust, 1987; Charimba, 2004 and Wijnker et al., 2006). According to Rust (1987), the preparation of sausages began with a simple process of salting and drying meats. The salt added was for preservation purposes. The meat was then flavored with spices to improve the flavor of the product. To make the product more convenient to eat, it was placed in a container made from the gastrointestinal tract of animals. Sausages have been produced from different meats such as beef, pork, chicken, fish and buffalo meat (Raju et al., 2003; Sallam et al., 2004 and Sachindra et al., 2005).

Food borne illness resulting from consumption of contaminated food with pathogenic bacteria and their toxins has been of vital concern to public health. More than 250 different food borne diseases (FBD) have been described, and the bacteria are the causative agents of two thirds of food borne disease outbreaks (Olsen et al.,2000).The most common bacteria causing food borne illness are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species , *Listeria monocytogenes*, *Clostridium botulinus*, *Vibrio parahaemolyticus* and others (Van et al.,2007).

Many types of pathogenic microorganisms isolated during the process of ground beef processing include most notably *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Campylobacter jejuni* (Farber et al., 1988 and Eisel et al., 1997).According to the

United States Department of Agriculture USDA (1999), sausage manufacturers should ensure that their products are not contaminated by pathogens such as *Listeria, Escherichia coli* O157, *Salmonellae* and *Staphylococcus* enterotoxin.

Staphylococci-contaminated food products that include red meat have been implicated in food-poisoning outbreaks (Shale et al., 2005). The presence of *Staphylococcus aureus* can be used as indicator of personal hygiene and also is known to produce harmful enterotoxins.*Escherichia coli* is a highly recognized food pathogen that causes gastro-intestinal diseases in humans, especially *Escherichia coli* O157:H7, which is frequently detected in the intestinal tracts and hide of cattle. This pathogen is also associated with ground beef products and other bovine products. The consumption of food and water contaminated with feacal matter of animals sometimes result in infections caused by *Escherichia coli* strains (Li and Logue., 2005; Arthur et al., 2008; Ateba and Bezuidenhout., 2008 and Wong et al., 2009).

The genus *Yersinia* comprises an important group of bacterial pathogens, *Yersinia enterocolitica, Yersinia pseudotuberculosis,* and *Yersinia pestis* representing the species of interest. *Yersinia enterocolitica* is the most common agent of this genus that is associated with a spectrum of clinical syndromes in man, like gastroenteritis as the most frequently encountered manifestation.

In this study we attempt to isolate *Staphylococcus aureus* , Enteropathogenic *Escherichia coli* , *Salmonellae*, and *Yersinia enterocolitica* from fresh beef sausage as well as serological identification of Enteropathogenic *Escherichia coli*.

2) MATERIAL AND METHODS:

2.1-Collection of samples:

A total of 100 random fresh beef sausages samples were collected as sold to consumers from different butcher shops at Alexandria governorate that were apparently normal including (odour, colour and consistency). The collected samples were transferred directly under hygienic conditions in an ice box (4±1°C) to the laboratory without any delay to be examined bacteriologically for isolation and identification of *Staphylococcus aureus,* Enteropathogenic *Escherichia coli, Salmonella* species and *Yersinia enterocolitica.*

2- Bacteriological examination (ICMSF,1978) **:**

2.1 Samples preparation:

Each sample was mixed thoroughly by the stomacher under sterile conditions then one gm from each sample was placed in 9 ml buffered peptone water for isolation of *Staphylococcus aureus,* Enteropathogenic *Escherichia coli,* and *Salmonella* species also another one gm from each sample was placed in 10 ml of Typticase soya broth for isolation of *yersinia enterocolitica*.

2.2. Isolation and Identification of some pathogenic bacteria:

2.2.1 Isolation and Identification of *Staphylococcus aureus* according to Harrigan (1998).

2.2.1 Isolation of Enteropathogenic *Escherichia coli* according to APHA (1992).

2.2.1 Isolation and Identification of *Salmonella* according to (SREN/ISO 6579/2003).

2.2.1 Isolation and Identification of *Proteus* species according to Holt et al.,(1994).

2.2.1 Identification of Enteropathogenic *Escherichia coli* by commercial kits (API 12A) (oxoid – Microbact GNB- Australia).

2.2.1 Isolation and Identification of *Yersinia enterocolitica* according to Wauters et al.,(1988).

3. Serological identification of enteropathogenic *Escherichia coli:*

Agar slants containing the most pathogenic generous growth of *Escherichia coli* (n= 12) isolates were submitted for agglutination tests using polyvalent and monovalent O *Escherichia coli* antisera , *Escherichia coli* strains were subjected to serological identification according to Ewing (1986).

3. RESULTS AND DISCUSSION

The increasing number and severity of food poisoning outbreaks worldwide has considerably increased public awareness about food (Forsythe, 2008)**,** especially meat and meat products which are one of the most important sources of human infections with a variety of food-borne pathogens (Norrung et al., 2009). However, meat and meat products continue to be an important food group in the diet for many consumers (Delgado,2003 and Speedy , 2003).

Staphylococcal food poisoning is caused by the consumption of food containing enterotoxins produced by certain strains of *Staphylococcus aureus*. Despite the wide - Spread association of *Staphylococcus aureus* with animals, humans are the main reservoir for *Staphylococcus aureus* involved in human disease (Jablonski and Bohach, 1997). Presence of *Staphylococcus aureus* indicates its contamination from food handlers and inadequately

cleaned equipments (ICMSF, 1978). Thus, the presence of *Staphylococcus aureus* in examined meat product samples may reflect the amount of handling when the conditions are favorable for growth and multiplication of such organism, enterotoxins are produced and subsequently the food is dangerous (NAS 1985).

Table (1) showed the incidence of *Staphylococcus aureus* isolated from 100 fresh beef sausage samples, The positive coagulase *Staphylococcus aureus* recovered from the examined fresh beef sausage samples was (65%), while the negative coagulase *Staphylococcus aureus* of the examined fresh beef sausage samples was (20%) .

Typical enteropathogenic *Escherichia coli* strains are diarrheagenic *Escherichia coli* historically associated with outbreaks of infantile diarrhea, particularly during the 1940s and 1950s. Although large outbreaks of infant diarrhea due to typical Enteropathogenic *Escherichia coli* have largely disappeared from industrialized countries, typical Enteropathogenic *Escherichia coli* strains remain an important cause of potentially fatal infant diarrhea in developing countries (Trabulsi et al., 2002) .

Table (2) showed that the incidence of enteropathogenic *Escherichia coli* isolated from 100 fresh beef sausage samples was (12%) which disagreed with the Egyptian Standard Organization that should be zero.

Serological identification illustrated in Table (3) revealed the presence of 12 serotypes {3 strains of O1 (VTEC) , 2 strains of O26 (EHEC) , O44 (EAggEC) , O55 (EPEC) , 2 strains of O125 (EPEC), O127 (EPEC) and 2 untypable strains}.

Verocytogenic *Escherichia coli* (VTEC) results in symptoms ranging from mild uncomplicated diarrhea to severe bloody diarrhoea. Complications including haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) can occur in some cases, both can result in death (John et al., 2007).

Enteropathogenic *Escherichia coli* (EPEC) causes either watery or bloody diarrhea, Low grade fever and vomiting are also associated with the infection. EPEC, unlike VTEC, do not produce any classic toxins (John et al., 2007).

Enteroaggregative *Escherichia coli* (EAggEC) is associated with acute or persistent diarrhea, Infection is typically followed by a watery, mucoid, diarrheal illness with little to no fever and an absence of vomiting (John et al., 2007).

Enterohemorrhagic *Escherichia coli* (EHEC) is a subset of pathogenic *Escherichia coli* that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults.

Salmonella typhimurium was the most frequently serotype and *Salmonella enteritidis* was the second one as a causative agents of human gastroenteritis throughout the world. An annual average, of 186 cases of *Salmonella* food poisoning was recorded in Norway by (Sharma et al.,1996). An outbreak of *Salmonella typhimurium* occurred in 1995 and involved 83 persons in Nothern Italy and the vehicle was sausage (Pontello et al., 1998), The presence of *Salmonella* in minced beef could be explained by contamination from the lymph nodes, viscera, skin and leather of animals, equipment or any manipulators healthy carriers (Reid et al.,2002). All the examined fresh sausage samples were free from *Salmonella* species which agreed with Egyptian Standard Organization.

Proteus species are the causative agent of a variety of opportunistic nosocomial infections including those of the respiratory tract, ear, nose, skin, burns, and wounds, it may also cause gastroenteritis, *Proteus* species (*Proteus mirabilis*, *Proteus vulgaris*, and *Proteus penneri*) are important pathogens of the urinary tract.(Jacobsen et al.,2008).

Table (4) showed the incidence of *proteus* species isolated from 100 fresh beef sausage samples was (10%) included: *Proteus vulgaris* (1%) and *Proteus mirabilis* (9%) which disagreed with the Egyptian Standard Organization that should be zero.

Yersinia enterocolitica shows good growth in the environment, with up to 5% of salt regardless of temperature, whereas it is inhibited at 7% salt concentration (Adams and Moss , 2000) also the storage of fish, fish products, meat, meat products, milk, and dairy products contaminated with *Yersinia enterocolitica* at 0–4 ◦C according to (Tudor et al.,2008) may lead to intensive multiplication of this bacteria, Food poisoning often results from the ingestion of food products in which a thermostable toxin has been produced.

Table (5) showed that the incidence of *Yersinia enterocolitica* isolated from 100 fresh beef sausage was (4%) which disagreed with the Egyptian Standard Organization that should be zero.

Table (1): Incidence of *Staphylococcus aureus* in the examined fresh sausage samples (N=100)

No. of examined sausage samples	*Staphylococcus aureus*			
	Coagulase positive		Coagulase negative	
	No.	%	No.	%
100	65	65	20	20

Table (2):

Escherichia coli serotypes	No.	%	Strain character**
O1	3	25	Verocytotoxigenic *Escherichia coli*
O26	2	16.66	Enterohaemorraghic *Escherichia coli*
O44	1	8.33	Enteroaggregative *Escherichia coli*
O55	1	8.33	Enteropathogenic *Escherichia coli*
O125	2	16.66	Enteropathogenic *Escherichia coli*
O127	1	8.33	Enteropathogenic *Escherichia coli*
Untypable	2	16.66	_
***Escherichia coli* isolates**	12	12	_

Incidence of Enteropathogenic *Escherichia coli* in the examined fresh sausage samples (_N=100)

Table (3): Serological typing of Enteropathogenic *Escherichia coli* in the examined fresh sausage (N=12)

**= Strain character according to (James et al.,2005) and (John et al.,2007).

No. of examined sausage samples	Enteropathogenic *Escherichia coli*	
	No.	%
100	12	12

Table (4): Incidence of identified enteric group (*Proteus* species) in the examined fresh Sausage samples (N=100)

No.of examined fresh sausage samples	*Proteus vulgaris*		*Proteus mirabilis*	
	No.	%	No.	%
100	1	1	9	9

Table (5): Incidence of *Yersinia enterocolitica* in the examined fresh sausage samples (N=100)

No. of examined sausage samples	*Yersinia enterocolitica*	
	No.	%
100	4	4

Finally, from the current study we conclude that implementation of a hygiene plan in slaughterhouses and in cutting and meat preparation companies are crucial. The possibility of contamination of meat products with such serious pathogen remains a public health hazard. Thus all precautions of proper sanitation during manufacture, handling and storage of such meat products should be adopted to control these serious pathogens and to obtain a maximum limit of safety to consumers.

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