

INCIDENCE OF COLIFORM AND STAPHYLOCOCCUS AUREUS IN READY TO EAT FAST FOODS

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ABSTRACT

A total of 100 random samples of ready- to – eat sandwiches of beef products of kofta, liver, shawerma, and sausage products (25 samples of each) were collected from different fast food services in different districts at Kaliobia governorate to be examined bacteriologically for detection of Coliform and *Staphylococcus aureus* microorganisms. The obtained results in the present study indicated that the mean value of coliform counts (cfu/g) in the examined samples of ready- to - eat meat products were $2.5 \times 10^3 \pm 0.74 \times 10^3$ for beef kofta, $8.85 \times 10^2 \pm 1.92 \times 10^2$ for beef shawerma, $8 \times 10^3 \pm 1.65 \times 10^3$ for beef sausage, $9.0 \times 10^4 \pm 2.30 \times 10^4$ for beef liver, further more the coliform were detected in 60% of beef kofta, 40% of beef shawerma, 52% of beef sausage and 80% of beef liver. The obtained results in the present study indicated that the *staphylococcus aureus* was detected in 32% of beef kofta, 44% of beef liver, 8% of beef shawerma and 16% of beef sausage . The obtained results in the present study indicated that the ready- to-eat liver sandwiches were more contaminated with *Staphylococcus aureus* as compared with those of kofta, shawerma and sausage. The examined samples of ready-to-eat liver sandwiches showed high incidence of coliform than those obtained by kofta, sausage and shawerma.

KEY WORDS: ready- to – eat sandwiches, Coliform, *Staphylococcus aureus*, kofta, liver, shawerma, sausage.

1. INTRODUCTION

Fast food is the term given to food that can be prepared and served very quickly. Typically the term refers to food sold in a restaurant or store with low quality preparation and served to the customer in packaged form for takeout/take-away. Due to the variety of ready-to-eat foods, the interpretation of microbiological results obtained from testing must be accounted for the method of processing and the individual components of the food (Food Standards Australia New Zealand, 2001). Therefore, the current study was planned out to evaluate the bacteriological status of some ready to eat meat meals sold at different districts and restaurants in Benha city Kaliobia Governorate.

3. MATERIAL AND METHODS

3.1. Collection of samples

A total of 100 random samples of ready to eat beef kofta, beef liver, shawarma and beef sausage are presented as (25 of each) were collected from different districts and restaurants in Benha city Kaliobia Governorate to be evaluated bacteriologically. Each sample was kept in a separate sterile plastic bag and put in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined bacteriologically to evaluate the bacterial quality of them and to evaluate the hygienic health hazard of contaminated with some food borne pathogens.

3.2. Bacteriological examination

3.2.1. Preparation of samples (APHA. 1992)

To 25 grams of the samples under examination were taken under aseptic condition to stomacher bag then add 225 ml sterile 0.1% peptone water, the contents were homogenized at Stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes, the mixture was allowed to stand for 5 minutes at room temperature. The contents were transferred into sterile flask and thoroughly mixed by shaking and 1 ml was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth- fold serial

dilutions were prepared. The prepared samples were subjected to the following bacteriological examination.

3.2.3. Determination of Coliform count (ICMS, 1996)

The same technique of the previous surface plating method was applied using Violet Red Bile agar medium. The plates were incubated at 37°C for 24 hours. All pink colonies measuring 0.5 mm or more in diameter on uncrowded plates were then counted and the average number of colonies were determined. Multiply the number of colonies by the dilution to obtain the number of Coliform organisms per gram of sample.

3.2.6. Determination of S.aureus Count (ICMSF, 1996)

Accurately, 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Mannitol agar using a sterile bent glass spreader. The inoculated and control plates were incubated at 37°C for 48 hours. The developed colonies (white, orange and yellow) were enumerated and the total Staphylococci count /g was calculated. Also, the colonies were picked up and purified on Semi-solid nutrient agar slopes for further identification. Moreover, yellow colonies surrounded by a halo zone (suspected *Staph.aureus*) were picked up and kept in Semi-solid agar slopes for morphological examination and biochemical identification.

Isolation of Staph.aureus (ICMSF, 1996)

Accurately, 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Baird Parker agar using a sterile bent glass spreader. The inoculated and control plates were incubated at 37°C for 48 hours. Black and shiny colonies with yellow halo zone around them (suspected *S.aureus*) were picked up and purified on Semi-solid agar slopes for morphological examination and biochemical identification.

3.2.6.1. Isolation and Identification of suspected S.aureus (Quinn et al., 2002)

3.2.6.1.1. Morphological identification

-Grams staining:

Smears from suspected pure colonies were stained with Gram-stain and examined microscopically. Gram-positive cocci, arranged in irregular clusters (bunches of grapes)

3.2.6.1.2. Detection of haemolysis

A loopful from inoculated Brain Heart Infusion (BHI) broth were streaked on the surface of 5% sheep blood agar plates and incubated at 37°C for 24 hours for detection of haemolysis. *Staph.aureus* positive for haemolysis.

3.2.6.1.3. Motility test

Inoculate the growth culture by stabbing the center of the semi-solid agar tubes and incubated at 25°C for 48 hours, Positive result: Motile organisms migrate from the stab line and diffuse into medium. Negative results: No migration from the stab line observed (*Staph.aureus* was non-motile).

3.2.6.1.4. Biochemical identification

The purified isolates of *S.aureus* were examined by different biochemical reactions according to (Quinn et al., 2002).

3.2.7. Statistical analysis: The obtained results were statistically evaluated by application of analysis of variance (ANQVA) test according to Feldman et al, (2003).

3-RESULTS

3.1. Coliform count (cfu/g) of the examined ready to eat food samples:-

Results achieved in table (1) declared that the mean value of coliform counts (cfu/g) in the examined samples of ready- to – eat meat products were $2.5 \times 10^3 \pm 0.74 \times 10^3$ for beef kofta, $8.85 \times 10^2 \pm 1.92 \times 10^2$ for beef shawerma, $8 \times 10^3 \pm 1.65 \times 10^3$ for beef sausage, $9.0 \times 10^4 \pm 2.30 \times 10^4$ for beef liver, further more the coliform were detected in 60% of beef kofta, 40% of beef shawerma, 52% of beef sausage and 80% of beef liver.

3.2. Staphylococcal aureus of the examined ready- to- eat food samples:-

As shown in table (3) indicated that the staphylococcus aureus was detected in 32% of beef kofta, 44% of beef liver, 8% of beef shawarma and 16% of beef sausage.

4. DISCUSSION

Ready-To-Eat foods of meat products are highly demanded due to their high biological value, reasonable price, agreeable taste and easily serving. But these constituents can be contaminated by many types of microorganisms due to bad hygienic measurements. Food borne pathogens may constitute health hazard to the consumers. Street vended meat have been incriminated in several out breaks of food poisoning (WHO, 1984 AND Mosupy et al., 1998).

Processed meats are subjected to be contaminated with several types of microorganisms from different sources during the period of slaughtering, preparation, processing and cooking. These microorganisms varied according to the method of manufacture, quality of used non-meat ingredients, and contamination level during the processing chain, packaging and storage (Narasimha and Ramesh, 1988).

Results achieved in table (1) declared that the mean value of total coliform counts (cfu/g) in the examined samples of ready- to – eat meat products were $2.5 \times 10^3 \pm 0.74 \times 10^3$ for beef kofta, $8.85 \times 10^2 \pm 1.92 \times 10^2$ for beef shawarma, $8 \times 10^3 \pm 1.65 \times 10^3$ for beef sausage, $9.0 \times 10^4 \pm 2.30 \times 10^4$ for beef liver, further more the coliform were detected in 60% of beef kofta, 40% of beef shawarma, 52% of beef sausage and 80% of beef liver.

The current results in table(1) agree with those recorded by El-Rayes (2008), who found that the mean value of total coliform was $2.83 \times 10^3 \pm 0.74 \times 10^3$ (cfu/g) in the examined samples of kofta sandwiches, Yassien (1992) who found that the mean value of coliform was 3.8×10^3 (cfu/g) in the examined cooked meat samples, Adam (2009) who found that the mean value of coliform was $9.3 \times 10^3 \pm 3.5 \times 10^3$ (cfu/g) for street vended cooked kofta samples.

While lower results were recorded by Elwi (1994) who found that the mean value of coliform was 45×10^2 & 22×10^2 (cfu/g) in the examined samples of cooked meat and cooked kofta respectively and Saadet al. (2011) who found that the mean value of coliform was $5.17 \times 10^2 \pm 1.2 \times 10^2$ (cfu/g) in the examined samples of grilled beef kofta. However, higher findings were obtained by Rafaie and Moustafa (1990) who found that the mean value of coliform was $33,9 \times 10^5$ (cfu/g) for shawerma samples, Hussien (1996) who found that the "mean value of coliform count was 1.8×10^5 (cfu/g) for kofta sandwiches & El-Mossalami (2003) who found that the mean value of coliform count was 1.9×10^5 (cfu/g) in the examined samples of kofta. The presence of coliforms group in meat has an epidemiological interest as some of members are pathogenic, and may result in serious infections and food poisoning. Thus, the total coliforms count may be used as a board base indicating fecal contamination of meat due to inadequate processing and / or post processing recontamination of meat (ICMSF., 1998).

Coliforms was significant organisms in meat as indicator of fecal contamination and had ability to grow well over wide range of temperature below 10^0 °C up to 46^0 °C (Gill et al., 1996), also the presence of coliform bacteria in great numbers may be responsible for inferior quality of meat products resulting in economic losses and the possibility of presence of enteric pathogens which constitute public health hazard (Trout and Osburn, 1997).

The high incidence of coliforms in the examined ready-to-eat -sandwiches indicates inadequate processing or post processing contamination (most probably from workers, dirty instrument, machinery and other contact surfaces), or from raw ingredients before processing which drive their contamination from various sources as human contact, polluted water, soil and manure, the presence of conforms indicates a probable faecal sources of contamination (Thatcher and Clark, 1975 ; ICMSF, 1978 and NAS, 1985).

Table (3) indicated that the staphylococci were detected in 32% of beef kofta, 44% of beef liver, 8% of beef shawerma and 16% of beef

sausage. *Staphylococcus* can be carried on hands, nasal passage or throats. Most food borne illness outbreaks are result of contamination from food handlers and production of heat stable toxins in food. Sanitary food handling and proper cooking and refrigerating should prevent staphylococcus food born illness (FSIS, 2003). The presence of *Staphylococcus aureus* in a food indicates its contamination from food handlers & in adequately cleaned equipments (ICMSF, 1996). *Staphylococcus aureus* intoxication is a worldwide problem where several food poisoning outbreaks were reported due to consumption of meat products contaminated with this organism. Accordingly, *Staphylococcus aureus* can be taken as index of sanitary conditions under which meat and its products are manufactured and handled (Potter, 2001). The symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea (never diarrhea alone). The onset of symptoms remission is observed after 24h (Le Loir, 2003).

Such organisms were previously isolated from ready-to-eat meat products by Soliman et al (2002) & Kirralla (2007) who isolate *Staphylococcus aureus* from cooked meat samples.

Staphylococcal food poisoning is the result of performed enterotoxins that are produced by certain strains of *Staphylococcus aureus* resulting in symptoms of an intoxication, not an infection. The most common symptoms appear approximately 3-8hrs after ingestion and include nausea, vomiting, abdominal cramps and diarrhea. Generally, symptoms are short in duration (approximately 24 - 48hrs) (Sandle and Mckillip, 2004).

5. REFERENCES

- 1-Adam, H.A.A (2009): Microbiological quality of street vended foods of animal origin. M.V.Sc, Thesis (Meat hygiene), Fac. Vet Med., KafrElshiekh University.
- 2-American Public Health Association (APHA) (1992): Compendium of methods for microbiological examination of Food. 3rd Ed. Brothers, Ann, Arb.
- 3-Bailey, W.R. and Scott, E.G. (1978): Diagnostic Microbiology. A text book for the isolation and identification of pathogenic microorganism. The C.V. Saint Louis.
- 4-El-Mossalami E.K. (2003): Risk assessment of ready prepared meat products, Ph, D., Thesis, Fac. Vet. Med., Cairo Univ.Egypt.
- 5-El-Rayes, A.M.A. (2008); Incidence of pathogenic *Escherichia coli* in fast foods. M.V.SC. Thesis, fac. Vet. Med, BenhaUniversity.Egypt.
- 6-Elwi, E.M. (1994): Sanitary improvement of meat meals in governmental hospitals in Assiut City. Ph. D. thesis, Meat Hygiene, Fac. of Vet.Med.; Assiut University .Egypt.
- 7- Feldman, D.; Canon, J.; Haffman, R, and Simpson, J. (2003): The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
- 8-Food and Drug Administration "FDA"(2001): Center for Food safety and Applied nutrition. (www.FDA.org).
- 9- Food Safety and Inspection service "FSIS" (2003):United states Department of Agriculture; Meat preparation: Beef from farm to table. Washington. DC. 20250-3700.
- 10-Gill, CO.; McGinnis, K.R. and A. Houde (1996): The hygienic condition of manufacturing beef destined for the manufacturing beef destined for the manufacture of hamburger patties. Food Microbiol, 13:391.

- 11-Hussein, M.I. (1996): Microbial evaluation of some meat meals of Assiut restaurants. M.V.Sc. Thesis, Fac. of Vet. Medicine, Assiut University. Egypt.
- 12-ICMSF *International commission of Microbiological Specification for foods* (1996): Microorganisms in Food. I-Their Significance and methods of enumeration. 3rd Ed. Univ. of Toronto, Canada.
- 13-*International Commission and Microbiological Specification for Foods "ICMSF"* (1978): Microorganisms in foods: Their Significance and Methods of Enumeration. Vol. 1st, 2nd Ed. University of Toronto Press, Toronto Ontario, Canada.
- 14-*International Commission and Microbiological Specification for Foods "ICMSF"* (1998): Microorganisms in Foods. Microbial Ecology of Foods Commodities. Blackie Academic and Professional, London, New York, Tokyo, Melbourne, Madress.
- 15-Kirralla, G.A. (2007): Sanitary status of meat meals of students of Tanta university. M.V. Sc. Thesis meat hygiene, Fac. Vet.Med., kafr El shickh university .Egypt.
- 16-Lachia, R.V.F.; Genigeogis, C. and Hoeplich, P.O. (1971): Meta chromatie agar-diffusion methods for detecting Staphylococcal nuclease activity. *Appl. Microbiol.* 21: 585:587.
- 17-Le Loir, K; Baron, F. and Gautier, M. (2003): *S.aureus* and food poisoning. *Genetics and Molecular Research*, 2(1): 63-76.
- 18-Mosupy, F.M.; Arntzen, L. and Van Holy, A, (1998): Microbiological survey of street-vended food in the Johannesburg metropolitan area of South Africa, *Food Sci*, 63(7): 842 – 846.
- 19-Narasimha, R.D. and Ramesh, U.S. (1988): Microbial profiles of minced meat. *Meat Science* 23:279-291.

- 20-NAS *National Academy of Science (1985)*:An evaluation of the role of microbiological criteria for foods and food ingredients. National Academy Press, Washington D.C.
- 21-Potter, N.N. (2001): Food Science. 3rd Ed.The AVI Publishing Co., INC. New York, USA.
- 22-Quinn, p.; Markey, B.; Carter, M.; Donnelly, W. and Leonard, F. (2002): Veterinary Microbiology and Microbial Disease. Black Well Science: chapters 26-36.
- 23-Rafaie, R.S. and Mostafa, S. (1990): Microbiological quality of shawarma in Assuit. Vet, Med. J. 24(47):135.
- 24-Saad M.S.; Hemat M.I. and Enas A.M.A. (2011): Microbial and chemical evaluation of fast foods, J. Benha vet. Med. S.E (1):44- 51.
- 25-Sandle, M.K. and Mckillip, J.L(2004): Virulence and recovery of *Staphylococcus aureus* relevant to the food industry using improvements on traditional approaches. J. Food Control. 15:5-10.
- 26-Soliman, M.R.; Abd El-Monem, KM, and Sand, S.M. (2002): Microbiological quality of ready-to-eat meat products and fishes in urban and rural areas. J. Egypt. Vet, Med. Assoc, 62(6); 39-51.
- 27-Thatcher, F.S. and Clark, D.S. (1975): Microorganisms in Food, I. International committee on microbiological specification for foods. Univ. of Toronto press, Tronto and Buffalo, Canada.
- 28-Trout, H. and Osburn, B. (1997): Meat from dairy cows: possible microbiological hazards and risks. Rev. Sci. Technol. 16(2): 405-414.
- 29-World Health Organization "WHO" (1984): The role of food safety in health development, Report of Joint FAO/WHO Expert Committee on Food Safety, Geneva.
- 30-Yassien, N.A. (1992): Enteropathogenic *E.coli* in a food serving establishment. Fleischwirtschaft.12:5.

Table (1): Frequency distribution of total coliform counts in the examined ready to eat foods samples (n = 25).

Count	Kofta sand.		Shawerma sand.		Sausage sand.		Liver sand.	
	No	%	No	%	No	%	No	%
0 - < 10	10	40	15	60	12	48	5	20
10 - < 10 ²	3	12	5	20	2	8	1	4
10 ² - < 10 ³	7	28	3	12	3	12	3	12
10 ³ - < 10 ⁴	4	16	2	8	2	8	1	4
10 ⁴ - < 10 ⁵	1	4	-	-	5	20	8	32
10 ⁵ - < 10 ⁶	-	-	-	-	1	4	7	28
Total	25	100	25	100	25	100	25	100

Table (2): Statistical analytical results of total coliform counts in the examined ready to eat food samples (n = 25).

Count	Positive samples		Min.	Max.	Mean ± S.E*
	No	%			
Kofta	15	60	6.0 x 10	8 x 10 ³	2.5 x 10 ³ ± 0.74 x 10 ³
Shawerma	10	40	3 x 10	2.1 x 10 ³	8.85 x 10 ² ± 1.92 x 10 ²
Sausage	13	52	1.5 x 10 ²	2.4 x 10 ⁴	8 x 10 ³ ± 1.65 x 10 ³
Liver	20	80	2.2 x 10 ²	5.1 x 10 ⁵	9.0 x 10 ⁴ ± 2.30 x 10 ⁴

*S.E: Standard error.

Table (3): Incidence of Staphylococcus aureus isolated from the examined ready to eat food samples (n = 25).

Products	Positive samples	
	No	%
Kofta	8	32
Shawerma	2	8
Sausage	4	16
Liver	11	44
Total	25	25

تواجد ميكروبات الميكروب العنقود الذهبي والكوليفورم في الوجبات السريعة الجاهزة للأكل

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الملخص العربي

تعتبر الوجبات المجهزه من اللحوم ومنتجاتها من اهم الاغذيه التي يقبل عليها عدد كبير من المستهلكين في مصر والعالم وذلك لاحتوائها على نسبة عاليه من البروتين الحيوانى ولطعمها الشهى ولسهوله اعدادها علاوة على تنوعها. وحيث انها عرضه للتلوث بالعديد من الميكروبات الممرضه والتي تشكل خطوره على صحه المستهلك اثناء تجهيزها وطهيها وقبل تناولها. لذا اجريت هذه الدراسة والتي اشتملت على مدى تواجد ميكروبات الميكروب العنقود الذهبي والكوليفورم في اللحوم الجاهزه للاكل بأماكن اعداد وتقديم الوجبات السريعة بمحافظة القليوبية.

تم جمع ١٠٠ عينه عشوائية من سندوتشات اللحم البقرى الجاهزه للاكل من اماكن تجهيز الوجبات السريعة بمحافظة القليوبية التي اشتملت اربعة انواع من السندوتشات هي كبدة، كفتة، شاورمة، سجق معدة من اللحم البقرى بواقع ٢٥ عينه من كل نوع ولقد تم نقل هذه العينات على وجه السرعة وتحت ظروف صحية مشددة الى المعمل لمعرفة الحالة البكتيرية لها من حيث نسبة الميكروب العنقود الذهبي والكوليفورم.

وقد دلت نتائج الدراسة على ان متوسط العدد الكلى للكوليفورم هو $0.74 \pm 2.5 \times 10^3 \times 10^3$ في عينات كفتة اللحم، $1.92 \times 10^2 \pm 8.85 \times 10^2$ في عينات شاورما اللحم، $1.65 \times 10^3 \pm 8 \times 10^3$ في عينات سجق اللحم، $2.30 \times 10^4 \pm 9.0 \times 10^4$ في عينات كبدة اللحم.

ولقد امكن عزل الميكروب المكور العنقودى الذهبي من ٢٥ عينه على النحو الاتى: ٣٢% لكفته اللحم، ٨% للشاورما البقرى، ١٦% لسجق اللحم، ٤٤% لكبدة اللحم.

هذا وقد تم مناقشة الاهميه الصحية للميكروبات التي تم عزلها من منتجات الاغذية الجاهزة للاكل ومدى تأثيرها على الصحة العامة والمصادر التي تسبب تلوث هذه الاغذية بهذه الميكروبات وكذلك المقترحات التي تؤدى الى تحسين جودة تلك الاغذية.