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Bacteria associated with some returned meat products

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Abstract

A total of 135 random samples of some returned meat and poultry final products were collected from market in Cairo governorate for which isolation and identification of *E. coli* O157: H7, *S. aureus* and *Salmonella* serovars.

The incidence of isolated *E. coli* O157 : H7 from some returned meat and poultry products were 9.1%, 18.2%, 18.2%, 9.1%,18.2% and 27.2% from beef burger, oriental sausage, minced meat, kofta, frozen hotdog and chicken burger, respectively and failed to be isolated from chilled hot dog, luncheon beef and smoked turkey.

As regards to salmonellae, Attempts to recover salmonella from returned meat and poultry products failed in all products except smoked turkey (Rusto),where two isolates were recovered and identified as *S.* Typhimuirium and *S.* Kentucky.

The incidence of *S. aureus* in beef burger, luncheon beef, minced meat, frozen hot dog, chilled hot dog, chicken burger and smoked cooked turkey meat were 4.4%, 39.1%, 13 %, 13%, 4.4%,13% and 13%, respectively and failed to be isolated from oriental sausage and kofta.

Keywords: *E. coli* , *Salmonella*, *S. aureus* , meat products, returned.

Introduction

Returned meat (beef or poultry) products are high risk products carrying several types of microorganisms, therefore in the present study were investigated as sources of pathogens contaminating products either during processing or even after exposure to contaminants due to leakages of containers, bad handling, etc.

Meat consumption is based largely on availability, price and tradition. Meat production is very complex operation depending not only on demand (which is usually based on price and income), but on many social and economic influences such as official policy, price support mechanisms, and interrelations such as the interaction between beef and milk production, the availability of animal feedstuffs and competition for food between man and animal.

Generally, the primitive manufacturing and improper handling of meat products constitute a public health hazard either due to the presence of spoilage bacteria responsible for unfavorable change, or pathogenic bacteria leading to harmful effects as food infection or intoxication among consumers. Food poisoning due to infection is caused by the consumption of live bacteria, while food intoxication occur due to presence of toxins in food produced by bacteria before food consumption. Toxins are very dangerous chemical compounds which may linger in food with no microbes growing in it (**Mastievskii** et al., **1971; Condera** et al., **2004 ; Stampi** et al., **2004).** Food borne diseases are more common than we know. Most of people think it is the kind of ailment that strikes 'other' people, and because hygienic eating habits are

usually kept, but not many of us know that food poisoning can be caused by various factors outside our control. Many of these factors are introduced in the food chain during its manufacture or cultivation, some others during its packing and preservation and many more during its retail handling, storing or even end use (which means consumers). The figures are awesome; the US exchequer alone bears between USD 5 and 17 billion according to conservative estimates.

An estimated 76 million cases of food borne diseases occur each year in the United States. The great majority of these cases are mild and cause symptoms for only a day to two. Some cases are more serious, and CDC (2005) estimated that there are 325,000 hospitalizations and 5,000 deaths related to food borne disease each year. Known pathogens account for an estimated 14 million diseases. 60,000 hospitalizations and 1,800 deaths. While unknown agents account for the remaining 62 million diseases, 265,000 hospitalizations, and 3,200 deaths. Surveillance and monitoring by number of countries indicates that food borne diseases are increasing around the world.

In 2011, the case counts confirmed by the German Robert Koch Institute included 520 patient with hemolytic uremic syndrome (HUS) – a type of kidney failure that is associated with *E. coli* or STEC infections and 11 deaths. In the United States, four suspected cases of STEC O104:H4 infections have been identified in persons who recently traveled to Hamburg, Germany, where they were likely exposed. (CDC, 2011).

Materials and Methods

The total number of random samples was 135 products. They were returned due to either near the expiry date or defected in packaging. Also may be due to excess amount of the product in the market or what is called "recall" either internally or externally from the market. Each sample was packed in plastic bag and transferred immediately to the laboratory in an icebox with minimum period of delay to be examined bacteriologically. Samples were prepared according to the technique recommended by ICMSF (1978). Determination of Aerobic plate count (APC) was done according to APHA (1992) using nutrient agar (Oxoid) plates.

Detection of *Escherichia coli*

Was done according to the method in ISO 16649-2:2001for the enumeration of beta glucuronidase positive *E. coli*

Salmonella isolation

was done according to ISO 6579:2002 : Horizontal method for detection of Salmonellae.

Detection of *Staphylococcus aureus*

was done according to ISO 688-1:1999/FDAM 1:2003: Horizontal method for enumeration of coagulase positive staphaylococci

- Baird-Parker agar medium for 24 hr. at 37 ° C.
- Using Blood agar medium for 24 hr. at 37 ° C.

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Results

The results given in Table (1) show that the highest Mean of the total colony counts for the examined samples was $5x10^6$ in chicken burger, but the lowest means were in smoked turkey and chilled hotdog ($1x10^5$).

Regarding the types and rates of isolated bacteria from the examined samples of the returned meat products, the result given in Table (2) and Fig. (1) show that the incidence of isolation of *E.coli* O157 :H7, *S.* Typhimuirium , *S.* Kentucky and *S. aureus* were 30.6%, 2.8%, 2.8% and 63.9%, respectively. And Numbers of islates were 11, 1, 1 and 23 respectively.

As shown in Table (3), it is evident that the highest level of the recovered *E. coli* O157 : H7 was detected in chicken burger (27.2%) and the lowest was in kofta and beef burger (9.1% each) but, it could not be isolated from chilled hot dog, rusto and luncheon beef.

As regards to salmonella, attempts to recover salmonella from returned products failed in all products except smoked turkey (Rusto), where two isolates were recovered and identified as *S.* Typhimuirium and *S.* Kentucky (Fig. 2)

From the current study it is shown in Table (4) and (5) that *S. aureus* were isolated from beef burger, oriental sausage, luncheon beef, minced meat, kofta, frozen hot dog, chilled hot dog, chicken burger and smoked cooked turkey with the incidence of 4. 34 %, 0%, 39.1 %, 13.04 %, 0%, 13.04 %, 4. 34 %, 13.04 % and 13.04 % and counts were 15 x 103, 0, mean 9.1 x 102, mean 6.4 x 103, 0 , mean 9 x 102, 2 x 103 , mean 11 x 103 and mean 1 x 103 , respectively.

- Data reveal that the highest Mean of isolated *S. aureus* was detected in beef burger (15 x 103) and the lowest Mean was detected in oriental sausage and kofta (< 10). The incidence of *S aureus* was the highest in luncheon beef (39.1%) and the lowest was in chilled hot dog and beef burger (4. 34 %).

Table (1): Aerobic plate count for the examined meat and poultry products (n = 15 for each product):

Results (Bact./ g)			Products
Mean	Max.	Min:	
2×10^6	6.4×10^6	8×10^4	Beef burger
2.1×10^6	4×10^8	3×10^4	Oriental sausage
1×10^6	3.1×10^6	1.2×10^3	Luncheon beef
1.1×10^6	6.4×10^6	6×10^3	Minced meat
2.1×10^6	8×10^8	1.1×10^4	Kofta
6×10^4	1×10^7	2×10^4	Frozen hotdog
1×10^5	2.5×10^8	2×10^4	Chilled hotdog
5×10^6	9×10^6	3×10^4	Chicken burger
1×10^5	2×10^8	3×10^2	Smoked turkey (Rusto)

Table (2): Occurrence *E. coli*, salmonellae and *staph. aureus* among the examined samples (135) :

Percentage	%	No. of isolates	Bacteria isolated
30.6	8.1	11	*E. coli* O157 :H7
2.8	0.7	1	*S. Typhimuirium*
2.8	0.7	1	*S. Kentucky*
63.9	17.03	23	*S. aureus*
100	26.7	36	Total

Table (3): Isolated *E. coli* O157 : H7 from the examined meat and poultry products :

Percentage to the total isolated *E. coli* O157: H7	Percentage to the total samples	No of isolated microbe	No. of examined samples	Products
9.1	6.7	1	15	Beef burger
18.2	13.3	2	15	Oriental sausage
0	0	0	15	Luncheon beef
18.2	13.3	2	15	Minced meat
9.1	6.7	1	15	Kofta
18.2	13.3	2	15	Frozen hot dog
0	0	0	15	Chilled hot dog
27.2	20	3	15	Chicken burger
0	0	0	15	Smoked turkey
100	8.1	11	135	Total

Table (4): Isolated *S. aureus* from examined meat and poultry products:

Counts (bacteria/g)			Meat products
Mean	Max.	Min:	
15×10^3	15×10^3	15×10^3	Beef burger
< 10	< 10	< 10	Oriental sausage
9.1×10^2	3×10^3	4×10^2	Luncheon beef
6.4×10^3	12×10^3	1×10^3	Minced meat
< 10	< 10	< 10	Kofta
9×10^2	3.3×10^3	5.5×10^2	Frozen hotdog
2×10^3	2×10^3	2×10^3	Chilled hotdog
11×10^3	4.3×10^4	8.2×10^3	Chicken burger
1×10^3	2×10^3	3×10^2	Smoked turkey (Rusto)

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Table (5): No of Isolated *S. aureus* from the examined meat and poultry products:

Percentage to the total isolated *S. aureus*	Percentage to the total No of samples	No of isolated microbe	No. of examined sampes	Products
4.34	6.7	1	15	Beef burger
0	0	0	15	Oriental sausage
39.1	60	9	15	Luncheon beef
13.04	20	3	15	Minced meat
0	0	0	15	Kofta
13.04	20	3	15	Frozen hot dog
4.34	6.7	1	15	Chilled hot dog
13.04	20	3	15	Chicken burger
13.04	20	3	15	Smoked turkey
100	17.03	23	135	Total

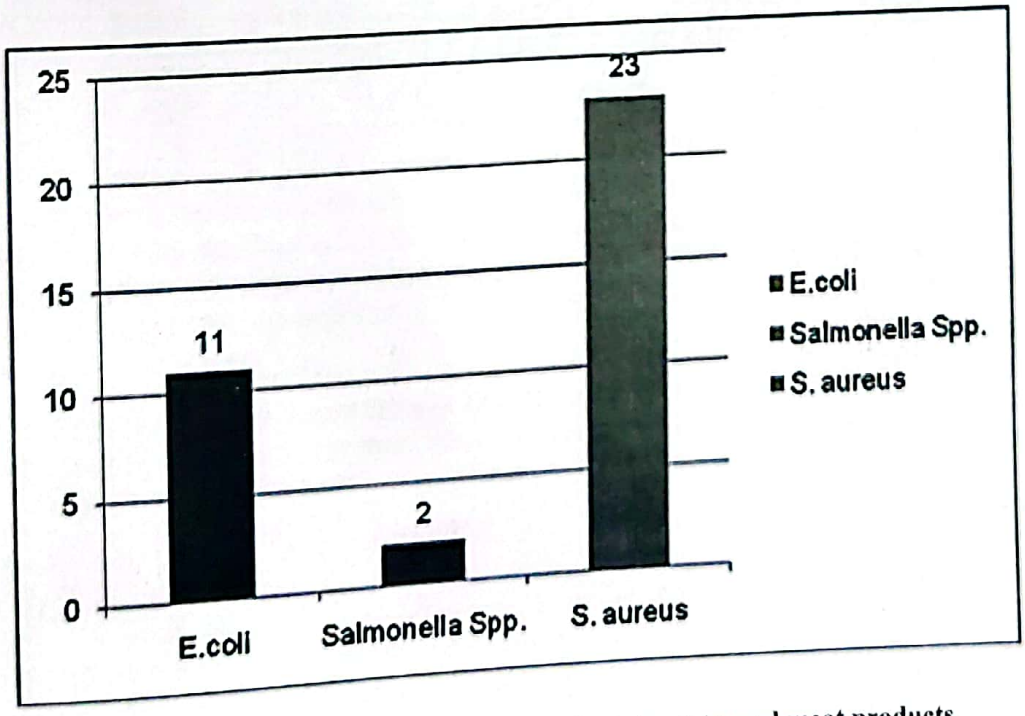

Fig. (1) : Types and Numbers of isolated bacteria from the returned meat products.

Discussion

Technological development in meat processing, preservation and handling has given the consumers a much greater choice over foods they can buy but also at the same time meat products are considered a major source of food born pathogens that cause food poisoning in humans and the major vehicle of most reported outbreaks. Returned met products can give full picture about the history of the product and its journey from gate to plate and play important role in food poisoning process.

In the present work, meat and poultry returned products were examined to detect the total bacterial colony count and the incidence of *E. coli* O157: H7, *Salmonella* spp. and *S. aureus* to evaluate the products. 135 samples of different 9 meat products each 15 samples of chicken burger, beef burger, frozen hotdog, chilled hotdog, frozen oriental sausage, beef luncheon, beef kofta, smoked turkey and minced meat including different 4 brand names of products which were collected from returned products from market and from stores distributed in Cairo .

As regard to the results recorded in Table (1) that the total aerobic plate count for the examined Beef burger samples ranged from 8×10^4 to 6.4×10^6 with a mean value of 2×10^6 organisms per gram, oriental sausage samples ranged from 3×10^4 to 4×10^8 with a mean value of 2.1×10^6 organisms per gram. Nearly similar results were obtained by El-Mossalami (2003) (9.3×10^6). While lower mean results were recorded by Ouf (2001) (6×10^5). In luncheon beef samples they ranged from 1.2×10^3 to 3.1×10^6 with a mean value of 1×10^6 organisms per gram, minced meat samples ranged from 6×10^3 to 6.4×10^6 with a mean value of 1.1×10^6 organisms per gram, beef kofta ranged from 1.1×10^4 to 8×10^8 with a mean value of 2.1×10^6 organisms per gram. It was noticed that the higher mean was obtained by El-Mossalami (2003) (3.2×10^7) and lower means was recorded by Tolba (1994) (2.9×10^5) and Hassan (2001) (2.1×10^4), frozen hotdog ranged from 2×10^4 to 1×10^7 with a mean value of 6×10^4 organisms per gram, chilled hotdog ranged from 2×10^4 to 2.5×10^8 with a mean value of 1×10^5 organisms per gram, chicken burger ranged from 3×10^4 to 9×10^6 with a mean value of 5×10^6 organisms per gram, smoked turkey ranged from 3×10^2 to 2×10^8 with a mean value of 1×10^5 organisms per gram.

The results recorded in table (2) and Fig (1) showed that the incidence of isolation of *S. aureus*, *Escherichia coli* O157 : H7 and Salmonella Spp. were 63.9% , 30.6% and 5.4% respectively.

The native habitat for *E. coli* is intestinal tract of man and animals. Thus its presence in foods generally indicates direct or indirect pollution of fecal origin (ICMSF, 1978). Meat products may be contaminated with *E. coli* from food handlers, food utensils, air, soil and water under incomplete hygienic circumstances during manufacturing packaging and marketing of these products (Frazier and Westhoff, 1988).

Presence of bacterial contamination in meat products was reported by Stampi et al. (2004) who stated that *E. coli* was detected in 45 (30.2%) of 145 samples examined, mainly in the hamburger samples mixed with vegetables and in the minced beef while *E. coli* O157 was found in one sample of hamburger collected

from different three butcher stores. From 77 examined samples of ground beef, *E. coli* was found in 0.51 log CFU/g (Le Jeune and Christie, 2004) .

Also isolation of *E. coli* O157 from meat has been reported by Dontorou et al. (2003). While Le Jeune and Christie, (2004) failed to isolate *E. coli* O157. Isolation of *E. coli* O157 :H7 from beef burger 6% from 18% positive samples and from kofta 4% from 12% positive samples by Fayed (2006) but he failed to isolate it from oriental sausage.

The incidence of *E. coli* O157:H7 in the examined beef burger samples was higher than the findings recorded by Chapman et al. (2000) (3.7%); Fayed (2006) (6%) and lower than results obtained by Saleh (2001) (35.7%); Ouf (2001) (31.3%). Dontorou et al. (2003) could not isolate *E. coli* O157:H7 from beef burger samples in north western Greece. The variation in the results obtained by different investigators may be due to differences in manufacturing practices, handling and difference in time of exposure.

The presence of *E. coli* O175: H7 may be due to poor labor hygiene, worker's hands contaminated with stool then handle food directly.

The present study also indicates that the incidence of *E. coli* O157: H7 in sausage 18.2% which is higher than the results recorded by Chapman et al. (2000) (4.1%); Chinen et al (2001) (4.8%); Dontorou et al. (2003) (1.3%) and Fayed (2006) failed to isolate *E. coli* O157: H7.

This high results may be due to bad handling during processing and significant contamination of hands and equipments.

The current study failed to isolate *E. coli* from luncheon samples which is agreed with those resulted by Sayed et al. (2001); Ouf (2001) results. That may be due to using excessive amount of preservatives exceed the allowed limit in low grade factories and also due to that luncheon process include cooking and exposure to high temperature.

The minced meat in the present study recorded 18.2% *E. Coli* O157 :H7 in examined samples which is higher than results recorded by Chapman et al. (2000) (1.1%); Chinen et al. (2001) (3.8%); Sayed et al. (2001) (4%); Kassam and Sabry (2003) (3.3%); Magwira et al. (2005) (5.22%); Samadpour et al. (2006) (1.1%) and Fayed (2006) (2%) but, Fantelli and Stephan (2001), Heredia et al. (2001), Uihtil et al. (2001) and Le Jeune and Christie (2004) failed to isolate *E. coli* O157: H7

The current study detected *E. coli* O157: H7 from beef burger and kofta by 9.1% which higher than result obtained by Fayed (2006) (4%).

Among the salmonellae the prevalence rate was 20% for the presence salmonella in beef burger by Mrema et al. (2006). But Ismail *(2008)* could not detect it in the examined samples.

In oriental sausage the isolated salmonella results recorded by Abrahim et al. (1998) were 20% and Casalinuovo et al. (2001) were 13.3%.

In minced meat White et al. (2001) were 20% ; Zhao et al. (2002) were 3.5% ; Malkawi (2003) were 87% and Rahimifard et al. (2008) were 9.5%.

The presence of *S.* Typhimuirium in meat products indicates human origin and therefore poor personal hygiene during handling of meat products. Phillips et al. (2008) reported that incidence of *Salmonella* Typhimurium was 1.1% out of 360 minced meat samples.

The results revealed that salmonellae could be isolated from smoked cooked turkey constituted (5.4 %) represented as *S.* Typhimuirium and *S.* Kentucky. But faild to isolate salmonellae in the other samples.

Bryan and Doyle (1995) stated that salmonellosis cases were caused by poultry meat with 6.6% from turkey and Mulder (1999) said that chicken products are documented as significant reservoir for salmonella and Altabari and Al-Dughaym (2002) recorded that poultry ranks first as cause in food poisoning with incidence of 92.32% and were mainly salmonellae with an incidence of 8.99% which nearly agreed with the results, as salmonella detected in poultry products (smoked cooked turkey) only. But in chicken burger, its results agreed with results of Siriken and Pamuk (2005) as they faild to isolate *Salmonella* spp.

The low incidence of salmonella detected in the local products as compared to the findings of other investigators may be attributed to the excessive amount of preservatives usually used illegally in some unlicensed and low grade factories.

Presence of *S. aureus* in beef burger in the present study constituted 4.34% with mean 15×10^3 which is similar with the results reported by El-Mossalami (2003) who found that the mean counts were 9×10^2 , Eleiwa (2003) recorded count ranging from 3.5×10^3 to 17.2×10^3 with an average number of $8.95 \times 10^3 \pm 0.95 \times 10^3$ which is lower than percentage stated by Abd El-Monem (1998) (15%) and Hassan (2001) (12%), and higher than the results recorded by Saleh and Abu-Khadra (2010) who detected a mean staphylococcal count in samples $2.17 \times 10^3 \pm 4.31 \times 10^2$ and an incidence of 36%.

The *S. aureus* failed to be isolated from examined sausage samples in the present study which agreed with Khalafalla (1996) but Casalinuovo et al. (2001) (10%) and Saleh et al. (2010) (40%) found the mean count was $2.20 \times 10^3 \pm 4.54 \times 10^2$. The failure in isolation of *S. aureus* from the Egyptian oriental sausage may be due to the excessive amount of the additives were added.

In current study incidence of *S. aureus* in minced meat was 39.13% with mean value of 9.1×10^2 which neary agreed with results recorded by Eleiwa (2003) who found that it ranged from 2.8×10^3 to 12.8×10^3 with an average of $7.45 \times 10^3 \pm 0.63 \times 10^3$ and nearly agreed with the results of Manfreda et al. (2005) who recorded a lower level of 34.6%.

In the present study, results showed that the incedince of *S. aureus* in the examined chicken burger was 13.04% with a mean value of 11×10^3.

The decision concerning returned products according to many factors. For examples, If the returned is from the end of production line or internal stores they under goes microbiological exams and chemical tests to ensure that it is fit for

human use, if it passed so it can be sold for labors with discount. But if the returned products get from out stores or the market (retails) they are examined for detecting the reason behind returning specially in case of high amount, the company form a committee to study the case and results in corrective actions to be added. Concerning the returned products in this case it is condemned under the supervising of quality, security and ministry of health representative.

The microbiological judgments in Egypt according to the International Standardization Organization (ISO) limits. Total bacterial count ISO 4833/ 2003 the products are not allowed to used by consumers in case of Cooked meat products $>10^4$ and for frozen products $> 10^6$. Concerning ISO 16649/2/2001 for *E. coli* , It should be Nil/g . About ISO 6579/2002 for salmonella it must be Nil/25 g as acceptable limit to be allowed for consumers and ISO 6888/1/2003 put a limits for *S. aureus* for cooked product should be Nil/g but for frozen products it must not exceed than 100/gm.

Returned products are a picture for the life time of the products from gate to plate , i recommend to give it more importance from the companies as it is need to be studied more to know how the products are handled inside the factory and also out by retails, to check about the hygiene status inside the planet, to discover problems and defects and to improve the products.

Conclusion

It is very important to study the returned to check about the hygiene statuse inside the plant, to discover problems and defects and to improve the products. In the examined meat and poultry returned products the incidence of isolation of *E.coli* O157 :H7, *S.* Typhimuirium , *S.* Kentucky and *S. aureus* were 30.6%, 2.7%, 2.7% and 63.9%, respectively.

The high results in current study may be due to poor hygienic practicing during process and the bad conditions of handling in market specially that minced meat as product not pass through a lot of control points and not exposure to any high temperature or cooking as in some other products. Frozen products have high count of *E. coli* O157 : H7 (frozen hot dog 18.2% and chicken burger 27.2%) which may be due to the direct contact with labors hands and also the bad sanitation in some low grade factories also frozen products during their process not include cooking which make it more exposure for contamination and growing of the organism in the products. But in chilled hot dog and smoked cooked turkey E. coli O157: H7 failed .to be isolated.

The transmission and survival of enteric pathogens in the food processing and preparation environment through human and raw food source. Pathogens in the food preparation area can originated from infected food workers, raw food or other environmental resources. The most frequent means of worker contamination is the fecal-oral route, and study results have indicated that toilet paper may not stop transmission of pathogens to hands.

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البكتريا المرتبطة ببعض مرتجعات منتجات اللحوم

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[1] أستاذ الرقابة الصحية على اللحوم و وكيل الكلية لشئون خدمة البيئة و المجتمع كلية الطب البيطري جامعة القاهرة ، [2] أستاذ الميكروبيولوجي كلية الطب البيطري جامعة القاهرة

تم جمع عدد 135 عينة من بعض مرتجعات منتجات اللحوم الحمراء و الدواجن لدراسة مدي تواجد وإنتشار بكتريا التلوث وخاصة بكتريا الايشريشيا كولاي و السالمونيلا والاستافيلوكوكس أوريس مع التصنيف لهذه البكتريات باستخدام الوسائل التقليدية ودراسة مصادر تلوث هذه المنتجات سواء خلال العملية الإنتاجية أو بعدها بالتعرض للميكروب نتيجة عيوب التغليف أو التعامل السيء مع المنتجات وكانت النتائج كالتالي:

كانت نسبة عزل بكتريا الايشريشيا كولاي : ١، ٩% من البيف برجر والكفتة كلٍ على حده ،٢%، ١٨من السجق الشرقي واللحمة المفرومة و الهوت دوج المجمد كلٍ علي حده ٢،٢٧% من البرجر الدجاج.

ولم يتم عزل بكتريا الايشريشيا كولاي من اللانشون البيف والهوت دوج المبرد و اللحم الرومي المدخن من الملاحظ أن قد تم عزل بكتريا الأيشريشيا كولاي من ٦ منتجات مختلفة من أصل عدد ٩ منتجات و جميعهم مجمدات ولم يتم عزل بكتريا الايشريشيا كولاي من المنتجات المبردة و أعلى نسبة كانت من بيرجر الدجاج وقد يرجع ذلك لتعرض المنتجات للتلوث أثناء العملية الإنتاجية و خاصة أن المنتجات المعزول منها جميعها لا تتعرض للحرارة.

لم يتم عزل ميكروب السالمونيلا من جميع المرتجعات فيما عدا منتج لحم رومي مدخن حيث قد تم عزل و تصنيف سالمونيللا تايفيموريم و سالمونيللا كنتاكي منه.

وكانت نسبة عزل بكتريا الستاف أوريس :

٤,٤% من البيف برجر و الهوت دوج المبرد كلٍ علي حده، و ١٣% من اللحم المفروم و الهوت دوج المجمد والبرجر دجاج واللحم الرومي المدخن كلٍ على حده، و ٣٩,٢ % من لانشون البيف.

ولم يتم عزل بكتريا الاستافيلوكوكس أوريس من الكفتة البقري و السجق الشرقي .ولكن لوحظ أن أعلي نسبة لبكتريا الستافيلوكوكس أوريس سجلت في منتج اللانشون البقري بالرغم من أن المنتج مطهي ومعرض لدرجات الحرارة و لكن قد يرجع ذلك لتلوث المنتجات بعد العملية الإنتاجية.