

Dept. of Food Hygiene,  
Faculty Vet. Med., Assiut University,  
Head of Dept. Prof. Dr. H. Youssef.

**COLIFORMS, ENTEROBACTERIACEAE AND TOTAL  
AEROBIC MESOPHILIC COUNTS IN SOME  
SELECTED MEAT PRODUCTS**  
(With 2 Tables)

By

**SH.M. FATHI and M.R.A. RASHWAN\***  
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الكوليفورم ، الميكروبات المعدية والميكروبات الحبيبة  
للتنمو على درجة حرارة معتدلة في بعض منتجات اللحوم

شركت فتحي ، محمد رشوان

شمل البحث على الفحص البكتريولوجي لعدد ٤٠ عينة مختلفة من منتجات اللحوم بواقع ١٥ عينة من كل من البرجر المجمد ، السجق المجمد و ١٠ عينات من اللانشون تم شراؤها من محلات السوبر ماركت المحلية بمدينة أسيوط لتعيين الكوليفورم والميكروبات المعدية والميكروبات التي تنمو على درجة حرارة معتدلة وذلك للوقوف على الحالة الصحية لمثل هذه المنتجات لأن هذه الميكروبات تعتبر مؤشراً لذلك . تم تعيين العدد الكلي للكوليفورم والميكروبات المعدية بواسطة طريقتين للأطباق وتغطية سطحها بواسطة فايوليت . رد بيل آجر (V.R.B.A) على التوالي . وكان متوسط العدد الكلي لميكروبات الكوليفورم ، الميكروبات المعدية بواسطة صب وتغطية الآجر ١٦ × ٩٠ و ١١ × ٩٠ ، ١٢ × ٩٠ و ٢٢ × ٩٠ و ٢٢ × ٩٠ و ٣٤ × ٩٠ / جرام لكامل عينات البرجر المجمد ، السجق والانشون على التوالي . في حين كان ٥٩ × ٩٠ و ٦٦ × ٩٠ ، ٦٠ × ٩٠ و ١١ × ٩٠ و ١٧ × ٩٠ و ٨١ × ٩٠ / جرام من العينات المفحوصة باستخدام زرع الميكروبات على سطح البيئات المذكورة . كما بين البحث أن زرع الميكروبات على سطح البيئات أعلى منها على أسطح البيئات المغطاة ، وبالنسبة لمتوسطات الميكروبات الهوائية والتي تنمو على درجة حرارة معتدلة كانت ٦٦ × ٩٠ ، ٢٧ × ٩٠ ، ١١ × ٩٠ / جرام من عينات البرجر المجمد ، السجق المجمد ، اللانشون على التوالي . وبناء على النتائج السابقة يمكن التوصية باستخدام طريقة الصب والتغطية بالآجر في الأغذية ذات الإصل الحيواني . التي يتوقع فيها أعداد كبيرة من الميكروبات المعدية بينما تعتبر طريقة زرع الميكروبات على سطح البيئات مناسبة في حالة الكوليفورم . كما وجد هناك فروق كبيرة في الفحص البكتريولوجي بين مختلف المنتجين لمثل هذه المنتجات ولذلك ينصح بمزيد من الفحص البكتريولوجي بالأخص في مصانع الإنتاج ومن خلال مراحل التصنيع المختلفة لضمان السلامة الصحية .

\* Dept. of Food Science & Technology, Faculty of Agriculture, Assiut University.

### SUMMARY

A total of 40 meat products samples, 15 each from frozen burger and frozen sausage and 10 luncheon samples were purchased from local retail markets and supermarkets in Assiut city for determination of coliforms, Enterobacteriaceae and total aerobic mesophilic bacteria populations. The enumeration of coliforms and Enterobacteriaceae was performed by two methods, pour plate with an overlay and surface plating of violet red bile agar (VRBA) and violet red bile glucose agar (VRBG) respectively. The mean values of coliforms and Enterobacteriaceae counts by pour plate with overlay method were  $16 \times 10^5$  and  $11 \times 10^5$ ,  $12 \times 10^5$  and  $22 \times 10^5$  and  $32 \times 10^5$  and  $34 \times 10^5$  per gram examined frozen burger, frozen sausage and luncheon respectively, while they were  $59 \times 10^5$  and  $66 \times 10^5$ ,  $60 \times 10^5$  and  $11 \times 10^6$  and  $97 \times 10^5$  and  $81 \times 10^5$  per gram examined sample by surface plating method respectively. It is evident that surface counts of both coliforms and Enterobacteriaceae were significantly higher than the overlay counts, where the pour plate with an overlay method is recommended in foods of animal origin with expected high Enterobacteriaceae level, while surface plating method is suitable in case of coliforms enumeration. The aerobic mesophilic bacteria mean counts were  $66 \times 10^6$ ,  $27 \times 10^6$  and  $11 \times 10^6$  per gram examined frozen burger, frozen sausage and luncheon samples respectively. The presence of numerous mesophilic bacteria often indicate contaminated raw materials or unsatisfactory processing from a sanitary point of view. Finally, it was found wide variation in microbiological quality of the examined meat products between different manufacturers.

### INTRODUCTION

The bacteriological quality of meat products has been of interest to a number of investigators in different geographical areas and resulted in the publication of several papers. In this respect, ROGERS and McCLESKEY (1957) found that 16 out of 24 samples of ground beef had a count more than  $10^7$  organisms per gram, and upon examination of 59 samples collected by a local inspector, 20 samples counted in excess of  $10^7$  organisms/g. and 33 over  $10^8$  organisms/g. SADEK (1963) recorded  $2 \times 10^{10}$  organisms/g examined raw sausage. In (1977) DUISCHAEVER and DUISCHAEVER *et al.* reported that 46.5% and about 19% of examined luncheon meat and frozen hamburger patties had total aerobic plate counts in excess of  $5 \times 10^6$ /g. and 10 million/g. examined sample respectively, while FOSTER *et al.* (1977)

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found that the range of aerobic plate count in raw ground beef was from  $6.9 \times 10^4$  to  $8.3 \times 10^7$  organisms per gram, and KARIM (1977) evaluated that 13% and 22% of raw hamburger had range of aerobic plate and coliform counts from  $10 \times 10^5$  to  $10 \times 10^6$  and from  $10 \times 10^5$  to  $10 \times 10^4$  /g. of examined sample respectively.

PIERSON *et al.* (1978) reported that the mean coliform counts in retail ground beef by using violet red bile surface-overlay plate and pour plate methods were  $3.97 \times 10^4$  and  $3.52 \times 10^4$  per gram examined sample respectively, whereas, OBLINGER and JR (1978) found that mean of total aerobic plate and total coliforms counts was  $2.3 \times 10^4$  and  $1.8 \times 10^4$  /g examined imported frozen lean beef pieces respectively. The total colony and Enterobacteriaceae counts in raw hamburger were more than  $10^8$  /g and  $10^7$  /g (TAMINAGA *et al.*, 1980);  $29 \times 10^7$  /g and  $17.8 \times 10^4$  /g (IBRAHIM, 1981), respectively. SRIVASTAVA *et al.* (1981) found that the average of total aerobic and coliforms counts from 24 samples of freshly bought frozen goat meat were  $8.3 \times 10^6$  and  $7.6 \times 10^7$  /g examined sample, respectively, whereas TIWARI and KADIS (1981) pointed out that geometric means of aerobic plate counts/g of examined beef luncheon, summer sausage and beef salami were  $4 \times 10^5$ ,  $15 \times 10^5$  and  $44 \times 10^5$ , respectively. MATES (1983) showed that the range of aerobic plate counts/g frozen ground meat was from  $10 \times 10^6$  to  $50 \times 10^6$  in examined 76 samples, while STEWART (1983) found that the geometric means of aerobic plate counts were  $1.0 \times 10^5$  and  $1.6 \times 10^5$  /g of examined loose and encased sausage, respectively, whereas, DARWISH *et al.* (1986) reported that the mean total colony counts of minced meat and beef burger were  $1.8 \times 10^6$  and  $5.3 \times 10^6$  /g, while the mean Enterobacteriaceae counts were  $1.1 \times 10^3$  and  $4.4 \times 10^3$  /g examined sample, respectively. SCRIVEN and SINGH (1986) recorded that the mean total plate count was  $5.0 \times 10^7$  per gram examined minced beef sample.

HAMDY and NOUMAN (1986) evaluated that the average count of Enterobacteriaceae by surface spread method with overlay and without overlay was  $3.66 \times 10^4$  and  $3.87 \times 10^4$  /g examined frozen meat cuts, respectively. LOTFI *et al.* (1986) noticed the Enterobacteriaceae count in manufactured raw meat, sausage and basterma with mean values  $9 \times 10^4$ ,  $9.1 \times 10^4$  and  $3.2 \times 10^4$  /g, respectively, while ABDEL-RAHMAN and EL-KHATEIB (1987) found the means of coliforms, Enterobacteriaceae and aerobic plate counts were  $5 \times 10^2$ ,  $2 \times 10^6$  and  $6 \times 10^7$  /g examined frozen ground beef respectively.

ELMOSSALAMI *et al.* (1988) pointed out that the total aerobic and Enterobacteriaceae mean counts in meat collected from open weekly markets and village butcher shops were  $17.807 \times 10^6$  and  $0.919 \times 10^6$ , and  $0.969 \times 10^6$  and  $0.049 \times 10^6$  per gram respectively, while KHALAFALA *et al.* (1989) found that mean values of Enterobacteriaceae count were  $10^3$ ,  $2 \times 10^4$ ,  $5 \times 10^5$  and  $8 \times 10^5$  organisms/g. of examined heart, liver, rumen and intestine respectively.

The present study reports the results of a comparative study of relative numbers of coliforms, Enterobacteriaceae by pour plate with overlay and surface plating, and total aerobic mesophilic bacteria count to evaluate the bacteriological quality of some selected meat products.

## **MATERIAL and METHODS**

### **Collection of samples**

Forty samples (about 500 g each) of different selected meat products, 15 each from frozen burger and frozen sausage and 10 from luncheon samples were collected and obtained from different local retailers and supermarkets in Assiut city and transported directly to the laboratory as soon as possible for enumeration of coliforms, Enterobacteriaceae and total aerobic mesophilic bacteria, where the subjected methods were recommended by ICMSF (1978), ISO (1979) and MURTHY (1984), and the ingredients were supplied by Oxoid, U.K.

### **Preparation of samples**

Twenty-five grams of each sample were blended in 225 ml of buffered peptone water for 1 min. in a pre-sterilized blender. Individual serial decimal dilutions were prepared in 90 ml volumes of buffered peptone water up to a  $1/10^6$  dilution of the original sample.

### **Coliforms and Enterobacteriaceae plate count:**

#### **a. By overlay method:**

Duplicate 1 ml volumes of suitable dilutions were dispensed in Petri dishes and ca. 15 ml of melted violet red bile agar (VRBA) in case of coliforms and violet red bile glucose agar (VRBG) for Enterobacteriaceae were added with thorough mixing. After solidification, the plates were overlaid with a second layer of 15 ml of VRBA and VRBG respectively.

#### **b. By surface plating method:**

Petri dishes containing ca. 20 ml of VRBA and VRBG were prepared and dried. Duplicate 0.1 ml volumes of each suitable dilution were placed on the surface of the agar medium and spread with glass spreaders.

### **Total aerobic mesophilic count (TAMC):**

0.1 ml volumes of suitable dilutions were spread onto the surface of the pre-poured and dried plates containing standard plate count agar.

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### Incubation and counting:

Plates were incubated at 35°C for 18-24. in case of plates containing selective media and for 48 h. in case of TAMC plates. On selective media, the number of red or purple colonies with a diameter 7 0.5 mm surrounded by a zone of precipitated bile were counted. Colonies judged to be border-line were also counted.

### Statistical analysis:

Data were analysed statistically for means, standard error and T test between two applied methods.

## RESULTS

The results of the present study were recorded in Tables (1 & 2).

## DISCUSSION

In fresh foods of animal origin, most Enterobacteriaceae stem from fecal contamination, their occurrence in high numbers may indicate insanitary handling and/or inadequate storage (HECHELMANN *et al.*, 1973; HUNYADY *et al.*, 1973 and COX *et al.*, 1975). Furthermore, the presence of Enterobacteriaceae indicates microbiological proliferation when allow multiplication of pahogenic and toxigenic bacteria in meat and lead to public health hazard (ICMSF, 1978).

The obtained results in table (1) revealed that the means coliform and Enterobacteriaceae count were  $16 \times 10^5$  and  $11 \times 10^5$  /g by overlay method and  $59 \times 10^5$  and  $66 \times 10^5$  /g by surface plating method in case of examined frozen burger, respectively.

Similar observations were obtained by TAMINAGA *et al.* (1980); IBRAHIM (1981); LOTFI *et al.* (1986) and ABDEL-RAHMAN & EL-KHATEIB (1987), while lower results were recorded by PIERSON *et al.* (1978) and HAMDY & NOUMAN (1986).

In addition to coliforms and Enterobacteriaceae count, the total aerobic bacterial count is believed to provide as much information as any other microbiological index in the routine monitoring of foods (MISSIKIMI *et al.*, 1976). The presence of numerous mesophilic bacteria which grow readily at or near body temperatur often indicated contaminated raw materials or unsatisfactory processing could facilitate the recognized food-borne pathogenic bacteria (ICMSF, 1978).

It is evident from Table (1) also that the mean total aerobic mesophilic bacterial counts in examined frozen burger, frozen sausage and luncheon were  $66 \times 10^6$ ,  $27 \times 10^6$  and  $11 \times 10^6$  /g, respectively. Similar findings were recorded by DUITSCHAEVER *et*

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al. (1977); IBRAHIM (1981); DARWISH *et al.* (1986) and ABDEL-RAHMAN & EL-KHATEIB (1987), while higher finding was reported by TAMINAGA *et al.* (1980).

As regarded to sausage samples, higher results were recorded by SADEK (1963) who showed that the total colony counts was  $2 \times 10^{10}$  /g examined raw sausage and lower results obtained by TIWARI and KADIS (1981) who revealed that the geometric means were  $15 \times 10^5$  and  $44 \times 10^5$  /g summer sausage and salami, respectively, while similar results were recorded by STEWART (1983) who found that the geometric mean was  $1.0 \times 10^7$  /g loose sausage.

Concerning luncheon samples the obtained results were higher than the results reported by DUITSCHEVER (1977) and TIWARI & KADIS (1981) who pointed out that 46.5% had total aerobic count in excess of  $5 \times 10^6$  per gram luncheon meat and geometric mean was  $4 \times 10^5$  /g examined beef luncheon, respectively.

The present results pointed that high level of coliforms and Enterobacteriaceae count and wide variation in total aerobic mesophilic counts in the examined meat products between different manufacturers because the source of Enterobacteriaceae in meats was associated with the meathandling work surface in the packing plants (STILES and NG, 1981). The addition of spices to meat products lead to a marked increase in the bacterial population including aerobic and coliform count (HEFNAWY and YOUSSEF, 1984), besides to contaminated raw materials, unsatisfactory processing, neglected and/or inadequate sanitary measures during slaughtering, evisceration, transportation, uncleaned equipment or improper handling.

It is concluded that surface counts of both coliforms and Enterobacteriaceae were significantly higher than the overlay counts in case of examined frozen sausage and frozen burger, which in acceptance to results obtained by MURTHY (1984), where the pour plate with an overlay method is recommended in foods of animal origin with expected high Enterobacteriaceae level, while surface plating method is suitable in case of coliforms enumeration (ISO, 1979 and HAMDY & NOUMAN, 1986).

Moreover, the results obtained allowed to conclude that the microbiological examination was useful as it gives the first aid in judging the fitness of the product. It is important to measure Enterobacteriaceae count, coliform count and total aerobic mesophilic count in meat products as to control the sanitary conditions under which the product had been produced and handled.

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Table (1): Statistical analytical results of coliform, enterobacteriaceae and total aerobic mesophilic counts/g. examined sample.

Type of sample	No. of samples	Results	Coliform count		Enterobacteriaceae count		TAMC
			overlay method	surface plating	overlay method	surface plating	
Frozen burger	15	Min.	45x10 <sup>3</sup>	30x10 <sup>4</sup>	35x10 <sup>3</sup>	13x10 <sup>4</sup>	14x10 <sup>5</sup>
		Max.	60x10 <sup>5</sup>	28x10 <sup>6</sup>	33x10 <sup>5</sup>	30x10 <sup>6</sup>	40x10 <sup>7</sup>
		Mean	16x10 <sup>5</sup>	59x10 <sup>5</sup>	11x10 <sup>5</sup>	66x10 <sup>5</sup>	66x10 <sup>6</sup>
		S.E.	±48x10 <sup>4</sup>	±19x10 <sup>5</sup> *	±30x10 <sup>4</sup>	±21x10 <sup>5</sup> **	±26x10 <sup>6</sup>
Frozen sausage	15	Min.	10x10 <sup>2</sup>	10x10 <sup>3</sup>	20x10 <sup>2</sup>	10x10 <sup>3</sup>	30x10 <sup>4</sup>
		Max.	80x10 <sup>5</sup>	28x10 <sup>6</sup>	14x10 <sup>6</sup>	11x10 <sup>7</sup>	15x10 <sup>7</sup>
		Mean	12x10 <sup>5</sup>	60x10 <sup>5</sup>	22x10 <sup>5</sup>	11x10 <sup>6</sup>	27x10 <sup>6</sup>
		S.E.	±58x10 <sup>4</sup>	±23x10 <sup>5</sup> **	±11x10 <sup>5</sup>	±73x10 <sup>5</sup>	±98x10 <sup>5</sup>
Luncheon	10	Min.	0	0	10x10 <sup>2</sup>	10x10 <sup>2</sup>	30x10 <sup>4</sup>
		Max.	96x10 <sup>5</sup>	47x10 <sup>6</sup>	92x10 <sup>5</sup>	35x10 <sup>6</sup>	80x10 <sup>7</sup>
		Mean	32x10 <sup>5</sup>	97x10 <sup>5</sup>	34x10 <sup>5</sup>	81x10 <sup>5</sup>	11x10 <sup>7</sup>
		S.E.	±11x10 <sup>5</sup>	±45x10 <sup>5</sup>	±11x10 <sup>5</sup>	±38x10 <sup>5</sup>	±78x10 <sup>6</sup>

\* Means are significantly different (P < 0.05).

\*\* Means are highly significantly different (P < 0.01)

TAMC: Total aerobic mesophilic count.

Table (2): Distribution of analytical results of coliforms and Enterobacteriaceae counts/g. examined sample by overlay & surface plating methods.

Type of sample	Range	Coliforms				Enterobacteriaceae			
		Overlay		Surface		Overlay		Surface	
		No.	%	No.	%	No.	%	No.	%
Frozen burger	10 <sup>2</sup> - 10 <sup>3</sup>	3	20	0	0	2	13.3	0	0
	10 <sup>4</sup> - 10 <sup>5</sup>	12	80	12	80	13	86.7	11	73.3
	10 <sup>6</sup> - 10 <sup>7</sup>	0	0	3	20	0	0	4	26.7
Frozen sausage	10 <sup>2</sup> - 10 <sup>3</sup>	6	40	3	20	7	46.67	3	20.0
	10 <sup>4</sup> - 10 <sup>5</sup>	9	60	8	53.3	7	46.67	9	60.0
	10 <sup>6</sup> - 10 <sup>7</sup>	0	0	4	26.7	1	6.66	3	20.0
Luncheon	0 - 10 <sup>1</sup>	1	10	1	10.0	0	0	0	0
	10 <sup>2</sup> - 10 <sup>3</sup>	1	10	1	10.0	3	30.0	2	20.0
	10 <sup>4</sup> - 10 <sup>5</sup>	8	80	5	50.0	7	70.0	6	60.0
	10 <sup>6</sup> - 10 <sup>7</sup>	0	0	3	30.0	0	0	2	20.0