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

DETERMINATION OF COLIFORMS AND ESCHERICHIA COLI IN SOME MEAT PRODUCTS USING MOST PROBABLE NUMBER TECHNIQUE

(With 4 Tables)

By

SH.M. FATHI; M.R.A. RASHWAN* and S.I. EL-SYIAD*

(Received at 25/7/1992)

تقدير ميكروب الكوليفورم والايشاريشياكولاي في بعض منتجات اللحوم باستخدام طريقة العد الاحتمالي الاجمالي

شوكت فتحي ، محمد رشوان ، سامي الصياد

الكشف عن تواجد ميكروب الكوليفورم والايشاريشيا كولاي عند تحليل ٤٧ عينه من بعض منتجات اللحوم الممتازه تم تقديرها باستخدام طريقة العد الاحتمالي الاجمالي ممثله لعدد ١٩ بيف برجر ، ١٦٠ عينة سجق ، ١٢٠ عينة لانشون . ثبت تواجد ميكروب الكوليفورم في جميع العينات المفحوصه بمتوسطات عدديه ٩٥ر١٠٠٩٥ ، ٣٨ر٧٦٦٥ ، ٩٣ر٧٤٠٩ / جرام من كل عينه مفحوصه بينما تمكن الكشف عن تواجد اليشاريشياكولاي في ٩(٣٧ر٤٧٪) ، ٤(٢٥٪) ، ٥(٦٧ر٤١٪) من عينات البيف برجر ، السجق ، اللانشون على التوالي بمتوسطات عدديه ٢٥ر٦١٧ ، ٤٤ر٧٠٣ ، ٦٨ر١٨/جرام من العينات التي تم فحصها على التوالي . وننصح بطريقة العد الاحتمالي الاجمالي لبعض المنتجات الغذائيه خاصة عندما تكون مكوناتها الطبيعيه يصعب تقديرها بواسطة طريقة العد القياسي النمطي أو عندما يكون ما تحويه العينه أقل من ١٠ ميكروبات المكونه للتجمعات البكتيريه لكل جرام ، ويعزي تواجد الكوليفورم في جميع العينات الى تلوث اللحوم أثناء تصنيعها الى منتجات بالاضافه الى أن من منتجات اللحوم تكون عرضه للتلوث بميكروب الايشاريشياكولاي من عمال التصنيع ، الأدوات ، الهواء ، التربه والماء بطرق

* Food Science and Technology Dept., Fac. of Agriculture.

غير صحيه ، ولهذا يجب مراعاة اتباع الطرق الصحيه والمواصفات القياسيه والاشراف على مصانع منتجات اللحوم على فترات متلاحقه أثناء التجهيز وتصنيع منتجات اللحوم .

SUMMARY

Recovery of coliforms and Escherichia coli from some selected meat products were determined using Most Probable Number (MPN) technique. A total number of 47 different meat products samples (19 beefburger, 16 sausage and 12 luncheon) were analysed. Coliforms were found in all examined samples with average counts of 10095.95, 7665.38 and 7409.93/g examined beefburger, sausage and luncheon samples, while E.coli were detected in 9(47.37%), 4(25%) and 5(41.67%) out of examined samples with average counts of 617.25, 703.44 and 18.68/g examined sample respectively. MPN technique is recommended for many food products particularly when the compositional nature of the sample makes it difficult to use standard plating procedures and when the microbial density of the sample is less than 10 organisms or colony forming units (CFU) per gram. The presence of coliforms in all samples is attributed to contamination of raw meat used for manufacture of such products. In addition, meat products may be also contaminated with E.coli from food handlers, food utensils, air, soil and water under incomplete hygienic circumstances during manufacturing, packing and marketing of these products. So, it is very important to control the hygienic measures periodically in such factories particularly during preparation and manufacturing of these meat products.

INTRODUCTION

The Most Probable Number (MPN) technique is a means of estimating the density of viable organisms particularly when found in less than 10 organisms or colony forming units (CFU) per gram in a sample. It is based on probability statistics and the results from any type of an MPN analysis are directly related to the frequency of occurrence of a series of positive results that are most likely to occur when given numbers of organisms are present in a sample (OBLINGER and KOBURGER, 1984). It was found to be

preferable to the MPN technique for enumeration of E.coli in raw meats, because of lower variability, better recovery of E.coli from frozen meats, rapidity and decreased cost of analysis (STILES and NG, 1980).

The presence of coliforms in meat is frequently a reliable indicator for faulty method of slaughtering carcases, preparation and handling. Such meat contamination with coliforms by several ways induces undesirable changes and economic losses of meat. Moreover, contamination with great number may be associated with increasing the number of E.coli and consequently may constitute the public health hazards (LIBBY, 1975 and ICMSF, 1980).

In fresh sausage, SADEK (1963) found that the coliform count was 10^2 /g, whereas, ABDEL-AZIZ (1979) reported that all examined 50 fresh sausage samples were positive for coliform and E.coli with an average of $11x10^4$ and $4x10^2$ organisms/g examined sample, respectively.

TAMMINGA, et al. (1980) examined 182 raw hamburger samples and found that coliforms and E.coli counts were ranged from 10^4 to 10^6 and from 10^3 to 10^5 /g examined sample, respectively, while, IBRAHIM (1981) found that the average count of coliforms was $15.5x10^2$ /g frozen hamburger. ABDEL-AZIZ (1987) showed that total coliforms count/g sausage and hamburger was log 5.06 and 6.25, while, he found the incidence of E.coli was 50% and 70% of the examined samples, respectively. IMAN (1989) found that coliform counts were 10^2 and $4x10^2$ /g beefburger and sausage, while the incidence of E.coli was 60% and 68% in examined samples, respectively.

The presence of E.coli in food as agents for foodborne enteritis or as indicators of fecal contamination have resulted in increasing concern and interest for estimation the organism from selected meat products.

KENNETH (1975) reported that the standard limit for E.coli is 50/g for fresh or frozen meat products and 10/g for processed meats. WESTHOFF and FELDSTEIN (1976) found that E.coli counts were ranged from zero to 10^2 /g examined hamburger, while DUITSCHAEVER et al (1977) isolated E.coli from 28(27.72%) out of 99 frozen hamburger, whereas REIS et al. (1980) isolated 9 strains out 1200 colonies of E.coli from examined sausage, hamburger and keebe. EL-KHATEIB (1982) and GOBRAN (1985) recorded that the incidence of E.coli in examined sausage samples was 36.11% and 50%, respectively, while in beefburger, it was 30% (DARWISH et al., 1986).

TOLBA (1986) detected E.coli in sausage and hamburger samples in incidence of 52.5% and 25%, respectively and NIAZI and REFAI (1988) isolated E.coli from 22(44%) out of examined 50 sausage samples, while,

EMAN (1990) found that the incidence of E.coli in fresh sausage and frozen beefburger was 48% and 16%, respectively.

By using direct plate count the incidence of E.coli in sausage and beefburger was 40% and 12%, while by using multiple tube fermentation technique (MPN), it was 48% and 16% in examined samples, respectively (DARWISH et al., 1991).

Therefore, the present study was intiated to determine coliforms and E.coli organisms in some selected meat products by using Most Probable Number (MPN) technique.

MATERIAL and METHODS

1. Collection of samples:

A total number of 47 random samples of different meat products, 19 beefburger, 16 sausage and 12 luncheon samples were collected from various shops and supermarkets at Assiut City. The collected samples were obtained in their intact original packages and transferred directly to laboratory for determination of coliforms and E.coli organisms by using Most Probable Number (MPN) techique.

2. Preparation of samples:

The samples were prepared according to the method recommended by ICMSF (1978). Twenty five grams of each sample were weighed aseptically into a sterile blender jar and 225 ml of buffered peptone water were added. The samples were homogenized at low speed (2000 r.p.m.) for 2 minutes. Subsequent 10 fold serial dilutions of the homogenate were prepared with buffered peptone water.

3. Laboratory technique:

The applied technique was recommended by ICMSF (1978).

3.1. Presumptive determination of coliforms and E.coli:

One ml from the previously prepared dilutions was inoculated separately into eachy of three Lauryl Sulphate Tryptose (LST) broth fermentation tubes with inverted Durham's tubes. The tubes were incubated at 35–37 C for 24 and 48 hours. After 48 h., tubes showing gas production reported as positive.

3.2. Confirmation test for coliforms:

A loopful from each positive tube in the presumptive test was transferred separately into each of three Brilliant Green Lactose Bile Broth 2% (BGLB) tubes with inverted Durham's tubes. The tubes were incubated at

35–37 °C for 24 and 48 hours. Tubes showing gas production were recorded as positive test and the MPN of coliforms per gram examined sample was determined (A.O.A.C., 1980).

3.3. Confirmation test for E.coli:

A loopful from each positive tube in the presumptive test was transferred separately into each of three E.C. broth tubes with inverted Durham's tubes. The tubes were incubated at 45.5 °C for 48 hours. Tubes showing gas production were considered positive. From each gas positive tube of E.C. broth, a loopful was streaked on Levine's Eosin Methylene Blue (EMB) agar plates. The plates were incubated at 35–37 °C for 24 ± 2 hours. Plates showing typical colonies or colonies most likely to be E.coli were recorded for determination MPN/g examined sample.

RESULTS

The obtained results were recorded in Tables (1,2,3 and 4).

DISCUSSION

Escherichia coli and related coliforms bacteria predominate among aerobic commensal flora present in the gut of man and animals. So, their presence in meat or other meat products is indicative of faecal contamination (ICMSF, 1978). On the other hand, the contamination of meat products with pathogenic bacteria like E.coli constitute a public health hazard in the form of infection or intoxication in human consumers (MATSIEVSKIII et al., 1971 and MEHLMAN & ROMERO, 1982).

Form the results achieved in Table (1), it can be concluded that coliform organisms were detected in all examined samples with average counts of 10095.95,7665.35 and 7409.93/g examined beefburger , sausage and luncheon samples, respectively. Lower results were reported by Sadek (1963) and Iman (1989) who found that coliforms counts were 10^2 and $4X10^2$/g of examined sausage samples, respectively. On the other hand, higher results were evaluated by Abdel–Aziz (1979) who detected coliforms in all examined sausage samples with an average count of $11X10^4$/g. It was also found that coliform counts recorded by Ibrahim (1981) and Iman (1989) were lower than obtained results in examined beefburger.

From Table (3), it is evident that E. coli were detected in 9(47.37%),4(25%) and 5(41.67%) of the examined beefbuger, sausage and luncheon samples with average counts of 617.25,703.44 and 18.68/g examined

sample, respectively. These results seen to be higher than the standard limit reported by KENNETH (1975). The incidence of E. coli in the examined sausage samples was 25 % which seen to lower than the findings reported by many investigators at variable percentages, ABDEL-AZIZ (1979) (100 %), EL-KHATEIB (1982) (36.11 %), GOBRAN (1985) (50 %), TOLBA (1986) (52.5 %). ABDEL-AZIZ (1987) (50 %), NIAZI and REFAI (1988) (44 %), EMAN (1989) (68 %), EMAN (1990) (48 %) and Darwish et al. (1991) who showed that the incidence was 40 % and 48 % by using direct plate count and MPN technique, respectively. On the other hand, the present results gave higher incidence of E.coli in examined beef-burger than the findings recorded by DUITSCHAEVER et al. (1977) (27.72%), DARWISH et al. (1986) (30 %), TOLBA (1986) (25 %), EMAN (1990) (16 %) and DARWISH et al. (1991) who reported that the incidence was 12 % and 16 % by using direct plate count and MPN method, respectively, while lower than the results pointed out by ABDEL-AZIZ (1987) (70 %) and IMAN (1989) (60 %).

It is concluded that presence of coliforms in all exmined meat products samples is attributed to contamination of raw meat, in addition to contamination of raw meat, in addition to lack of hygienic circumstances during manufacturing of such products. Many workers have stated that E.coli should be taken into account when considering the sanitary standards and hygiene of food handling particularly minced meat, sausage, beefburger and local manufactured meat products either frozen or fresh (Stiles & Ng, 1980; Gobran, 1985 Niazi and Refai, 1985 and Niazi and Refai, 1988). So it is recommended also that hygienic measures must be applied periodically in such factories particulary during preparation and manufacturing of these meat products.

REFERENCES

Abdel-Aziz, A.A. (1979): Studies on hygienic quality of manufactured, fresh sausage. M. of public Health Sci., Thesis, High Inst. Public Health, Alexandria University.

Abdel-Aziz, A.T. (1987): Microbiological load of some meat products as influenced by the hygienic status of the producing plant. M.V.Sc. Thesis, Fac. Vet. Med., Cairo University.

A.O.A.C. (1980): Official methods of analysis of the Association of official analytical chemistis PO. Box 540, Benjamin Franklin station 13th Ed., Washington ,DC 20044.

Daraish, A.; Hamdy, M.and Nouman, T.M. (1986): Quality evaluation of market

Daraish, A.; Hamdy, M.and Nouman, T.M. (1986): Quality evaluation of market meat pastes. Vet. J. 34,No. 1,37–48.

Darwish, A.M.; Niazi, Z.M, and Zaki, E.M. (1991): Escherichia coli in meat products. Vet. Med. J. Giza, 39, No.3,841–851.

Duitschaever, C.L.; Bullock, D.H. and Arnott, D.R. (1977): Bateriological evaluation of rerail ground beef, frozen beef –patties, and cooked hamburger. J.Food Prof. Vol.40:No. 6: 378–381.

El–Khateib, T. (1982): Sanitary condition of sauage in Assiut. M.V.Sc., Fac. Vet. Med., Assiut University.

Eman M. Zaki (1990): Escherichia coli meat products with special referance to enteropathogenic strains. M .V.Sc. Thesis, Fac.Med., Cairo University.

Gobran, R.A. (1985): Enterobacteriaceae in meat products in Upper Egypt. M.V.Sc. Thesis, Fac.Vt.Med., Assiut University.

Ibrahim, A.M. (1981): Sanitray codition of locally of locally produced hamburzir, M.V.Sc.Thesis, Cairo Universitiy.

ICMSF (1978): Internationl Nommission on Microbiological Specification for Food. Microorganisms in foods. Their significance and methods of enumeration ,Vol. I, 2nd Ed. Univ. of Toronto press, Toronto. Ontario, Canada.

ICMSF (1980): Microbial ecology of food, Vol. I. Univ. of toronto Press. Toronto. Canada.

Iman, A.A.F. (1989): Microbiological status of packed meat products. M.V.Sc. Thesis, Academemy,Cairo.

Kenneth, C.(1975): Oregon,s experience with microbiological standards for meat.J. Milk food Technol. Vol.38, No. 8:483–486.

Libby,J.A.(1975): Meat Hygiene, 4th Ed.Lea and Febiger, Philadlphia. Matsievskii, V.; Logachev, A.; Fedorina, A. and Risklova, A.(1971): Outbreakof food poisoning caused by E.Coli 0124:K 72 (B17). Epidemiology Immunobilogy 48, 137. Dairy Sci. Abst., 35:388.

Mehlman, J.J. and Romero, A.(1982): Enteropathogenic E.coli, Methods for recovery from food.J.Assoc. of Anal.Chem.59: 67–80.

Niazi, Z.M. and Refai, M.(1988): Isolation of enteropathogenic and enterotoxigenic. escherichia coli from meat and cheese. Vet. Med. J. 36: 121–134.

Oblinger, J.L. and Koburger, J.A. (1984): The most probable number technique. Compendium of methods for the microbiological examination of food. 2nd Ed. American Pbulic Health Association, Washington, D.C.

Reis, M.H.L.: Vasconcelos, J.C. and Trabulsi, L.R. (1980): Prevalence of Enterotoxigenic Escherichia coli in some processed raw food from animal origin. Appl. Environ. Microbiol. Vol. 39, No. 1: 270–271.

Sadek, M.I. (1983): Studies on locally manufactured fresh sausaze. M.V.Sc. Thesis, Cairo University.

Stiles, M.E. and Ng, Lai–King (1980): Estimation of Escherichia coli in raw ground beef. Appl. Environ, Microbiol. 40: 346–351.

Tamminga, S.; Benner, R. and Kampelmacher, E.H. (1980): Bacteriological evaluation of hamburger. 1. Studies on raw, heat treated and pre–.fried hamburger. Voedingsmiddlen–Technolozie, 13: 29.

Tolba, K.S. (1986): Antibiotic resistant microorganisms in some meat products. M.V.Sc. Thesis, Fac. Vet. Med., Cairo University.

Westhoff, D. and Feldstein, F. (1976): Bacteriological analysis of ground beef. J. Milk Food Technol., Vol. 39: 401.

Table 1: Statistical analytical results of coliforms count (MPN/g) in examined meat products.

Type of samples examined	No. of samples examined	Positive samples No.	Positive samples %	Min.	Max.	Average
Beefburger	14	14	100	43	$46x10^3$	10095.95
Sausage	16	16	100	93	$11x10^3$	7665.38
Lunchen	12	12	100	91	$11x10^3$	7409.93

Table 2: Frequency distribution of coliforms count (MPN/g) in examined meat products.

Range	Beefburger Frequency No.	Beefburger Frequency %	Sausage Ferquency No.	Sausage Ferquency %	Luncheon Frequency No.	Luncheon Frequency %
91 - 93	1	5.26	2	12.50	1	8.33
150 - 210	1	5.26	2	12.50	1	8.33
240 - 460	1	5.26	-	-	2	16.67
$11x10^2$-$11x10^3$	16	84.22	12	75.00	8	66.67
Total	19	100.00	16	100.00	12	100.00

Table 3: Statistical analytical results of E.coli count (MPN/g) in examined meat products.

Type of samples examined	No. of samples examined	Positive samples No.	Positive samples %	Min.	Max.	Average
Beefburger	19	9	47.37	0	$11x10^3$	617.25
Sausage	16	4	25.00	0	$11x10^3$	703.44
Luncheon	12	5	41.67	0	$11x10^3$	18.68

Table 4: Frequency distribution of E.coli count (MPN/g) in examined meat products.

Range	Beefburger Frequency No.	Beefburger Frequency %	Sausage Frequency No.	Sausage Frequency %	Luncheon Frequency No.	Luncheon Frequency %
/ 3.0	10	52.63	12	75.00	7	58.33
3 - 36	6	31.58	2	12.50	2	16.67
61 - 75	-	-	-	-	3	25.00
210 - 460	2	10.53	1	6.25	-	-
11×10^3	1	5.26	1	6.25	-	-
Total	19	100.00	16	100.00	12	100.00