**INTERNATIONAL JOURNAL OF RESEARCH –
GRANTHAALAYAH**
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Science

THE MICROBIOLOGICAL STUDY OF MINCED MEAT IN SOME MARKETS IN ALEXANDRIA WITH REFERENCE TO ESCHERICHIA COLI O157 H: 7

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Abstract

This cross sectional study was carried out during a four months period from December, 2012 to March, 2013. A total of 140 fresh and frozen ground beef samples were purchased from local butchers and supermarkets in Alexandria. Each of the ground beef sample was analyzed for its microbiological quality (Aerobic plate count, total coliform count and E. coli count) as well as for the presence of E. coli O157:H7. Out of the 140 studied ground meat samples, 75(53.6%) proved to be unsatisfactory according to the three tested parameters. None out of 140 examined ground meat samples showed E. coli O157:H7.

Keywords: Microbiological Quality; Ground Meat; APC; TCC And E. coli O157:H7.

Cite This Article: Aiada Daw Mohamed (2017). "THE MICROBIOLOGICAL STUDY OF MINCED MEAT IN SOME MARKETS IN ALEXANDRIA WITH REFERENCE TO ESCHERICHIA COLI O157 H: 7." *International Journal of Research - Granthaalayah*, 5(9), 26-35. https://doi.org/10.5281/zenodo.999193.

1. Introduction

Meat is considered as the most nutritive source of protein consumed by humans [1]. Most meat have high water content corresponding to the water activity of approximately 0.99, which is suitable for microbial growth [1]. It also contains sufficient nutrients needed to support the growth of microorganisms [2].Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic environmental factors; [3]. However the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability [4].

In fact, tissues from healthy animal are sterile, however it has been pointed out that during slaughter, dressing and cutting, microorganisms come chiefly from the animal and its intestinal tract, but more are added from knives, cloths, air, carts and equipment in general. [5]. Turtura (1991) reported that the most frequently Gram negative bacilli identified on meat were *Citrobacter freundii (C. freundii), Escherichia coli (E. coli), Enterobacter agglomerans (En. agglomerans*), and the less frequently isolated strains are of the genera *Klebsiella, Shigella* and

Proteus. E. coli and *Staphylococcus aureus (S. aureus)* are normal flora in human and animals, their presence in foods are indications of excessive human handling [6].

Ground meat is not only highly susceptible to spoilage, but also is frequently involved in the spread of pathogens, [7].which are major causes of illness and death worldwide [8]. It is resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food [6]. Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety [9].The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication [10].

Aerobic plate counts (APC) often are chosen as an indicator of the effectiveness of Hazard Analysis Critical Control Point (HACCP) plans, because data for all aerobic bacteria are more easily collected than data for pathogens of concern or other indicator organisms [11]. A common practice is to use tests for coliform, including *E. coli* for screening and if there is reason to determine the likelihood of fecal contamination, the total coliform count (TTC) or other *Enterobacteriaceae* are subjected to further tests to establish whether any of them are *E. coli* [12].

2. Material and Methods

Sample collection: One hundred grams of ground meat were obtained from worker in collection sites and placed in the food collection bags. Sample source and sample number was identified on sample form. Refrigeration of sample was used during transport. Frozen samples were kept frozen. A submission form for each sample was filled out including: type of meat and its color; date and locality of the collection site; name of butcher and its personal hygiene and sanitary condition of the shop.

Sample preparation: Ten gram of each sample were weighted and transferred to a stomacher bag under aseptic conditions. The sample was then diluted to a 10^{-1} dilution with 90ml of peptone water and homogenized for 2 min by using a Stomacher. Following homogenization, ten-fold serial dilutions for each sample were made in sterile peptone water up to 10^{-4}, by transferring 1 ml of previous dilution to 9 ml of peptone water**.**

2.1. Microbiological Analysis

Aerobic Plate Count: One ml of each dilution was pipette into separate, appropriately marked Petri dish and to each plate, 12-15 ml plate count agar (cooled to 45 ± 1°C) was added within 15 min of the original dilution. After Petri dishes solidified were incubated promptly for 48 ± 2 h at 35°C. Plates with colony number between 30-300 colony-forming units (CFU) were selected, coun++ted and multiplied by the dilution factor to calculate CFU/gram of ground meat.

Determination of coliform and *E. coli* using the Most Probable Number (MPN) method: One ml of each dilution was cultured in the Lauryl Sulphate Tryptose broth (LST) tubes for determining the presence of coliform and the positive LST tubes showing gas were subcultured into Brilliant Green Bile (BGB) broth and EC broth. All positive BGB tubes recorded as confirmed MPN of coliform bacteria per gram of ground meat, however gas production in the

EC tubes was considered a confirmed test for fecal coliform organisms and subcultured onto Eosin Methylene Blue (EMB) agar plates which incubated for 24h at 35°C and examined for green metallic sheen colonies and then transfered to IMViC (Indole, Methyl red,Vogues-Proskauer and Citrate) tubes. MPN of *E*. coli per gram were computed considering producing ++-- or-+-- IMViC patterns.

Determination for *Escherichia coli* O157:H7 (*E. coli* O157:H7): The presence of *E. coli* O157:H7 were detected in ground meat samples by preenriched with modified novobiocin EC broth at 37C^0 for 24h. The dilution was inoculated onto Sorbitol MacConkey's Agar (SMA) and incubated at 37°C for 24 h. At the end of incubation, colorless, sorbitol negative colonies were subjected to *E. coli* O157:H7 test latex.

3. Results

The results of this study were recorded according to the Commission of the European Communities [13] and the Egyptian guidelines [14,15] as satisfactory, acceptable and unsatisfactory. Out of the 140 studied ground meat samples, 75(53.6%) proved to be unsatisfactory according to the 3 tested parameters. None out of 140 examined ground meat samples showed *E. coli* O157:H7. The following tables and figures illustrate the results of this study:

Table (1) shows the distribution of the 140 examined ground meat samples according to their microbiological quality**.**

Regarding the microbiological quality of the studied 140 examined ground meat samples, the APC parameter showed that 57(40.7%) were satisfactory, 48(34.3%) were acceptable and 35(25.0%) were unsatisfactory. The corresponding figures according to the TCC parameter were 37(26.4%), 49(35.6%) and 54(38.6%) respectively and those according to the *E. coli* count 67(47.9%), 51(36.4%) and 22(15.7%) respectively.

The collective means ± standard deviation of APC, TCC and *E. coli* count were $4.1 \times 10^6 \pm 7.2 \times 10^6$ cfu\g, $1.02 \times 10^4 \pm 2.2$ x 10^4 cfu\g and $1.9 \times 10^3 \pm 1.2$ x 10^4 cfu\g respectively.

Table 1: Distribution of the 140 examined ground meat samples according to their microbiological quality, Alexandria, 2013.

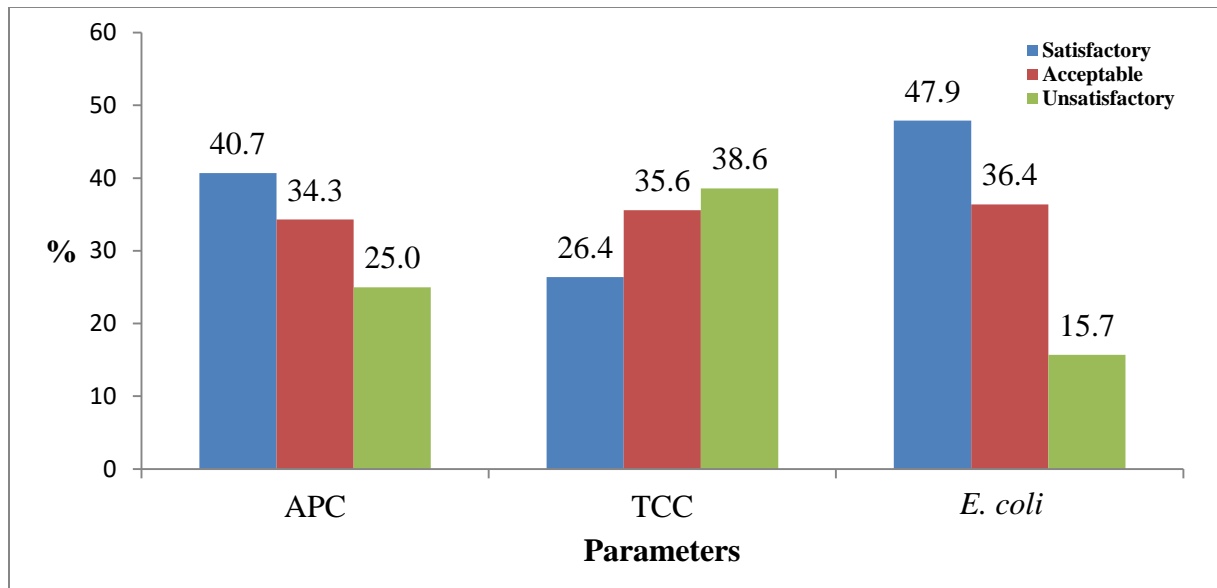| Parameter	Microbiological quality of ground meat						
	Satisfactory		Acceptable		Unsatisfactory		Mean ± SD CFU\g
	No.	%	No.	%	No.	%	
APC	57	40.7	48	34.3	35	25.0	$4.1 \times 10^6 \pm 7.2 \times 10^6$
TCC	37	26.4	49	35.6	54	38.6	$1.02 \times 10^4 \pm 2.2 \times 10^4$
E. coli	67	47.9	51	36.4	22	15.7	$1.9 \times 10^3 \pm 1.2 \times 10^4$

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Figure 1: Distribution of the 140 examined ground meat samples according to their
microbiological quality, Alexandria, 2013.

Tables 2 a, b and c show the comparison between microbiological quality of the examined
ground meat samples according to their APC ,TCC and *E. coli*.

Table 2 a shows that both the APC and TCC parameters agreed in the results of 68(48.6%)
samples as either satisfactory or acceptable, and 17(12.1%) samples as unsatisfactory. On the
other hand, 37(26.4%) samples which proved to be unsatisfactory according to their TCC were
either satisfactory or acceptable according to their APC; and 18(12.9%) samples that proved to
be either satisfactory or acceptable by the TCC were unsatisfactory by the APC. The difference
between these results was found to be statistically significant. **(p=0.014)**.

Table 2 b shows that both the APC and *E. coli* parameters agreed in the results of 91(65.0%)
samples as either satisfactory or acceptable, and 8(5.7%) samples as unsatisfactory. On the other
hand, 14(10.0%) samples which proved to be unsatisfactory according to their *E. coli* were either
satisfactory or acceptable according to their APC; and 27(19.3%) samples that proved to be
either satisfactory or acceptable by the *E. coli* count were unsatisfactory according to their APC.
The difference between these results was found to be not statistically significant. **(p=0.060).**

Table 2 c shows that both the TCC and *E. coli* parameters agreed in the results of 83(59.3%)
samples as either satisfactory or acceptable, and 19(13.6%) samples as unsatisfactory. On the
other hand, 3(2.1%) samples which proved to be unsatisfactory according to their *E. coli* were
either satisfactory or acceptable according to their TCC; and 35(25.0%) samples that proved to
be either satisfactory or acceptable by the *E. coli* count were unsatisfactory according to their
APC. The difference between these results was found to be statistically significant. **(p=0.000)**.

Table 2: Comparison between microbiological qualities of examined ground meat samples according to their parameters.

a. APC versus TCC parameters.

APC / TCC	Microbiological quality of ground meat					
	Satisfactory + Acceptable		Unsatisfactory		Total	
	No.	%	No.	%	No.	%
Satisfactory + Acceptable	68	48.6	37	26.4	105	75.0
Unsatisfactory	18	12.9	17	12.1	35	25.0
Total	86	61.4	54	38.6	140	100.0

b. APC versus *E. coli* parameters.

APC / *E. coli* Count	Microbiological quality of ground meat					
	Satisfactory + Acceptable		Unsatisfactory		Total	
	No.	%	No.	%	No.	%
Satisfactory + Acceptable	91	65.0	14	10.0	105	75.0
Unsatisfactory	27	19.3	8	5.7	35	25.0
Total	118	84.3	22	15.7	140	100.0

c. TCC versus *E. coli* parameters.

TCC / *E. coli* count	Microbiological quality of ground meat					
	Satisfactory + Acceptable		Unsatisfactory		Total	
	No.	%	No.	%	No.	%
Satisfactory + Acceptable	83	59.3	3	2.1	86	61.4
Unsatisfactory	35	25.0	19	13.6	54	38.6
Total	118	84.3	22	15.7	140	100.0

4. Discussion

Because of its high bacterial level, and its likelihood of keeping pathogenic bacteria, ground meat is an important meat product that threatens human health [16].

Contamination with spoilage microorganisms may lead to product and economic losses, while presence of pathogens or their toxins may be the cause of foodborne disease that may lead to loss of human life [17].

Narasimha and Ramesh [18]. Suggest that minced meat of better microbiological quality might be obtained if animals were processed on a small scale with further care during mincing of the meat. The retail grinder was assumed to randomly mix the pathogen throughout the 5 kg lot of ground beef. Extensive contamination, or abusive conditions of handling and storage that allow

microbial proliferation, increase the potential for presence of pathogenic bacteria and formation of toxins, and may lead to product spoilage and public health problems [19,20].

APC are a widely accepted measure of the general degree of microbial contamination.(21) Meat is generally considered of poor hygienic quality or unfit for consumption when the APC exceeds 10^6 cfu\g. Mohammad et al [22] in Pakistan pointed out the bacterial count exceeding 10^5\g in delicatessen food products are indicative of dangerous contamination. However Gil et al [23] proved that samples of minced meat contaminated with APC of 6 $\log_{10}$ cfu\g indicates a deterioration process with off odor and reduction of shelf life and when contamination reach 7 $\log_{10}$ cfu\g slime formation is already evident.

In the present study the mean of total aerobic plate count of the140 examined ground meat samples were 4.1×10^6 cfu\g. Almost similar results at the average levels of 10^6 cfu/g were reported by Khalafalla et al [24] Higher results of 7.2 x 10^8 cfu\g were reported by Mousa et al (25).On other hand much lower results were reported by Elmali and Yaman (26) at the average of 3.4x104 cfu\g.

According to the Commission of the European Communities and the Egyptian guidelines, to be unsatisfactory, ground meat samples should contain > 10^6cfu/g, this figure was recorded in this study in only 25.0% of the studied samples. This percentage was relatively low when compared to those reported in Istanbul, Turkey by Cetini et al [16] who found the APC to be above the given limits in 66% of their samples. Also in a study reported by Duitschaever et al [27], they found APC in 64% of ground meat samples with counts more than >10^6 cfu/g. Even higher percentages reaching 79% and 75% were reported by Siriken et al [28] in Turkey and Heredia et al [29] in Mexico respectively, however these studies took the 10^5 limit as being unsatisfactory. APC provides an estimate of overall bacterial populations. Higher APC usually relates to poorer quality and a reduced shelf life, but the relationship between APC and concentration of foodborne pathogens in raw meats is unclear [30].

Microbial indicators are more often employed to assess food safety and sanitation than quality. They may be employed to reflect the microbiological quality of foods relative to product shelf life or their safety from foodborne pathogens [31,32]. The collective mean of microbial contamination of ground beef with TCC in this study was 1.02x 10^4 cfu\g denoting low sanitary level. These include 38.6% recorded as unsatisfactory according to the European Communities and the Egyptian guidelines. Similar results to this mean value was reported in Oklahoma in United States by Davidson et al [33], who found the TCC mean level of 1.2x10^4cfu\g. Much lower results were reported in Turkey by Elmali and Yaman [26] at an average of 2.0x10^1 cfu\g. While higher results also in Turkey were reported by Sancak et al [34] who found that the average numbers of TCC were 4.0 x10^6 cfu\g. The previous unsatisfactory percentage is considered much lower than the 64.3% reported in Turkey by Siriken et al [28] even when their unsatisfactory criteria was >1100 cfu\g, which is higher than the >1000 adopted in the present study.

According to Gill et al [23], the counts of *E. coli* increase steadily (more than 3 log unit) during the various stages of carcass handling and deboning process. Such increases in contamination become further aggravated with poor hygienic conditions during processing. In the present study

the collective mean of *E. coli* count were 1.9×10^3 cfu\g. This result come in agreement with Davidson et al (33) in Oklahoma, United States who reported *E. coli* at the level of 4.8×10^3 cfu\g, while much lower results of 5.0×10^0 cfu\g were reported by Westhoff and Feldstein [35].

Much higher results in Ankara, Turkey were reported by Tekinsen et al [36] who examined 20 ground meat samples and found *E.coli* at the average levels of 4.2×10^6. However high counts of *E. coli* in foods are not always alarming because most strains are harmless and opportunistic in nature. [37]. Nevertheless, the bacterium is well recognized as a fecal indicator that provides a fair estimate of the level of fecal contamination and the hygienic conditions during handling and processing [30].

Meat samples containing > 500 *E. coli*\g are considered to be unsatisfactory by the Commission of the European Communities and the   Egyptian guidelines; this level was recorded in only 15.7% of the studied samples. This percentage was relatively low when compared to those reported in Istanbul, Turkey by Cetini et al [16], who observed 36.22% of their samples to be at the critical limit for their *E. coli* counts.

The results of this study showed that none of used parameters agreed completely with the other, meaning that the use of only one parameter may show a sample as satisfactory or acceptable, which in the another parameter may forbid its consumption. Therefore, microbiological monitoring using several parameters appears to be a must. Moreover 53.6% of the total 140 examined ground meat samples were above the safety limits and unfit for human consumption. Higher percentage (80.7%) were reported in Morocco by Cohen et al [38].

The potentially high mortality associated with *E. coli* O157: H7 strain infection, make its presence in any food material worrisome and of serious public health concern, as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *E. coli* 0157:H7 strain [39].

In order to assess the risk to human health from *E. coli* O157:H7 associated with the consumption of ground beef, we examined all samples for prevalence of *E. coli* O157:H7 and revealed none out of the 140 examined ground meat samples showing it. This result comes in agreement with those of Hinton et al [40] and Hussein [39], they did not detect any *E. coli* O157:H7 in frozen and fresh meat. However Noveir et al [41] reported that   *E. coli* O157 was isolated in only 0.4 % of their samples, but none of these isolates were found to be H7 serotype.

Schroeder et al [42] mentioned that *E. coli* O157:H7 outbreaks due to plants and animal produce have become increasingly common. While half of produce associated outbreaks were due to kitchen-level cross-contamination, which calls for further prevention efforts targeting food preparers, the other half were due to produce already contaminated with *E. coli* O157:H7 before purchase. [43] So the absence of these bacteria in this study may indicate that the Egyptian animals do not serve as source of contamination with *E. coli* O157:H7.

5. Conclusion

The ground meat samples were analyzed for determination of their microbiological quality by using APC, TCC and *E. coli* count as bacteriological parameters. In addition detection of *E. coli* O157:H7 was performed. None out of 140 examined ground meat samples showed *E. coli* O157:H7. About 53.6% of the examined ground meat samples were unfit for human consumption.

The difference between APC and *E. coli* was found to be not statistically significant. **(p=0.060).**

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