1. INTRODUCTION

Meat and meat products are the most palatable and desirable foods for human being, as they are an important source of animal protein, fat, essential amino acids, minerals, vitamins and other nutrients (Zafar et al., 2016). On the other hand, they are considered as ideal culture medium for growth of many organisms because of the high moisture, high percentage of nitrogenous compounds, plentiful supply of minerals, some fermentable carbohydrates (glycogen) and favorable pH for most microorganisms resulting in their spoilage, economic losses, foodborne infections in human and health risk (Komba et al., 2012).

The bacterial contamination and hygienic measures during meat production can be measured using the aerobic plate count and coliforms (Hamed et al., 2015). Aerobic plat count is used as indicator for bacterial population, but it cannot differentiate types of bacteria. Aerobic plate counts can be useful to indicate quality and shelf life of certain food items. Sources of coliforms in meat are solid hands, knives used for cutting and contaminated water. Fecal coliforms had been used as indicator for fecal contamination of the meat (Shaltout et al., 2019).

Meat products may be contaminated with fungi which may occur during the transportation, storage and handling processes of meat (Nasser, 2015).

Contaminated meat products may constitute a public health hazard (Datta et al., 2012 and Hamed et al., 2015). Fungi may cause three basic types of diseases which are mycosis, allergy and mycotoxicosis. A mycosis is defined as invasion of living tissue by fungi, while an allergy is a hypersensitivity to fungal antigen and mycotoxicosis is a toxic manifestation resulting from ingestion or exposure to fungal metabolites (El-Tawab., 2014). Mycotoxins, particularly aflatoxin (AF), fumonisins (FUM), and deoxynivalenol (DON), may impair child growth. Although these toxins have distinct actions, they all mediate intestinal damage through inhibition of protein synthesis (AF, DON), increase in systemic pro-inflammatory cytokines (DON) and inhibition of ceramide synthesis (Laura et al., 2012).

Chronic exposure to aflatoxin well above the FDA guideline 20 (ppb) affects many organs; however, the major targets is the liver. Aflatoxins are hepatotoxic in humans and animals, food exposed to AFs and resulting in aflatoxicosis can ranged from acute to chronic, and illness can ranged from mild to severe, including development of cirrhosis (severe liver damage ) and may result in development of liver cancer. Aflatoxin B1 is the most potent known natural carcinogen (FDA, 2013).

As the level of meat contamination of meat and its products with different foodborne pathogens constitutes serious problems for consumers, so, the current study was conducted...
to microbial evaluation of some meat products with special attention to detection of aflatoxins.

2. MATERIAL AND METHODS

2.1. Sample collection
A total of 80 random samples from sausage and kofta (40 of each) were purchased from different shops at Kalobia governorate. Each sample was kept in a separate sterile plastic bag and put in an icebox then transferred to the laboratory under complete aseptic conditions without undue delay and subjected to bacteriological and mycological examination.

2.2 Methods
2.2.1. Bacteriological examination

2.2.2.1. Determination of Aerobic Plate Count APC/g using standard plate count following (FDA, 2001)

2.2.2.2. Determination of coliform count by the surface plating method of using Violet Red Bile agar medium (ICMSF, 1996)

2.2.2.3. Mycological examination

2.2.2.2.1. Determination of total mould and yeast count
The collected samples were prepared according to the technique recommended by ISO. 217-1-2:2008. The isolated fungi were identified according to macro and microscopic characteristics as described by Pitt and Hoching (2009), while yeast isolates were identified according to some complementary tests used for final identification of the isolates as recommended by Kurtzman et al. (2003) and Pitt and Hoching (2009).

2.2.2.2.2. Detection of aflatoxins residues in sausage and kofta samples by using fluorimeter method: (AflaTest Fluorometer Instruction Manual, 2014).

3. Statistical analysis
Data were analyzed using the descriptive statistic SPSS (Version 20). Differences in mean of analyzed data were considered significant at P < 0.05.

3. RESULTS

Results showed in Table (1) indicated the total aerobic bacterial count in the examined kofta samples, where the total aerobic bacterial count ranged from 10×10^3 to 5×10^7 with a mean value of 2.04×10^6 ± 0.12×10^6 cfu/g , while sausage samples ranged from 2.4×10^3 to 15×10^3 with a mean value of 11×10^3±4.5×10^3 cfu/g.

Results achieved in table (3) indicated that the incidence of mould in the examined meat samples were (62.5%) and (82.5%) for the examined samples of Kofta, and sausage, respectively. Furthermore, the mean value of total mould count (cfu/g) in the examined sausage, and Kofta samples were 1.1×10^3 ± 0.14×10^3 and 1.4×10^3 ± 0.27×10^3, respectively.

Regarding the results recorded in the tables (3), it revealed that the incidence and total mould count of examined samples of sausage had a minimum count 1.0×10^2 and maximum 4.2×10^3 with a mean value of 1.1×10^3 ± 0.14×10^3 (cfu/g).

The results achieved in table (4) revealed that the incidence of yeast contamination in the examined kofta, and Sausage samples were (28%) and (62.5%). Furthermore, the mean value of total yeast (cfu/g) in the examined kofta and Sausage samples were: .47×10^3 ± .07×10^3 and .52×10^3 ± .08×10^3.

In Table 2, the results showed that coliform count ranged from 7.9×10^2 to 0.17×10^3 with a mean value of 1.2×10^3 ± 0.16×10^3 in kofta samples, while in sausage sample ranged from 8.9×10^2 to 3.5×10^3 with a mean value of 6.7×10^2 ± 0.03×10^3 cfu/g.

Results achieved in table (5) declared that 7 mould genera could be isolated and identified from the examined samples. The identified mould genera were Acremonium, Aspergillus, Cladosporium, Chaetomium, Geotrichum, Penicillium, and Talaromyces species. The incidence of identified mould isolated from the examined sausage and kofta was (40 and 25%) for A.flavus, (32 and 18%) for A.niger, (15 and 5%) for A. ustus, (5 and 2.5%) for A.terreus, (2.5 and 0 %) for A.
flavipes, (and 0 and 7.5%) for A. clavatus, (10 and 0%) for A. fumigatus, (0 and 2.5%) for A. nidulens, (7.5 and 5%) for Acremonium spp, (2.5 and 7.5 %) for Cl. Cladosporidiae, (0 and 5%) for Chaetomium globosum, (12.5 and 7.5%) for P. citreonigrum, (5 and 2.5%) for P. oxalicum, (5 and 7.5%) for P. fellutanum, and (0 and 12.5%) for P. chrysogenum, (0 and 7.5%) for T. trachycpermus, (5 and 15%) for Geotrichum candidum.

The incidence of identified yeast isolated from the examined sausage and kofta samples were (5 and 2.5%) for Candida pseudotropicalis, (17.5 and 10%) for C. homlili, (12.5 and 17.5%) for C. famata, (5 and 10%) for C. gullermondii, (25 and 22.5%) for Rhodotorula spp., (12.5 and 2.5%) for Saccharomyces spp., (5 and 7.5%) for Torulopsis spp., C. valida was only present in the examined sausage sample with incidence of 10% (Table 6).

Table 5 Incidence of the identified fungal strains isolated from the examined samples of meat products (n=40).

| Mould spp. | Sausage | Kofta |
|------------|---------|-------|
| No. | % | No. | % |
| A. Aspergillus spp. | 16 | 40.0 | 10 | 25.0 |
| - A. flavus | 1 | 2.5 | 0 | 0 |
| - A. flavipes | 32 | 80.0 | 18 | 45.0 |
| - A. niger | 6 | 15.0 | 2 | 5.0 |
| - A. terreus | 2 | 5.0 | 1 | 2.5 |
| - A. clavatus | 0 | 0.0 | 3 | 7.5 |
| - A. fumigatus | 4 | 10.0 | 0 | 0.0 |
| - A. nidulens | 0 | 0 | 1 | 2.5 |
| B. Acremonium spp. | 3 | 7.5 | 2 | 5.0 |
| C. Cladosporium spp. | 1 | 2.5 | 3 | 7.5 |
| - Cl. Cladosporidiae | 0 | 0 | 2 | 5.0 |
| D. Chaetomium spp. | 0 | 0 | 3 | 7.5 |
| - Chaetomium globosum | 2 | 5.0 | 6 | 15.0 |
| E. Penicillium spp. | 5 | 12.5 | 3 | 7.5 |
| - P. citreonigrum | 2 | 5.0 | 1 | 2.5 |
| - P. oxalicum | 2 | 5.0 | 3 | 7.5 |
| - P. fellutanum | 0 | 0 | 5 | 12.5 |
| - P. chrysogenum | 0 | 0 | 3 | 7.5 |
| F. Talaromyces spp. | 2 | 5.0 | 6 | 15.0 |
| - T. trachycpermus | 0 | 0 | 3 | 7.5 |
| G. Geotrichum spp. | 2 | 5.0 | 6 | 15.0 |

Table 6 Incidence of the identified yeast strains isolated from the examined samples of meat products (n=40).

| Yeast spp. | Sausage | Kofta |
|------------|---------|-------|
| No. | % | No. | % |
| A. Candida spp. | 2 | 5 | 1 | 2.5 |
| - C. pseudotropicalis | 2 | 5 | 1 | 2.5 |
| - C. holmii | 7 | 17.5 | 4 | 10.0 |
| - C. famata | 5 | 12.5 | 7 | 17.5 |
| - C. valida | 4 | 10.0 | 0 | 0.0 |
| - C. gullermondii | 2 | 5.0 | 4 | 10.0 |
| B. Rhodotorula spp. | 10 | 25.0 | 9 | 22.5 |
| C. Saccharomyces spp. | 5 | 12.5 | 1 | 2.5 |
| D. Torulopsis spp. | 2 | 5.0 | 3 | 7.5 |

Table (7) revealed that the incidence and average concentration of aflatoxins (µg/kg) in the examined samples of meat products. The average conc. of aflatoxins in kofta was 11.5 ±3.3* with frequency and incidence of 12 (30%), while in sausage with an average concentration of 19.8±2.5* with frequency and incidence of 13(32.5%).

4. DISCUSSION

Meat and meat products are considered as major vehicles of most reported food poisoning outbreaks. Therefore, it is important to use the microbiological criteria to determine the quality of such products.

Processed meats may be contaminated with several types of microorganisms from different sources during the period of slaughtering, preparation, processing and cooking (Narasimha and Ramesh, 1988).

Concerning kofta samples, nearly similar results were obtained by El-Taher- Amna (2009) who recorded that the mean value of APC was 1.2×10⁴ for kofta. Sausage samples ranged from 2.4×10³ to 15×10² with a mean value of 1×10² ±5.4x10^⁰. Nearly similar results were obtained by Elmossallami (2003) who recorded that aerobic plate count of sausage samples was 9.3 x10⁵ organisms/g. Also, these results counts were higher than those reported by El-Maghrary-Marwa (2014); Ahmed- Alyaa (2015) and Hamed et al. (2015). Lower results were recorded by Hamouda (2005) who said that the mean value of aerobic plate count of sausage samples was 5x10².

The total Aerobic bacterial count of any food articles is not only a sure indicator of its safety for consumption, yet it is of importance in judging the hygienic conditions under which it has been processed and handled (Saad, 1976).

The results of coliforms count are higher than those reported by El-Maghrary-Marwa (2014); Ahmed- Alyaa (2015) and Hamed et al. (2015). The presence of Coliforms in meat and meat products indicates a potable faecal source of contamination which begins from slaughterhouse as a result of skinning of animals by knives and workers also during evisceration. Contamination may come from animal intestine, air and water used for washing and rinsing of carcasses (Gaalaf, 2009).

Incidence of mould in the examined meat samples were (62.5%) and (82.5%) for the examined samples of Kofta, and sausage respectively. The obtained results were nearly similar to those recorded by El-Diasty and Wahba (2008) who recorded that the incidence of mould in sausage samples was (80%), Sausage had a higher incidence as they are raw not heat treated beside the addition of some meat additives. The obtained results were nearly similar to that obtained by Lamada and Nassif (2008) who reported an incidence of 60% in kofta. However, the incidence was lower than Hussein (2008) who reported that the incidence of mould in kofta (93.3%).

These results were nearly higher than Shaltout and Salem (2000); Abu Zaid (2015) and Morshdy et al. (2015) who
mentioned that mean value of total mould count of sausage samples was $11\times10^3 \pm 3\times10^4$. While higher figure were reported by Shaltout (1996); Maha and Sohad (2005); El-Diasty and Wahba (2008) and Naas et al. (2009), who mentioned that examined fresh beef sausage samples had mould count $2.3\times10^2 \pm 2.7\times10^1$ CFU/g.

The obtained results of the examined sausage samples were nearly similar to those recorded by Shaltout and Salem (2000); and Hussein (2008). Meanwhile, the results of our present study were higher than those reported by El-Tabiy (2006) who found that the mean value of total mould counts in examined sausage samples were $1.0 \times 10^3 \pm 0.33 \times 10^0$ (CFU/g). The obtained results were lower than those reported by Eleiwa and El-Diasty (2014). According to the permissible limits stipulated by EOS (2005) for total mould and total yeast total yeast count (Free) in case of kofta, and sausage. (Table 8, Fig. 1).

| Product | EOS standard No. | EOS legislations | Accepted samples | Rejected samples |
|---------|------------------|------------------|------------------|------------------|
| Kofiga  | 19732005         |                   |                  |                  |
|         |                  | APC $>10^8$      | 29               | 72.5             |
|         |                  | No.              | 11               | 27.5             |
|         |                  | Coliform $>10^7$ | 34               | 85.0             |
|         |                  | No.              | 6                | 15.0             |
|         |                  | Mould & Yeast    | Free             | 12               |
|         |                  |                  | 30.0             | 28               |
|         |                  |                  |                  | 70.0             |
| Sausage | 19722005         |                  |                  |                  |
|         |                  | APC $>10^8$      | 28               | 70.0             |
|         |                  | No.              | 12               | 30.0             |
|         |                  | Coliform $>10^7$ | 30               | 75.0             |
|         |                  | No.              | 10               | 25.0             |
|         |                  | Mould & Yeast    | Free             | 7                |
|         |                  |                  | 17.5             | 33               |
|         |                  |                  |                  | 82.5             |

PL: Permissible Limit.

The results of mould identification agreed with those obtained by Seham- Ismail et al. (2013) who reported that 7 mould genera were identified in the examined samples. The identified mould genera were Aspergillus, Penicillium, Expeicillium, Cladosporium and Bysschalmys nivea. The predominant species were Aspergillus, and Penicillium. The frequencies of isolated mould genera in examined samples were A. niger 10 (26.3 %), and A. flavus 7 (18.4%).

Yeasts contribute a small but permanent part of the natural microbiota on meat. The results of identified yeast isolated from the examined sausage and kofta samples come in accordance with those recorded by Shaltout (1996), Samaha (2013), and Eleiwa and El-Diasty (2014) whom reported that mentioned that 7 species belonging to 4 yeast genera were isolated from examined sausage samples were Candida albicans, Candida krusei, Candida neoformans, Candida tropicalis, Cryptococcus spp., Rhodotorula spp. and Saccharomyces cerevisiae. While C. famata, C. pelliculosa, C. tropicalis, C. paraparastesis, Cryptococcus spp., Rhodotorula spp. and Torulopsis spp. were isolated from minced meat (Salem et al., 2015).

These findings may be attributed to use of unsterilized spices (untreated food additives) which usually carry mould spores used in manufacture of this meat products specially both of oriental sausage and kofta as this fresh products usually manufacture under unhygienic conditions in addition to use of inferior quality raw materials.

Regarding the incidence and average concentration of aflatoxins ($\mu$g/kg), higher values were detected by Refai et al. (2003) and El-Diasty (2013). These different mean values of aflatoxins residues may be related to the amount of additives contamination with aflatoxin and the amount of aflatoxin residues which may be present in animal muscles. At the same time, the mean values of detected aflatoxins in the examined samples were lower than the maximum permissible limit recommended by commission regulation (EC), the maximum levels of aflatoxins (aflatoxins B1, B2, G1, G2, and M1) are 10-15$\mu$g/kg and B1 is 5 $\mu$g/kg in as commission Regulation (EC) No. 1881/2006.

5. REFERENCES

1. Abu Zaid, K.E.A. (2015): Trials for Improving Mycological Quality of some Meat Products Using Essential Oils. M.V. Sc. Thesis, Meat Hygiene, Fac. Vet. Med. Benha Univ.
2. Ahmed-Alyaa, S. O. S. (2015):"Quality of Native and Imported Meat in the Egyptians Markets". M. V. Sc. Thesis (Meat Hygiene) Fac. Vet. Med. Cairo Univ.
3. Datta, S. A.; Akter, A.; Shah, I. G.; Fatema, K.; Islam, T. H.; Bandypadhyay, A.; Khan, Z. U. M. and Biswas, D. (2012): "Microbiological Quality Assessment of Raw Meat and Meat Products and Antibiotic Susceptibility of Isolated Staphylococcus aureus". J. Agric. Food Anal. Bacteriol. 2: 187-195.
4. El-Diasty E. M. and Wahba A. K. A. (2008): Proteolytic activity of some microorganisms isolated from meat products. J. Egyptian veterinary Medical Association, 68 (3): 257-270.
5. El-Diasty, E.M.; Ismail, A. S. and Shehata, A. A. (2013): Microbiological quality of some meat products in local markets with special reference to mycotoxins. Global Veterinaria 10 (5): 577-584.
6. Eleiwa, N.Z. and El-Diasty, E.M. (2014): Antifungal activity of dill essential oil (Anethum graveolens L.) in minced meat. VRPhymother., 2(1) 6-12.
7. El-Maghraby-Marwa, S. M. (2014): "Bacteriological evaluation of meat and some meat products at consumer level". M. V. Sc., Thesis (Meat Hygiene), Fac. Vet. Med.,Sudat City University.
8. El-Mossalam-Tahar, K. (2003): Risk assessment of ready prepared meat products, Ph. D., Thesis, Fac. Vet. Med., Cairo Univ.
9. El-Tabiy, A. A. (2006): Mycological study on some processed meat products exposed for sale in markets. Assiut Vet. Med. J. 52 (110) 121-131.
10. El-Taher-Amma, M. (2009): Impact of temperature abuse on safety of food offered in a University Student Restaurant. M.V.Sc.(Meat Hygiene), Fac. Vet. Med., Benha Univ. Egypt.
11. El-Tawab, M.M. (2014): Studies on mycotoxins in some meat products. M.V.Sc. (Meat Hygiene), Fac. Vet. Med., Benha Univ. Egypt.

12. “FDA” Food and Drug Administration (2001): Foodborne illness, what consumer need to know. USDA Food Safety and Inspection Service.

13. “FDA” Food and Agriculture Organization of the United Nations and World Food and Drug Administration (2013): Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring, P. 87-92: 232-292.

14. Gafaar, R.M.H. (2009): Quality evaluation of ready to eat meat products in Alexandria governorate. B.V.Sc. Fac. Vet. Med., Alexandria University.

15. Hamed, E. A.; Ahmed, A. S. and Abd El-Aaty, M. F. (2015): Bacteriological hazard associated with meat and meat products. Egypt. J. Agric. Res., 93, 4 (B): 385-393.

16. Hamouda, M.N. 2005: Microbiological risk assessment of some meat products. Ph.D. Thesis Meat Hygiene, Fac. Vet. Med., Beni-Suef University.

17. Hussein, M. A. M. (2008): Mycological Aspect of Fresh and Processed Meat Products with Special Respect to Proteolytic and Lipolytic Mold. Ph.D. Thesis (Meat Hygiene) Fac. Vet. Med., Zagazig Univ., Egypt.

18. "ICMSF” International commission of Microbiological Specification for Foods (1996): "Microorganisms in Food. Their Significance and Methods of Enumeration". 3rd Ed. Univ. of Toronto, Canada.

19. ISO (217-1-2:2008) “International Standards Organization EAST AFRICAN STANDARD: Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination-Part 1-3: Specific rules for the preparation of meat and meat products, 2008.

20. Komba, E. V. G.; Komba, E. V.; Mkpasi, E. M.; Mbuzi, A. O.; Mshamu, S.; Luwumba, D.; Busagwe, Z. and Mzula, A. (2012): Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzania. J. Health Res., 14 (2):1-12.

21. Kurtzman, C.P.; Boekhout, T.; Robert, V.; Fell, J.W. and Deak, T. (2003): Methods to identify yeasts. In: Yeasts in Food, T. Boekhout, V. Robert, eds.CRC Press, Germany. Pp. 69-121.

22. Lamada, H.M. and Nassif, M.Z. (2008): Mycological profile of meat product, with special reference to aflatoxins. 9th Vet. Med.Zag. Conference (20-22 August 2008) Port-Said.

23. Laura, E.S.; Rebecca, J.S. and Andrew, P. (2012): Food chain mycotoxin exposure, Gut health and impaired growth A conceptual framework. Adv Nutr 1;3(4):526-31.

24. Maha, I. A. E. and Sohad, H.E.E. (2005): Some chemical and mycological examinations of meat and fish products. Vet. Med. J. Giza. 55: 941-948.

25. Morshdy, A.E. M.A.; Hussien, M. A. M.; El-Abbasy M. T. and Elzawahery, R. R.M. (2015): Aflatoxins Residues in Some Meat Products. 2rd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine, I: 90-95.

26. Naas, H.T.; Garbit, A.M., Eshamah, H.L. and Abolghait, S.K. (2009): Microbial status of fresh beef sausage sold in Tripoli. SCVMJ, IVX (2):111-118.

27. Nasser, L.A. (2015): Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences 22(5): 513-520.

28. Narasimha, R.D., and Ramesh, U.S (1988): Microbial profiles of minced meat. Meat Science 23, 279-291.

29. Pitt, J.I. and Hocking, A.D. (2009): Fungi and Food Spoilage, 3rd Ed. Published by Blackie Academic and Professional Academic Press New York, London.

30. Refai, M.K.; Niazi, Z.M.; Aziz, N.H. and Khafaga, N.E. (2003): Incidence of aflatoxin B1 in the Egyptian cured meat basterma and control by gamma-irradiation. Nahrung; 47(6): 377-82.

31. Saad, S. M. (1976): Studies on sanitary condition for locally manufactured pastera. M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Zag. University.

32. Salem, R. M.; El-mosallamy, M.M and El-Saery, M.I. (2015): Quality and extent of minced meat is free of fungus with an attempt to reduce fungal contamination by using some vegetable oils. Ani. Health Res. J. 3 (1): 20-27.

33. Samaha, H. A. M. (2013): Frequency Rates of Fungal Contaminants in Imported Meats from Alexandrian Retail Markets. Life Sci. J. 10(4) 158-165.

34. Seham- Ismail, A.; Amal- Shehata, A. and El-diasty, E.M. (2013): Microbiological quality of some meat products in local markets with special reference to mycotoxins. Global Veterinaria 10 (5): 577-584.

35. Shaltout (1996): Mycological and Mycotoxicoxidological profile Of Some Meat products, Ph. D. Thesis, (Meat Hygiene), Fac. Vet. Med., Zag, Univ, Benha branch.

36. Shaltout, F.A. and Salem, R.M. (2000): Moulds, Aflatoxin B1 and Ochratoxin A in frozen livers and meat products. Vet. Med. J. Giza, 48 (3) 341-346.

37. Shaltout, F.A.; Nassif, M.Z.; Lotfy, L.M. and Gamal, B.T. (2019): Microbiological status of Chicken cuts and its products. Benha Vet. Med. J. 37 (1): 57-63.

38. Zafar, A.; Ahmed, E.; Wajiha, H. and Khan, A. (2016): Microbiological evaluation of raw meat products available in local markets of Karachi, Pakistan. Pakistan Academy of Sciences B. Life and Environmental Sciences. 53 (2): 103–109.