Vet.Med.J.,Giza. Vol.42,No.2.(1994):99-105.

MIOROFLORA IN LOCALLY PROCESSED FROZEN MEAT

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Received: 20/4/1994

SUMMARY

One hundred and sizty frozen meat, minced meat, sausage, beefburger, basterma, luncheon, frankfurter and kofta (20 samples each) were examined for the presence or absence of microflora. Aerobic plate count, MPN of Coliforms and Staph. aureus count were investigated. Neither *E. coli nor* Salmonellae could be isolated. The public health importance of the isolated microorganisms as well as the suggestive measures to minimize microflora in processed meat products and also to safeguard consumers were mentioned.

INTRODUCTION

Human food poisoning is commonly associated with pathogenic bacteria originated from animal sources. In most cases, consumption of contaminated meat and their products may lead to food poisoning (Report, 1970). Such contamination may occur during processing and handling before sale (Gilbert and Watson, 1971).

Food borne disease outbreaks are often associated with mishandling of ground beef in food service establishments, it was responsible for 67 % of food poisoning outbreaks, while mishandling in the food processing industry accounted for 3 % of such outbreaks. On the other, hand mishandling in private homes was implicated in 30 % of such outbreaks (U.S. Department of Health, Education and Welfare Public Health Service, 1975).

However, meat and meat products are considered a good media for growth of many organisms including E. coli, Salmonellae and *Staph. aureus*, the bacterial load of such organisms depends upon the rate of contamination during handling, processing, and storage. Moreover, the presence of indicator organisms in meat and their products is commonly accepted as an index of pollution and may lead to intestinal disturbances and food poisoning cases (Sadek and El-Afifi, 1965 and Roushdy, 1971).

On the other hand, high total Mesophilic bacterial count in food of animal origin is indicative of a great risk of pathogens being present, the presence of coliform group of microorganisms indicate unsatisfactry hygienic conditions and therefore, potential pathogenicity (ICMSF, 1980).

The objectionable heavy contamination of meat products with different types of Enterobacteriacae, Coliforms, *E. coli, Staph. aureus* and other contaminants may be responsible for the undesirable changes in the meat products that render them unfit for human consumption, and may at time, constitute a public health hazard (Al-Cherif, 1983 and Davey, 1985).

The collected data exatending over many years pointed out that many cases and outbreaks of food intoxication have been attributed to consumption of contaminated meat and their products with Staph. aureus (Sedik, 1982; Roushdy et al., 1983; Niazi *et al.*, 1986 and Tolba, 1991).

In the last years, trials were made to determine the correlation between the presence of Salmonellae as a potential food poisoning microorganisms and keeping quality of frozen meat (Abd El-Rahman *et al.*, 1992) as well as to improve the quality of packed meat by raising the hygienic measures of meat and meat contact surfaces (Abd El-Aziz, 1993).

Technological development in meat processing, preservation and handling have given consumers a

much greater choive over the foods they can buy. Consequently meat eaters are more aware of and sensitive to spoilage, off odours, strong flavour, discolouration and other indications of lack of freshness. Therefore product quality has become a more significant factor in marketing meat and meat products.

The present study was conducted to assure the presence of the microflora in locally processed frozen meat collected from different areas.

MATERIAL AND METHODS

Atotal of one hundred and sixty random samples of frozen meat, minced meat, sausage, beefburger, basterma, luncheon, frankfurter and kofta (20 samples each) were collected from the finished products of different areas in Cairo and Giza Governorates, collected in a sterile plastic bags and then transferred directly to the laboratory.

Handling and preparation of collected samples were carried out according to the techniques recommended by Lachica *et al.* (1971) and ICMSF (1980), before they were subjected to the following bacteriological examinations.

1. Determination of Aerobic Plate count (APC/g):

The drop plate method recommended by ICMSF (1978) was used. Inoculated plates were incubated at 3 ± 1°C for 48 hours for enumeration of Aerobic mesophilic bacteria.

2. Determination of Most Propable Number of Coliforms (MPN/g):

MPN/g was determined using three tubes Most Propable Number technique recommended by ICMSF (1978) and ISO (1975). Inoculated tubes were incubated at 37°C for 24 hours for enumeration of Most Probablr Number of Coliforms.

3. Isolation and identification of Coliform grganisms:

Suspected colonies on Eosin Methylene Blue (EMB) agar plates were isolated, purified and identified according to Kreig and Holt (1984).

4. Determination of Staph. aureus count/g:

The surface spread plate method recommended by ICMSF (1978) was used. Inoculated Baird-Parker agar plates with control were incubated at 37 °C for 24-48 hours for counting Staph. aureus.5.

5. Isolation and identification of Staph. aureus:

Separate conlonies of typical growth were purified on Brain Heart Infusion (BHI) slant slope and identified morphologically (Cruickshank et al., 1975) and biochemically using mannitol fermentation (Hugh and Lelfson, 1953) and coagulase production either bound (Flandrois and Carret, 1981) or free coagulase (ICMSF, 1978).

Enterotoxigenicity of isolated Staph, aureus strains:

Isolated Staph, aureus strains were tested for the ability to produce enterotoxins using sac culture method recommended by Donnelly et al. (1967). The detection and serological typing of enterotoxins were carried out by Reversed Passive Latex Agglutination (RPLA) technique recommended by Dda et al. (1979) and Shingaki et al. (1981) using Oxoid EST-Rpla Kits.

6. Detection of E. coli:

The technique recommended by ISO (1975) was applied.

7. Detection of Salmonellae:

The technique adopted was that recommended by Harvey and Price (1981).

RESULTS AND DISCUSSION

It is evident from the achieved results (Table 1) that the mean + SE of Aerobic mesophilic, Staph. aureus and Coliforms counts of examined frozen meat samples were $1.2 \times 10^5 \pm 2.9 \times 10^4$, 3.3 ±

Table (1) Statistical analytical results of the microbiological quality of examined samples.

Type of examined samples	No of samples	APC	Staph. count	Coliform count
Frozen meat Min Max Mean+S.E	20	$14x10^2$ $4.0x10^5$ $1.2x10^5+2.910^4$	10^2 $2.0x10^3$ $3.3x10^2+98.9$	<3 $3x10^2$ 66.1+17.9
Minced meat Min Max Mean+S.E	20	$22x10^3$ $3.0x10^7$ $2.2x10^6+2.910^6$	10^2 $6.0x10^3$ $9.9x10^2+3.9x10^2$	15 $6x10^3$ $7.94x10^2+2.96x10^2$
Sausage Min Max Mean+S.E	20	$22x10^2$ $14x10^6$ $1x10^6+7x10^5$	10^2 $3x10^3$ $2.5x10^2+1.310^2$	<3 $11x10^3$ $4.41x10^3+9.61x10^2$
Kofta Min Max Mean+S.E	20	$2.0x10^3$ $4.0x10^6$ $2.9x10^5+1.9x10^5$	10^2 $9x10^3$ $1.0x10^3+5.5x10^2$	10^2 $6x10^3$ $1.1x10^3+3.4x10^2$
Frankfurter Min Max Mean+S.E	20	$6.0x10^3$ $3.0x10^5$ $6.9x10^4+1.9x10^4$	10^2 $9x10^2$ $1.8x10^2+10^2$	<3 15 5.2+0.98
Beefburger Min Max Mean+S.E.	20	$13x10^3$ $12x10^5$ $2.0x10^5+7x10^4$	10^2 $8x10^3$ $7.6x10^2+3.8x10^2$	10^2 $4x10^3$ $1.19x10^3+2.79x10^2$
Luncheon Min Max Mean+S.E.	20	$3.0x10^2$ $8.0x10^5$ $1.3x10^5+4.3x10^4$	10^2 $8x10^2$ $2x10^2+49.5$	<3 15 3.9+0.62
Basterma Min Max Mean+S.E.	20	$8.0x10^2$ $3.0x10^6$ $3.9x10^5+1.7x10^5$	$2x10^2$ $2x10^3$ $5.5x10^2+10^2$	<3 90 17.1+5.9

$10^2 \pm 98.9$ and 66.1 ± 17.9 respectively. These findings agree with that reported by oblinger and Kennedy (1978) who found that the mean value of Aerobic mesophilic mesophilic and Coliforms counts were 2.3 x 104 and 1.8 x 10 respectively, while the Staph. aureus count ranged from O to 2.3 x 10. Higher results were recorded by Refai et al. (1991) who found that the meanvalue of APC of examined frozen meat samples in Assiut was 2.7×10^6 where Staph. aureus count was 1.9×10^4 / g.

In examined minced meat samples, the mean + SE of Aerobic mesophilic, Staph. aureus and Colifroms counts were $2.2 \times 10^6 \pm 1.3 \times 10^6$, $9.9 \times 10^2 \pm 3.9 \times 10^2$ and $7.9^4 \times 10^2 \pm 2.9^6 \times 10^2$ respectively. These findings were in agreement with that reported by Rao (1973) Smith *et al.* (1975): Foster *et al.* (1977) and Darwish et al. (1986). The comparatively higher findings reported by Roushdy (1971), Elmossalami and Roushdy (1973), Duitschaever *et al.* (1973), Roushdy *et al.* (1983) and Youssef et al. (1985) may be due to contamination of minced meat during processing and handling.

Concerning sausage, the mean value of Aerobic mesophilic, Staph. aureus and Coliforms counts were $10^6 \pm 10^5$, $2.5 \times 10^2 \pm 1.3 \times 10^2$ and $4.41 \times 10^3 \pm 9.61 \times 10^2$ respectively. These findings are consistant with that reported by Sorour (1978). Foda *et al.* (1984) and El-Kotary *et al.* (1986) while higher records were obtained by Sadek (1963) and Sadek and El-Afifi (1965). Lower findings were recorded by Ameen (1976). Table

(1) illustrate the mean value of Aerobic mesophilic count, Staph. aureus and Coliforms counts of examined kofta samples. They were 2.9 x 10^5 ± 1.9 x 10^5, 10^3 ± 5.5 x 10^2 and 1.1 x 10^3 ± 3.4 x 10^2 respectively. In this respect Darwish *et al.* (1986) found that the mean value of Aerobic mesophilic count of rice kofta was 107 while it was 2.7 x 10^6 for kofta panee and 7.2 x 10^5 of kofta for grill.

The examined frankfurt samples showed that the mean Aerobic mesophlic, Staph. aureus and Coliforms counts were 6.9 x 10^4 ± 1.9 x 10^4, 1.8 x 10^2 ± 10^2 and 5.2 ± 0.98 respectively.

Concerning examined beefburger samples the mean Aerobic mesophilic, Staph. aureus and Coliforms counts were 2 x 10^5 ± 7 x 10^4, 7.6 x 10^2 ± 3.8 x 10^2 and 1.19 x 10^3 ± 2.79 x 10^2 respectively. These results are consistant with that obtained by Ibrahim (1981) and Tolba (1991) while higher counts obtained by Taminga *et al.* (1980).

Regarding the examined luncheon meat samples, the mean Aerobic mesophilic, Staph. aureus and Coliforms counts were 1.3 x 10^5 ± 4.3 x 10^4, 2 x 10^2 ± 49.5 and 3.9 ± 0.62 respectivel. Tolba (1991) concluded that the mean Staph. aureus conts of luncheon meat samples collected from urban areas during Winter and Spring months as well as during Summer and Autumn months were 1 x 10^2 and 1.9 x 10^2 respectively. This substantiates the findings in the present investigation.

The achieved results showed that Aerobic mesophilic, Staph. aureus and Coliforms counts of examined basterma samples were 3.9 x 105 ± 1.7 x 105, 5.5 x.102 ± 102 and 17.1 ± 5.9 respectively. Al-Cherif (1983) and Tolba (1986) found that the mean Staph. aureus counts of examined basterma samples were 1.8 x 104 and 4.4 x 106 respectively.

Concerning isolates of public health siginficance, neither *E. coli* nor Salmonellae could be detected from any of the examined meat and meat products samples. This may be attributed to the rapid death of *E. coli* during storage (Elliott and Michner, 1984). On the other hand Citrobacter freundii, Enterobacter agglumerans, Enterobacter aerogenes, Proteus vulgaris, Proteus mirabilis,

Table (2) Incidence of colifors organisms isolated from examined samples.

Isolated organisms	Frozen meat		Minced meat		Saueage		Frank-futer		Beef-burger		Luncheon		Basterma		Kofta	
	+ve		+ve		+ve		+ve		+ve		+ve		+ve		+ve	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Citrobacter Freundii	3	15	7	5	3	15	1	5	4	20	-	20	1	5	-	0
Enterobacter agglumerans	1	5	2	10	-	0	1	5	-	0	-	0	-	0	-	0
aerogenes	-	0	4	20	2	10	1	5	4	20	-	0	-	0	1	5
Proteus Vulgaris	1	5	1	5	2	10	-	0	1	5	1	5	-	0	2	10
mirabilis	1	5	-	0	1	5	-	0	-	0	1	5	1	0	-	0
morgani	-	0	1	5	-	0	1	5	1	5	-	0	-	0	-	0
Shigella boydii	-	0	-	6	1	5	-	0	-	0	-	0	-	0	-	0
Klebsiells aerogenes	1	5	-	0	4	20	1	5	-	0	1	5	-	0	2	10
Cloacae	-	0	1	5	1	5	1	5	2	10	-	0	-	0	-	0

Table (3): Incidence and distribution of Staphylococcal enterotoxins in examined meat products.

Type of examined samples	Coagulase +ve Staph.aureus	Enterotoxigenic Staph. aureus isolates									
		Single producer								Multiple producer	
		A		B		C		D		A+D	
		No	%	No	%	No	%	No	%	No	%
Minced meat	1	-	0	-	0	-	0	-	0	-	0
Sausage	1	-	0	-	0	-	0	-	0	-	0
Basterma	1	1	100	-	0	-	0	-	0	-	0
Kofta	2	-	0	-	0	-	0	-	0	1	50

Proteus morgani, Shigella boydii, Klebsiella aerogenes and Klebsiella cloacae could be isolated at different percentages from the examined samples ranged from 0.13 % to 8.12 %(Table 2).

Presence of Coliforms in meat products may be indicative of faulty methods of preparation, handling, processing and storage, which may lead to economic losses through the development of undesitable changes rendering the quality of the product unfit for human consumption or even constitutes a public health hazard (ICMSF, 1978). Moreover, some of the isolated microorganisms have been implicated in food illness e.g. Proteus spp., Citrobacter freundii, Enterobacter species Shigella boydii and Klebsiella species (Krieg and Holt 1984 and Abdel-Aziz, 1993).

It is evident from the results achieved in Table (3) that five Staph. aureus coagulase positive isolates were tested for their enterotoxigenicity. Only one isolate from basterma was able to produce enterotoxin type A. the second was isolated from Kofta and it was type A and D toxin producer.

The human race is considered to be the most important source of Staph. aureus in food as it has been found that approximately 40% of humanbeing harbour normaly Staph. aureus in their nose and throat, hence the fingers tips are often contaminated with Staph. aureus. This substantiates the findings reported by (Williams, 1963).

Baird-Parker (1971); Dempester *et al* (1973) and Tolba (1991) found that 50% of isolated Staph. aureus strains were enterotoxigenic and this substantiates the findings reported in the present investigation (50 - 100%).

Many investigators dealt with enterotoxigenic Staph. aurus; Casman (1965) found that 45 (90%)out of 50 tested Staph. aureus strains were enterotoxigenic and produced enterotoxin type A. While Sedik (1982) found that 36 % of totally 100 Staph. aureus strains examined were enterotoxigenic.

Imporoving the sanitary status of meat products and safeguarding the consumers from recieving contaminated meat products can be acheived by prolonging the durability of the product through the reduction of its bacterial load which in-turn protect the products from being spoiled in the market. Moreover, it protects the consumer from pathogens which may be present in meat products.

Good manufacturing practice (GMP) should be followed in order to assure safety and quality of the products. The manufacturer should be also referred for meat handling to Codex Alimentarius Commission (CAC) (1976): Code of hygienic practice for processed meat products.

Moreover, effective control of food borne diseases require that the (HACCPS) the Hazard Analysis Critical Control Point System which was

developed in USA should be employed.

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