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Microbial Contamination of Meat Products Produced in the Factories of West Azerbaijan Province, North West of Iran

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Abstract: Increasing reports of food borne diseases especially secondary contamination of foods after processing, enhanced the issue of food safety concerns about consumers, producers and others in the food industry. Meat and meat products are the main food sources in daily diets of people in developing countries that infected easily by different microorganisms. This study aimed to investigate the prevalence of food infections and microbial contamination of meat products. Forty two samples of meat products including sausages, burgers, kebabs and cutlets were taken from 12 factories since the years 2011 until 2013. Microbial contamination of samples were examined according to Iranian national standards No. 5272, 9263, 2197, 10899-1 and 3, 1810, 6806-1 and 3 and 2946 for total count of microorganisms, coliform, Clostridium, Mold and yeast, *Salmonella*, *Staphylococcus aureus* and *Escherichia coli*, respectively. The obtained results showed that the microbial quality of meat products in West Azarbaijan province was good and 5% of samples were unacceptable according to the Iranian national standards. Subsequently 5% prevalence of non-consumable meat products and low pollution shows proper sanitation and good hygienic quality of the production process of meat products in the farm, slaughtering, cold storage and sanitary production process is in West Azerbaijan province.

Key words: Meat Products · Microbial Standards · Microbial Contamination · West Azerbaijan Province · Iran

INTRODUCTION

Meat and meat products are the main food sources in daily diets of people in developing countries that affected by several factors. Meat products diversity and their ease to use and do not require for cooking of them increased their using, especially in young people [1,2]. Producing and Consumption of 200000 tons of meat products in Iran, requires careful monitoring of the production and supply of these products Meat and meat products may be infected by different microorganisms, easily. Not suitable conditions of transportation and storage, leading to the growth of spoilage and pathogenic microbes, reducing the meat quality and jeopardizing public health [3].

Increasing reports of food borne diseases especially secondary contamination of foods after processing, enhanced the issue of food safety concerns about consumers, producers and others in the food industry [4].

It is estimated that 30 percent of people in industrialized countries suffered at least once a year from food borne diseases [6,5].

Food borne diseases and food poisoning has always been a major problem worldwide. Due to increasing in consumption of meat products, sausages in particular and developing of industrial manufacturers, great efforts take place to produce health products with good quality.

Various types of meat products, including sausages, bergers, Kababs, cutlets and etc are produced in Iran like other countries. Use of meat products have increased due

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to changing in people's daily life and lifestyle changing to modernization. Meat producing and consumption increased in West Azerbaijan province (North West of Iran), therefore, good monitoring on their production is important. In this study, it's tried to investigate microbial contamination of meat products in West Azerbaijan province of Iran.

MATERIALS AND METHODS

Sampling: Forty two samples of meat products including sausages, burgers, kebabs and cutlets were taken from 12 factories since the years 2011 until 2013. Microbial contamination of samples was examined according to Iranian National Standards. Tests used for assessment various meat products are showed in the following tables.

Tests used for bacterial and fungal assessment of burgers, sausages, kababs and cutlets, acceptable limit and reference methods are shown in the following tables.

Total Count of Microorganisms (Standard No. 5272): From 1/10 diluted sample 1 mL was poured into a sterile plate and then 15 cc of PCA medium (with a temperature of 44°C) was added and stirred. After hardening of medium, plates were incubated upside down at 30°C for 24-48 hours. After incubation period, colonies were counted. In the absence of colony growth result was reported in less than dilution was cultured [7].

Coliform Count (Standard No. 9263): From 1/10 diluted sample 1 mL was poured into in a sterile plate and then 15 cc of VRBA medium (With a temperature of 44°C) was added and stirred. After hardening of medium, plates were incubated upside down at 37°C for 24-48 hours. After incubation period, colonies were counted. In the absence of colony growth result was reported in less than dilution was cultured [8].

Clostridium Count (Standard No. 2197): From 1/10 diluted sample 1 mL was poured into in a sterile plate and then 15 cc of Sulfite agar medium (With a temperature of 44°C) was added and stirred. After hardening of medium, plates were incubated upside down at 37°C for 24-48 hours. Colonies were counted after incubation period. In the absence of colony growth result was reported in less than dilution was cultured. Black colonies are due to reduction of sulfite by Clostridium [9].

Mold and Yeast Count (Standard No. 10899 and 3): On sterile plates containing DRBC, 0.1 mL of desired dilution distributed. Molds colonies were counted after 3-5 days of incubation at 25 °C [10].

From desired dilution of sample, 1 mL was poured on sterile plate containing DRBC and after that 15 mL of Y coca medium (With a temperature of 44°C) was added and stirred. After hardening of medium plates were incubated upside down at 25 ° C for 3-5 days [10].

Table 1: Tests, acceptable limits and reference methods for bacterial and fungal assessment of burgers

NO	Reference method	Acceptable limit	test
1	Iranian national standard no. 5272 [7]	Max 10^6	Total count (CFU/ˆg)
2	Iranian national standard no. 6806 1 and 3 [12]	Max 10^3	Coagulase Positive *Staphylococcus* (CFU/g)
3	Iranian national standard no. 10899 [10]	Max 10^3	Mold (CFU/g)
4	Iranian national standard no. 1810 [11]	Negative	*Salmonella* per 25 g

Table 2: Tests, acceptable limits and reference methods for bacterial and fungal assessment of Sausages

No	Reference method	Acceptable limit	Test
1	Iranian national standard no. 5272 [7]	Max 10^5	Total count (CFU/g)
2	Iranian national standard no. 9263 [8]	Max 10	Coliforms (CFU/g)
3	Iranian national standard no. 2946 [14]	Negative	*Escherichia coli* (CFU/g)
4	Iranian national standard no. 6806-1 and 3 [12]	<10	Coagulase Positive *Staphylococcus* (CFU/g)
5	Iranian national standard no. 2197 [9]	Max 50	*Colistridium perferingens* (CFU/g)
6	Iranian national standard no. 10899 [10]	Max 10^2	Mold and yeast (CFU/g)
7	Iranian national standard no. 1810 [11]	Negative	*Salmonella* per 25 g

Table 3: Tests, acceptable limits and reference methods for bacterial and fungal assessment of Kababs

NO	Reference method	Acceptable limit	test
1	Iranian national standard no. 5272 [7]	Max 10^6	Total count (CFU/g)
2	Iranian national standard no. 6806-1 and 3 [12]	Max 10^3	Coagulase Positive *Staphylococcus* (CFU/g)
3	Iranian national standard no. 10899 [10]	Max 10^3	Mold and yeast (CFU/g)
4	Iranian national standard no. 1810 [11]	Negative	*Salmonella* per 25 g

Table 4: Tests, acceptable limits and reference methods for bacterial and fungal assessment of Cutlets

No	Reference method	Acceptable limit	Test
1	Iranian national standard no. 5272 [7]	10^5	Total count (CFU/g)
2	Iranian national standard no. 9263 [8]	10^2	Coliforms (CFU/g)
3	Iranian national standard no. 2946 [14]	Negative	*Escherichia coli* (CFU/g)
4	Iranian national standard no. 1810 [11]	Negative	*Salmonella* per 25 g
5	Iranian national standard no. 6806-1 and 3 [12]	Negative	Coagulase Positive *Staphylococcus* (CFU/g)
6	Iranian national standard no. 10899 [10]	10^2	Mold and yeast (CFU/g)

***Salmonella* Isolation (Standard No. 1810):** Test was done in three days:

- First day: 5 g of sample is added in 45 ml and mixed well (0.1 dilution).
- Second day: 1 mL of diluted sample is added into tubes containing 10 ml tetrationate broth.
- Third day: 0.1 ml of prepared solution of second day are cultured on SS agar medium and incubated at 37 ° C for 24-48 hours [11].

***Staphylococcus aureus* Count (Standard No. 6806-1 and 3):**

- Standard No. 6806-1 is used if the limit upper than 100: 1 ml of prepared dilution distributed on Baired-Parker agar medium containing egg yolk and incubated at 37 ° C for 1-2 days. Black colonies with a colorless zoon are *Staphylococcus aureus*. To confirm the colonies coagulase test is used. *Staphylococcus aureus* colonies are coagulase positive [12].
- Standard No. 6806-3 is used if the limit is 1-100: test was done in three sections with nine tubes.
  - Section 1: 10 ml of diluted sample (0.1 dilutions) is added to 3 tubes containing giolet Cantonee broth
  - Section 2: 1-2 ml of diluted sample (0.1 dilutions) is added to 3 tubes containing giolet Cantonee broth
  - Section 3: 0.1 ml of diluted sample (0.1 dilutions) is added to 3 tubes containing giolet Cantonee broth

Tubes were incubated in anaerobic conditions at 37°C. After 24-48 hours tubes with black bottom cultured on Baired-Parker agar medium at 37°C for 2-1 days. Black colonies with colorless halo suspected as *Staphylococcus aureus* that confirmed with coagulase test [13].

Escherichia coli count (Standard No. 2946) [14].

RESULTS

Results of the bacterial and fungal assessment of meat products including burgers, sausages, kababs and cutlets showed in Tables 6, 7, 8 and 9, respectively. As shown in Graph 1, 92% of produced burgers are acceptable in point of view microbial standards. All samples (100 %) of sausages, kababs and cutlets are consumable (Tables 7, 8 and 9).

Table 6: Bacterial and fungal test results for samples burgers

No	Total count (CFU/g)	Coagulase Positive *Staphylococcus* (CFU/g)	Mold and yeast (CFU/g)	*Salmonella* per 25 g	Acceptability
1	$1880\pm10^4\times2$	Less than 10	$285\pm10^3\times1.34$	Negative	Non acceptable
2	$1800\pm10^4\times1.76$	<10	$285\pm10^2\times8.35$	Negative	Acceptable
3	$10^3\times8.78$	<10	$10^2\times5.4$	Negative	Acceptable
4	$1880\pm10^2\times1.44$	<10	<10	Negative	Acceptable
5	$1880\pm10^4\times1.02$	$10^2\times6.8$	$28.5\pm10^2\times1.44$	Negative	Acceptable
6	$1880\pm10\times1.4$	$10^2\times 3.83$	$28.5\pm10^2\times3.95$	Negative	Acceptable
7	$10^2\times2.16$	<10	<10	Negative	Acceptable
8	$1880\pm10^4\times1.14$	<10	$28.5\pm10^2\times2.1$	Negative	Acceptable
9	$1880\pm10^4\times2$	<10	$285\pm10^3\times1.34$	Negative	Non acceptable
10	$1800\pm10^4\times1.76$	<10	$285\pm10^2\times8.35$	Negative	Acceptable
11	$10^3\times 2.43$	<10	<10	Negative	Acceptable
12	$10^2\times 2.45$	$10^2\times1.66$	$10^2\times 4.27$	Negative	Acceptable

Table 7: Bacterial and fungal assessment results of Sausage sample

NO	Total count (CFU/g)	Coliforms (CFU/g)	*Escherichia coli* (CFU/g)	Coagulase Positive *Staphylococcus* (CFU/g)	*Colistridium perferingens* (CFU/g)	Mold and yeast (CFU/g)	*Salmonella* per 25 g	Acceptability
1	$4.4\times10^{2}\pm1880$	<10	Negative	<10	<10	<10	Negative	Acceptable
2	$2.75\times10^{2}\pm28.5$	<10	Negative	<10	<10	<10	Negative	Acceptable
3	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
4	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
5	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
6	5.64×10^{2}	<10	Negative	<10	<10	<10	Negative	Acceptable
7	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
8	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
9	<10	<10	Negative	<10	<10	<10	Negative	Acceptable
10	<10	<10	Negative	<10	<10	<10	Negative	Acceptable

Table 8: Bacterial and fungal assessment results of Kabab samples

No	Total count (CFU/g)	Coagulase Positive *Staphylococcus* (CFU/g)	Mold and yeast (CFU/g)	*Salmonella* per 25 g	Acceptability
1	$1.3\times10^{2}\pm1880$	<10	<10	Negative	Acceptable
2	$6.9\times10^{2}\pm1880$	<10	<10	Negative	Acceptable
3	$2.18\times10^{4}\pm1880$	<10	$285\pm10^{2}\times7.45$	Negative	Acceptable
4	$2.34\times10^{4}\pm1880$	<10	$28.5\pm10^{2}\times8$	Negative	Acceptable
5	$2.18\times10^{4}\pm1880$	<10	$285\pm10^{2}\times7.45$	Negative	Acceptable

Table 9: Bacterial and fungal assessment results of Cutlet samples

No	Total count (CFU/g)	Coliforms (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* per 25 g	Coagulase Positive *Staphylococcus* (CFU/g)	Mold and yeast (CFU/g)	Acceptability
1	$3.35\times10^{3}\pm1880$	<10	Negative	Negative	Negative	<10	Acceptable
2	2.75×10^{3}	<10	Negative	Negative	Negative	7.01×10	Acceptable
3	<10	<10	Negative	Negative	Negative	<10	Acceptable
4	<10	<10	Negative	Negative	Negative	<10	Acceptable
5	<10	<10	Negative	Negative	Negative	<10	Acceptable

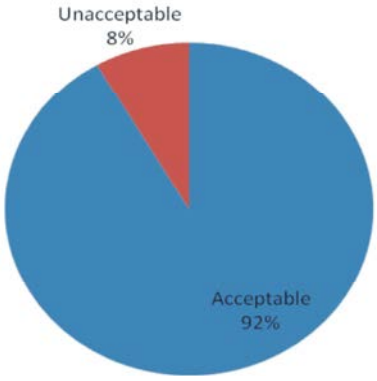

Fig. 1: Acceptability of Burger samples

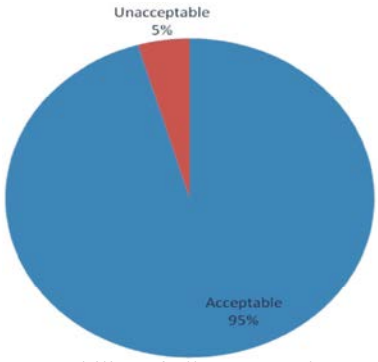

Fig. 2: Acceptability of all meat products samples in this study

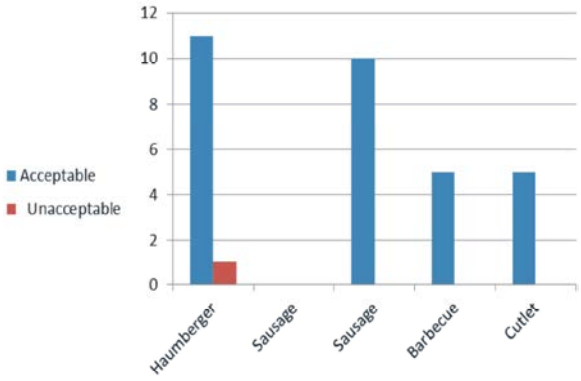

Fig. 3: Comparison of acceptable and non-acceptable samples of this study

DISCUSSION

Compare the present study results with previous studies examined the same products.

According to the obtained results, 95% of the meat products had acceptable bacterial and fungal quality and microbial numeration and only 5% of the samples was high (Graph 2).

In most countries, Staphylococcal food poisoning produced by the enterotoxin ingestion of bacteria that is the second most common food poisoning that results in

epidemic or sporadic [15-17]. *Staphylococcus aureus* is one of pathogenic microorganisms in meat products, especially those has contacts with the hands of producers or consumers [18] and currently has endangered public health worldwide [19].

In a research on 1047 food sample, Sultan Dalal and coworkers showed that 100 sample (9.6%) were contaminated with *Staphylococcus aureus* [20]. In other study, 4 of 360 samples of kababs and cooked burgers contaminated with *Staphylococcus aureus* in south area of Tehran [21]. An investigation on 1634 samples of meat and dairy products to assess *Staphylococcus aureus* showed contamination in 12.8 % of all samples [22]. Contamination of the meat products of this study was very low.

Escherichia coli is the most common aerobic microorganism in the intestinal tract of humans and animals which is known as indicator of fecal contamination of food and water. According to the reports *Escherichia coli* can be transmitted to humans through meat, milk and their products. Cooked foods are free from contamination because of destroying of the bacteria at 55° C temperatures [23-26].

Revelli and coworkers declared that mean value of total count in 6998 milk samples collected in the Alpine regions of Spain was 1.2×10^5 - 2.4×10^5 CFU/mL [27]. Holm and coworkers reported microbial population lesser than 10^6 CFU/mL [28].

E. coli is important due to toxicity [29]. In an outbreak in France, 69 cases of *Escherichia coli* O157: H 7 were seen in the ground meat [30]. Contamination of beef carcasses with *Escherichia coli* have been reported from Turkey and Bangladesh [31-32].

Salmonellosis is one of the major food borne diseases that has led to a public health concern, recently [33]. Salmonellosis is second food borne disease caused by consuming contaminated foods with *Salmonella* spp. (With over 2,500 serotypes of bacteria) in the United States [34] and has a large outbreak in Asia (Korea, Japan, Thailand, etc) [35]. Soltan dalal *et al*. reported 17.81% contamination with *Salmonella* spp. in packed and non-packed meat and poultry meat in Tehran [36].

Beef contamination with *Salmonella* was 2.6% in 1970 (37). In 2009, the Center for Disease Control, reported *Salmonella* Typhimurium as one of the causes of food borne diseases from beef [38].

In a study performed in South Korea, *Salmonella* serotypes were isolatedfrom 25.9% of grilled chicken. Isolated serotypes were *Salmonella* Entritidis, *Salmonella* Warsaw, *Salmonella* Virginia [39].

West Azarbaijan province is one of the centers of livestock and poultry production in Iran, Therefore, it's necessary to prevent the spread of microbial contaminants such as *Salmonella*, *Escherichia. coli*, coliforms, *Staphylococcus* and Clostridium in order to providing consumer health. As regards to obtained results of this study in comparison with previous studies, 5% prevalence of non-consumable meat products and low contamination shows proper sanitation and good hygienic quality of the production process of meat products in the farm, slaughtering, cold storage and sanitary production process is in West Azerbaijan province of Iran.

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