

Full Length Research Paper

Study and comparison of the bacterial contamination outbreak of chicken meat consumed in some cities of Mazandaran Province (Iran)

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Since meat is the richest protein source and plays an important role in the transmission of bacteria, especially *Zoonosis* to humans, the pathogenic bacteria outbreaks such as *Salmonella*, *Campylobacter*, *Yersinia*, and *Aeromonas* in the packed and unpacked chicken meat supplied to some cities of Mazandaran province of Iran performed, which have not been examined and analyzed yet. 200 samples of chicken meat from the early summer of 2010 to late winter of the same year were prepared and tested for 9 months. Sampling was conducted in the places where the meat were packed as well as the suppliers which had no packing and sold their materials in bulk in different areas of the cities of Mazandaran (Amol, Tonekabon, and Noor cities). All needed tests done exactly based on the instructions No. 2394 of Iranian National Standards Institute. Among the 200 samples of chicken meat prepared from Amol, Tonekabon, and Noor, the contamination of 75 samples were positive. 33 cases of the chicken were contaminated with *Salmonella* (21.5%), 53 samples with *Campylobacter* (26.5%), 18 samples with *Yersinia enterocolitica* (9%) and 14 samples were contaminated with *Aeromonas* (7%). The observed differences among the samples taken from different areas of the given cities, both in packed and unpacked forms, were statistically significant ($P < 0.05$) and also the unpacked and packed meat product supplying centers in Mazandaran were not significantly different for having some differences in the type of contaminations.

Key words: Pathogenic microbes, chicken meat, Mazandaran province, Iran.

INTRODUCTION

Nowadays, regarding the advances in food industry and science technology, the food related diseases is still an unsolved and inexplicable problem, which has not been unraveled. Many people from all around the world are suffering from the food borne diseases. This is a significant issue in the developing countries especially the individuals whose immune system is weak or those

with malnutrition (Egli et al., 2002). In the last decade, there has been an increase in the number of diseases, *Yersinia* have been reported to be the factors, which instigate the disease outbreak in different kinds of food which are caused by poisoned food (Todd, 1997). Among the pathogenic bacteria, the *Salmonella*, *Listeria*, and such as fish, dairy product, vegetable, meat, and its related product. The epidemiologic studies and analysis have shown that foods with animal product origin are abundant source of diseases, which are spread and transferred through foods (Todd, 1997; Tauxe, 1997).

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Other sources of human infection with the given organisms includes the product contamination and being in contact with the livestock in the farm especially the dogs and person-to-person contamination. Studies conducted on the meat product contamination have shed lights on the fact that the percentage of the contamination to the *Salmonella*, *Listeria*, and *Yersinia* is different from countries to countries (Logue, 1996). The food contamination to such pathogens could be triggered in different food preparation cycles including the producing, processing, distributing, retailing and preparing (Petersen, 1998).

One way to refrain from such contamination is distributing and supplying the food in packs. Nowadays, in most industrialized countries, protein enriched foods, vegetables, fruits, and so many other kinds of food, cooked or uncooked, will be packed to observe the people's health. Unfortunately, most kinds of foods are produced and supplied through traditional processes since meat products are a major source of food for people, it can serve as an important factor in transferring the pathogenic factors to human beings (Petersen, 1998; Dominguez et al., 2002; Capita, 2002; Neyts, 2000; Takeamaru, 1991; Zhao et al., 2001). Because no similar studies have focused on the issue and lack of access to the data for planning, supporting, and encouraging the packing industry and healthy supply of food with protein, the need for conducting the study in the case of those living in Tehran was imperative.

MATERIALS AND METHODS

This is a descriptive-analytical case study, which was conducted from the early summer of 2010 to late winter of the same year. 200 samples of packed and unpacked chicken meat supplied in the cities of Mazandaran which were tested on 80% test with the reliability level of 95% were selected randomly in 9 months from different wholesalers and retailers in the mentioned cities of Mazandaran and after being kept in refrigerator, they were transferred to Dr. Shohreh laboratory on the same day for preparing the sample to be examined and analyzed for the microbial evaluation based on the Iran national standard of 2394 and other resources. After collecting the data, they were analyzed through a windows-based SPSS software version 11.5 by using the CHI 2 square analysis and the OR values of the reliability level of 95% were used. The significance level considered at ($P < 0.05$).

The technique used for *Salmonella*

Pre- enriching stage

25 g of the meat (red meat: grated, chicken meat: sliced) is added to 225 ml of Lactose broth in sterile condition and is mixed and homogenized with stomacher and will be kept at 37°C for 24 h.

Enriching stage

(i) 1 cc of the last stage mixture is added to 9 cc of Tetrathionate

10863 broth, Merck, and was kept at 43°C for 24-48 h.

(ii) 1 cc of the previous stage condition was added to Selenite cystine broth, HiMedia, M 1079 and was kept at 37°C for 24-48 h.

(iii) One loop of the last two enriched stages were separately cultured on *Salmonella- Shigella* Agar Biolife, 402075 and were kept at 37°C for 24-48 h.

(iv) Lactose negative colonies (colorless) were regarded as open to question and unresolved colonies containing *Salmonella* either producing SH₂ or not.

(v) Selecting the unresolved or suspicious colonies and inoculating them into differential condition that Coligragar SIM, Broth uric was kept for 18-24 h and was transferred into differential condition of Simon Citrate agar, Lysine Iron Agar, Methyl red, and Malonate Broth.

(vi) For confirming and serotyping, the *Salmonella* strains with the Biomerieux kit were conducted.

The technique used for the *Campylobacter*

The enriching stage

25 g of chicken meat (both packed and unpacked) was added to 100 ml of Brucella Broth.

Homogenization stage

(i) They were mixed for 60 s by a stomacher and were kept at 42°C for 48 h.

(ii) 1 cc of the last stage mixture was added to Peptone water or diluted Ringer solution.

(iii) 0.1 cc of the given mixture was cultured on the *Campylobacter* selective agar, Merck 2248 and the plates were kept in anaerobe jar at 42°C at micro-aerophil for 48 h.

(iv) The questionable colonies were selected and after Gram coloring and observing the gram-negative, complicated and bent Bacillus, they were analyzed using the oxides, Catalase and hyporate hydrolyze tests.

The technique used for the *Yersinia*

The cold temperature enriching stage

25 g of meat was added to 225 Phosphate Buffer Salina with the pH of 7.2 and was totally mixed and freeze for 21 days.

(i) After freezing the microbes were cultured directly and indirectly on 7, 14, and 21 days.

Direct culturing

One loop of the first stage was inoculated on the selective condition of CIN agar, Biolife ,401302 and then the plated were kept at 25°C for 24-48 h.

Indirect culturing

0.5 cc of the first stage mixture was added to 4.5 cc of 25% KOH and after 30 s exposure, one loop was selected to be inoculated on the CIN condition and was kept at 25°C for 24-48 h.

(i) All the red colonies, positive Monitol colonies, were considered as suspicious colonies, which were analyzed and then confirmed using the Uricaz, lactose fermentation, Urnithin, ONPG, Oxides, Deckerboxiles, and movement tests at 25 and 37°C.

Table 1. The contamination rate of the consumed chicken meat samples to pathogenic bacteria.

Meat type	Sample size	The number of contamination to <i>Salmonella</i>	The number of contamination to <i>Campylobacter</i>	The number of contamination to <i>Yersinia</i>	The number of contamination to <i>Aeromonas</i>
Packed chicken meat	96	19 (19.79)	22 (22.91)	8 (8.33)	6 (6.25)
Unpacked chicken meat	104	24 (23.07)	31* (28.84)	10 (9.61)	8 (7.69)

* Significant level at $p < 0.05$.

Table 2. comparing the bacterial contamination rate on the consumed chicken meat samples in different seasons.

Meat	Summer	Fall	Winter
Packed chicken meat	57	54	50
Unpacked chicken meat	66	62*	55

* Significant level at $p < 0.05$.

The technique used for *Aeromonas*

Pre-enriching stage

First 10 g of each sample was added to 90 ml of 1% Buffered Peptone Water, ATD, 043622 and were mixed and homogenized with a Stomacher for 2 min.

(i) In order to increase the separation probability for the direct and enriched forms, the culturing was conducted in appropriate condition.

Direct culturing

A loop from the first stage was transformed to Blood agar base, Merck 10886 with 30 mg/l Amphotericin and Defibrinated sheep blood and MacConkey agar, Merck, 5465 and was kept at 37°C for 18 to 24 h.

Enriching stage

1 ml of the first stage mixture was added to 9 ml of Buffered Peptone water with Sephadex pH= 8.4, 10 mg/l and was kept at 22-28°C for 18 h and 1 loop of the

enriched base was transferred to Blood agar base, Merck 10886 with 30 mg/l Amphotericin and Defibrinated sheep blood and MacConkey agar, Merck, 5465 and was kept at 37°C for 18 to 24 h.

(i) In order to test the suspicious colonies, the oxidase test was performed and the smears with gram coloring were prepared from the oxidase positive colonies.

(ii) Then the oxidase positive colonies which were also Gram negative Bacillus were analyzed regarding the Andol, movement, Glucose and Monitol fermentation and growth on TCBS base (TCBS agar, HiMedia, M189).

RESULTS

Among the 200 samples of chicken meat prepared from different cities in Mazandaran, the microbial contaminations of 75 samples were positive. The statistical analysis showed that the microbial contamination in chicken meat samples were significantly higher at ($P < 0.01$). Among the 200 samples of chicken meat prepared from Amol, Tonekabon, and Noor, the contamination of

75 samples were positive. 33 cases of the chicken were contaminated with *Salmonella* (21.5%), 53 samples with *Campylobacter* (26.5%), 18 samples with *Yersinia enterocolitica* (9%) and 14 samples were contaminated with *Aeromonas* (7%). The observed differences among the samples taken from southern areas of Tehran, both in packed and unpacked forms, were statistically significant ($P < 0.05$). Statistically, the results showed that the unpacked and packed meat product supplying centers in Mazandaran were not significantly different though having some differences in the type of the microbe contaminations (Tables 1 and 2).

DISCUSSION

Foods, especially chicken meat, are supplied in two ways that (i) Through traditional approach that is retailers and local shops offer the products

in an unpacked form exposing them to the open air because of which the hygienic standards cannot be met, and (ii) the industrialized approach in which megastores and department stores would appropriately deliver the meat and its related products in packed and sterilized forms (Tackett et al., 1984; Linnan, 1983; Bean and Griffin, 1990; Tauxe, 1997; Logue, 1996; Kazuaki and Katsuhiko, 1999; Jorgen, 2002).

Different countries have reported to experiences a degree of chicken meat contamination to *Salmonella* swinging from 5 to 25 of which the developing countries have shown to strike the higher end (Jorgen, 2002; Dominguez et al., 2002; Takeamaru, 1991; Zhao et al., 2001). CDC receives more than 40000 cases of *Salmonella* contamination annually, which partly shows the contamination rate in the US that soars up to 2 million cases per year. Some cases of epidemics, which are rooted in *Salmonella*, happen to be in hospitals, kindergartens, and prisons, which occur despite observing the food health related issues in the kitchens and among the staffs (Mayrhofer et al., 2004; White et al., 2004; Nortje, 1990; Karib et al., 1999; Siriken, 2004).

A study was conducted in Switzerland in 1993 on 829 samples of (red meat, poultry, fish, and fish product, which showed 24.1% contamination to *Aeromonas* (Gobat and Jemmi, 1993). The results showed that the *Aeromonas* bacteria would be separated if they were noticed in foods. Totally, 2.9% meat samples under study showed a degree of contamination to *Aeromonas*. Although *Aeromonas* had the lowest degree of contamination after *Salmonella*, *Campylobacter* and *Yersinia* in chicken meat samples based on the pathogens distribution, it is required to consider this bacterium more than the others do because it is a very important bacterium in children's Gastroenteritis (Soltan and Moez, 2004; Hudson, 1992; Gobat and Jemmi, 1993; Kumar, 2000; Majeed, 1996; Regula et al., 2003; Hanna and Zink, 1976).

Regarding the packed and unpacked samples, the contamination rates were 59.3 and 45.7%, respectively, and the statistical test showed that there is just a significant difference in chicken meat samples ($P < 0.05$). The results also reveal the fact that 13.6% of the separated *Salmonella* were taken from the packed samples and 25.6% from the unpacked samples which are congruent with the findings of other studies which reported the *Salmonella* contamination to be about 5-25 (Dominguez et al., 2002; Takeamaru, 1991; Zhao et al., 2001).

Considering the *Campylobacter* parting in Switzerland, the contamination for the packed samples was 26 and 74% for the unpacked ones (Regula et al., 2003; Hanna and Zink, 1976). The same measures were reached in the South Africa of which the packed sample case was about 6.7% and for the unpacked one it was about 48.9%.

In chicken, plucking with warm water will kill the bacteria, but the contamination after the washing and some bacteria reproduction and multiplication at freezing and storing stage even 72 h after being sold to the customers will be thoroughly contaminating all the packed chicken. The amount of water used to wash the chicken can also be a source of contamination. Our results indicate that chicken supplying centers, both in packed and unpacked forms. In different cities of Mazandaran have different degrees of microbial contaminations which are significantly different ($P < 0.05$).

The present study focused on *Salmonella* among different pathogenic bacteria since the pathogen has the potentiality to instigate the Gastroenteritis pathogens, especially in children, the elderly and people with immune system issues, therefore, it is required that when the diarrhea with food origin occur, we should consider the existence of the given bacteria and these bacterial pathogens should be recorded and listed as pathogens which must be considered when examining people with diarrhea.

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