



## Prevalence of Foodborne Pathogens in Cooked Meat and Seafood from 2010 to 2013 in Shandong Province, China

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(Received 11 Mar 2016; accepted 23 Aug 2016)

### Abstract

**Background:** Current food safety issues are deleteriously reshaping the lifestyle of the population in the developing world. The globalization of food supply impacts patterns of foodborne disease outbreaks worldwide, and consumers are having increased concern about microbiological food safety.

**Methods:** A total of 2305 samples including sauced meat, sausage, smoked meat, shrimp, sashimi and shellfish were collected from different farmer's markets and supermarkets. The prevalence of selected foodborne pathogens was evaluated in cooked meat and seafood from 2010 to 2013 in Shandong Province, China.

**Results:** The average contamination rate was 6.39% (93.1456) for the selected pathogens in cooked meat and 16.84% (143.849) for *V. parahaemolyticus* in seafood. For the selected pathogens, 0.55%, 1.03%, 1.17%, 3.64% and 16.84% samples were contaminated with *E. coli* O157: H7, *Salmonella* spp., *L. monocytogenes*, *S. aureus* and VP, respectively. There was a significant ( $P < 0.05$ ) difference in the contamination rate between the farmer's markets and supermarkets.

**Conclusion:** The contamination was decreasing in cooked meat and maintaining a relatively high level in seafood from 2010 to 2013. *E. coli* O157: H7, *S. aureus*, *L. monocytogenes* and *Salmonella* spp. existed at a relatively low rate in retail foods. For VP, the contamination rate has been maintained at a relatively high level in Shandong Province in China. Moreover, cooked meat and seafood obtained from farmer's markets are more susceptible to be contaminated compared to those from supermarkets.

**Keywords:** Food safety, Foodborne pathogens, Cooked meat, Seafood

### Introduction

Food production and consumption is now making an important contribution to development of countries, food safety associated with foodborne pathogens is undoubtedly facing great challenges (1). Annually, one-third of the world population is infected by foodborne pathogens (2). Even in the United States, 9.4 million foodborne episodes, 55961 hospitalizations and 1351 deaths were due to 31 major pathogens every year (3-5). Therefore, the importance of monitoring foodborne pathogens from various sources was necessary, including cooked meat (6, 7) and seafood (8, 9).

With the improvement of living standards, the consumption of cooked meat and seafood is increasing for the higher value and richer in micro-nutrients (10). Food safety has become a global challenge, and most countries are victims of foodborne illnesses (11). The incidence of foodborne illnesses do increase in the every summer and autumn, and mainly for two reasons. First, there are the natural causes, foodborne pathogen grow faster in the warm summer and autumn months. Second, outside activities do increase

and the safety controls do implement difficulty (12, 13).

Being convenient and nutritious, the cooked meat and seafood are very popular worldwide. However, for inadequate hygienic measures during preparation, retailing and consumption, foods can be easily contaminated by foodborne pathogens such as *Escherichia coli*, *Salmonella* spp., *Listeria*, and *Staphylococcus aureus* (*S. aureus*) (14). Due to culture, climate and economic status, every country and region has its own unique food safety issues, but foodborne diseases caused by pathogens are similar. For cooked meat, the common foodborne pathogens include *Escherichia coli*O157:H7 (*E. coli* O157: H7), *S. aureus*, *Listeria monocytogenes* (*L. monocytogenes*) and *Salmonella* spp. (15, 16); for seafood, the most important pathogen is *Vibrio parahaemolyticus* (*VP*) (17, 18).

In the United States, each year more than 63000 illnesses, 2138 hospitalizations, and 20 deaths were caused by the food infected with *E. coli* O157: H7 (4). It associated with products ranging from ground beef to processed foods. *L. monocytogenes* is foodborne pathogen that can cause listeriosis in humans. The foodborne listeriosis outbreaks have been reported and recognized worldwide as a major public health concern (19). Salmonellosis and staphylococcal food poisoning are two of the main food-borne diseases (20). Salmonellosis is often caused by the contaminated foods such as meat products, eggs and dairy products (21, 22). Moreover, the existence of *S. aureus* in ready to eat (RTE) food has been reported by many countries, such as Korea, Brazil, and Greece (23- 25). Moreover, the foodborne disease outbreak caused by *VP* mainly was associated with different kinds of seafood and occurs in summer and autumn (26).

In Shandong Province, a populous and major tourism province, food safety is particularly important. However, the contamination status of foodborne pathogens is not clear for cooked meat and seafood. In light of their importance, the aim of this research was to assess to what extent the selected foodborne pathogens are prevalent in cooked meat and seafood in Shandong Province. The results of this study may provide

useful information for updating and assessing the risks for concerned departments, which oversee food safety.

## Methods

### *Collection of food samples*

There are two types of retail markets in China: supermarkets and farmer's markets. Supermarkets are indoors, often air-conditioned, typically offer controlled-temperature environments. In contrast, farmer's markets are traditional open-air markets where foodstuffs are sold without air-conditioned. Cooked meat products and seafood products were purchased randomly from geographically different farmer's markets and supermarkets in Shandong Province from 2010 to 2013. Shandong Province consists of 17 prefectures. The sampling sites included 12 supermarkets and 6 farmer's markets in 6 prefectures from 2010 to 2011. According to our real works, the sites were enlarged to 34 supermarkets and 17 farmer's markets in 17 prefectures respectively in 2012 and 2013.

The sample size was determined by following *Manual for Risk Surveillance on Foodborne Pathogens* issued by National Health and Family Planning Commission of the People's Republic of China. Three categories of cooked meat were collected including smoked meat, sausage and sauced meat. Three categories of seafood were collected including shrimp, sashimi and shellfish. All samples were transported to the laboratory in the insulated box with ice to maintain the temperature at 4 °C–6 °C. After removing the substandard samples, the actual number of tested samples was shown in Table 1 and Table 2.

### *E. coli* O157: H7

25g sample was homogenized with 225 mL of sterile mEC+n enrichment broth (Luqiao, China) and incubated at 36 °C for 20 h. Following incubation, one loop of broth was streaked onto Chromogenic Agar O157 (Chromagar, France) and incubated at 36 °C for 20 h. Suspected colonies was picked and confirmed by GN cards (BioMérieux, France) (27).

***S. aureus***

Twenty-five g sample was homogenized with 225 mL of 7.5% NaCl broth (Huankai, China) and incubated at 36 °C for 20 h. One loop of broth was

streaked onto *S. aureus* chromogenic agar (Chromagar) and incubated at 36 °C for 20 h. Suspected colonies were picked and confirmed by GP cards (BioMe'rieux, France) (28).

**Table 1:** The samples of cooked meat were collected in Shandong Province from 2010 to 2013

Years	Seasons	Supermarkets						Farmer's markets					
		Smoked meat		Sausage		Sauced meat		Smoked meat		Sausage		Sauced meat	
		Packages	Sliced forms	Packages	Sliced forms	Packages	Sliced forms	Packages	Sliced forms	Packages	Sliced forms	Packages	Sliced forms
2010	Summer	0	4	5	2	0	16	0	0	0	4	0	15
	Autumn	6	10	14	7	3	73	0	4	0	0	0	3
	Total	6	14	19	9	3	89	0	4	0	4	0	18
2011	Summer	0	11	14	11	12	27	0	0	0	0	0	9
	Autumn	4	7	8	8	8	36	0	5	0	1	1	15
	Total	4	18	22	19	20	63	0	5	0	1	1	24
2012	Summer	5	15	30	17	11	137	0	15	4	5	1	77
	Autumn	4	3	43	20	23	121	2	16	7	13	2	51
	Total	9	18	73	37	34	258	2	31	11	18	3	128
2013	Summer	20	14	48	5	65	74	4	6	10	5	7	61
	Autumn	19	6	10	8	42	55	0	3	0	0	0	29
	Total	39	20	58	13	107	129	4	9	10	5	7	90

**Table 2:** The samples of seafood were collected in Shandong Province from 2010 to 2013

Years	Seasons	Supermarkets			Farmer's markets		
		Shrimp	Sashimi	Shellfish	Shrimp	Sashimi	Shellfish
2010	Summer	2	8	0	0	4	1
	Autumn	16	28	42	9	1	13
	Total	18	36	42	9	5	14
2011	Summer	18	33	25	8	6	7
	Autumn	21	45	18	5	5	3
	Total	39	78	43	13	11	10
2012	Summer	22	36	30	7	23	41
	Autumn	18	46	30	18	20	32
	Total	40	82	60	25	43	73
2013	Summer	13	40	27	5	6	8
	Autumn	8	26	6	29	18	22
	Total	21	66	33	34	24	30

***L. monocytogenes***

25g food sample was homogenized in 225 mL of listeria enrichment broth (Luqiao) with listeria selective enrichment supplement and incubated for 48 h at 30 °C. After incubation, one loop of broth was streaked onto listeria chromogenic agar (Chromagar) and incubated at 36 °C for 24-48 h. Suspected colonies were picked and confirmed by GP cards (BioMe'rieux, France) (29).

***Salmonella* spp.**

25g food sample was homogenized in 225 mL of buffered peptone water (BPW) (Huankai) and incubated at 36 °C for 20 h. Then, 0.1 mL of the broth was added to 10 mL of selenite cystine broth (Huankai) and was incubated at 42 °C overnight. Following incubation, one loop of broth was streaked onto Salmonella chromogenic agar (Chromagar) and incubated at 36 °C for 24

h. Suspected colonies was picked and confirmed by GN cards (BioMe´rieux, France) (30).

**Vibrio parahaemolyticus**

25g food sample was homogenized in 225 mL of alkaline peptone water (Huankai) with 3% NaCl and incubated at 36 °C for 20 h. One loop of mixture was streaked onto vibrio chromogenic agar (Chromagar) and incubated at 36 °C for 20 h. Typical suspected colonies was picked and confirmed by GN cards (31).

**Statistical analysis**

The relationship between the contaminated samples and the different kinds of samples was analyzed using chi-squared analysis. All statistical and chi-squared analyses were performed using SPSS 19.0. A  $P < 0.05$  was used for statistical significance.

**Results**

From 2010 to 2013, 2305 samples including sauced meat, sausage, smoked meat, shrimp, sashimi and shellfish were collected from different farmer's markets and supermarkets in Shandong Province. 236 positive strains were identified by the method of chromogenic media and biochemical test among them, *E. coli* O157:H7 accounted

for 8, *S. aureus* for 53, *L. monocytogenes* for 17, *Salmonella* spp. for 17 and *VP* for 143.

For cooked meat, the average contamination rate was 6.39% (93.1456) for the selected pathogens in the past four years. According to the annual statistics, the prevalence of the selected pathogens decreased (3.26%, 16.491) in 2013, have a significant differences when it compared to the year 2010 (9.04%, 15.166), 2011 (7.34%, 13.177) and 2012 (7.88%, 49.622).

According to the statistics of tested food, the detection rate of tested pathogens in sausage (3.01%, 9.299) was significantly lower compared with in bacon (6.01%, 11.183) and in sauced meat (7.49%, 73.974) (Fig. 1).

For seafood, the average contamination rate was 16.84% (143.849) for *VP* in the past four years. According to the annual statistics, the contamination rate was lower in 2010 (1.61%, 2.124), with significant differences compared to the year 2011 (12.37%, 24.194), 2012 (26.01%, 84.323) and 2013 (15.87%, 33.208). According to the statistics of tested foods, the positive rate of shrimp (23.12%, 46.199) was the highest compared to sashimi (11.59%, 40.345) and shellfish (18.69%, 57.305) (Fig. 2).

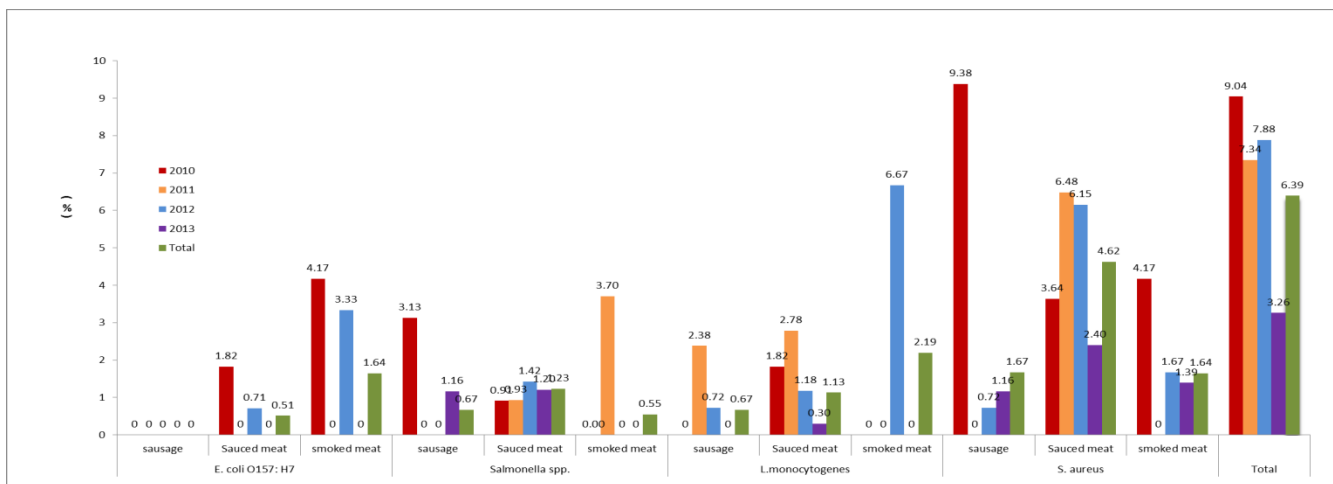


Fig. 1: The contamination rate of foodborne pathogens in cooked meat in Shandong Province from 2010 to 2013

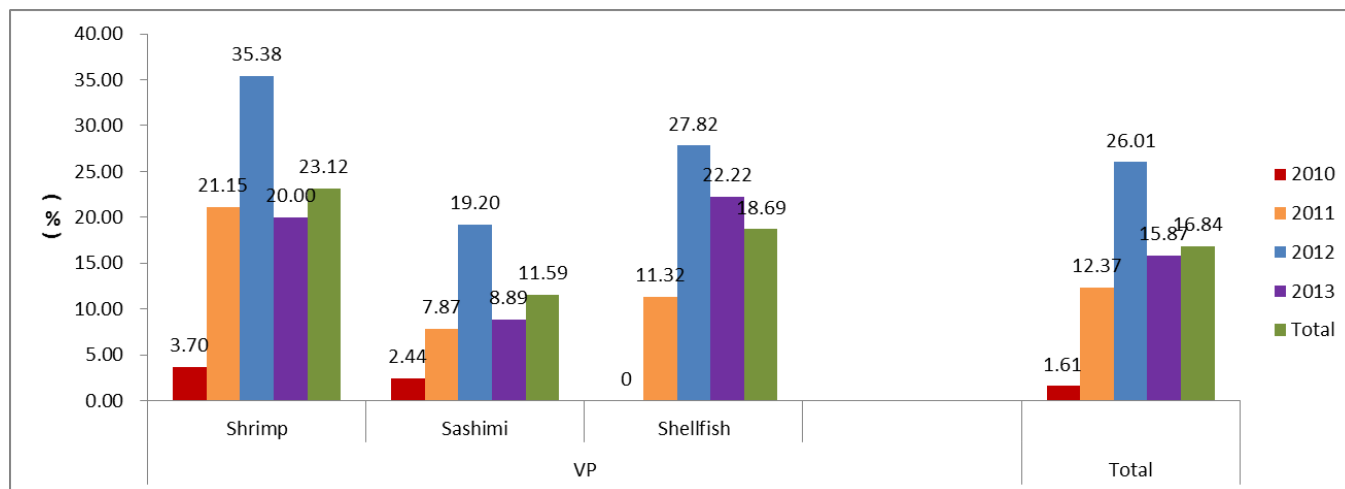


Fig. 2: The contamination rate of foodborne pathogens in seafood in Shandong Province from 2010 to 2013

For the selected pathogens, 8 (0.55%) samples were contaminated with *E. coli* O157: H7, and among them, 5 (0.51%) and 3 (1.64%) sauced meat and smoked meat samples were contaminated with *E. coli* O157: H7, no *E. coli* O157: H7 was detected in 299 samples of sausage; 15 (1.03%) *Salmonella* spp. isolates were detected, and among them, 12 (1.23%), 2 (0.67%) and 1 (0.55%) were detected in sauced meat, sausage

and smoked meat samples, respectively; 17 (1.17%) samples were contaminated with *L. monocytogenes*, and among them, 11(1.13%), 2 (0.67%) and 4 (2.18%) sauced meat, sausage and bacon samples were contaminated with *L. monocytogenes*, respectively; 53 (3.64%) *S. aureus* isolates were detected, and among them, 45 (4.62%), 5 (1.67%) and 3 (1.64%) were detected in sauced meat, sausage and bacon samples, respectively (Fig. 1).

Table 3: The contamination rate of cooked meat in different markets in Shandong Province from 2010 to 2013

Years	Super markets	Farmer's markets	Total	Contaminated samples						
				Supermarkets		Farmer's markets		Total		P-value
				number	(%)	number	(%)	number	(%)	
2010	140	26	166	10	7.14	5	19.23	15	9.04	0.06
2011	146	31	177	6	4.11	7	22.58	13	7.34	0.00
2012	429	193	622	18	4.20	31	16.06	49	7.88	0.00
2013	366	125	491	6	1.64	10	8.00	16	3.26	0.00
Total	1081	375	1456	40	3.70	53	14.13	93	6.39	0.00

Table 4: The contamination rate of seafood in different markets in Shandong Province from 2010 to 2013

Years	Super markets	Farmer's markets	Total	Contaminated samples						
				Supermarkets		Farmer's markets		Total		P-value
				number	(%)	number	(%)	number	(%)	
2010	96	28	124	1	1.04	1	3.57	2	1.61	0.40
2011	160	34	194	15	9.36	9	26.47	24	12.37	0.02
2012	182	141	323	47	25.82	37	26.24	84	26.01	1.00
2013	119	89	208	12	10.08	21	23.6	33	15.87	0.00
Total	557	292	849	75	13.91	68	21.94	143	16.84	0.00

Based on the cooked meat and seafood in the four years, generally, there was a significant ( $P < 0.05$ ) difference in the selected contamination rate of the pathogens between the farmer's markets and supermarkets (Tables 3, 4). For cooked meat products, the contamination was decreasing from 2010 to 2013 in farmer's markets and supermarkets (Table 3). For seafood, however, the contamination was maintaining a relatively high level in every year from 2010 to 2013 (Table 4).

## Discussion

Cooked meat can provide a source of readily available and nutritious meals for the consumers, but as these foods do not receive any heat treatment before consumption, the first priority should be their safety and microbiological quality. For cooked meat, 3.64% of samples were positive for *S. aureus*, which was lower than the contamination rate of RTE foods (5.98%) reported in Korea (32) and post-cooked samples (16.7%) in Trinidad (33); 1.17% of samples were positive for *L. monocytogenes*, which was higher than the reported incidences (0.37%) in United Kingdom (34) and significantly lower than Brazil where the rates was 42.50% (35); 1.00% of samples were positive for *Salmonella* spp., which was consistent to the rate of cooked ham samples (1.89%) reported in Spain (36); the rate of *E. coli* O157: H7 (0.55%) was lower than other selected pathogens. The fact that the selected pathogens were isolated from samples of cooked meat products suggests that either the heat treatment had been inadequate, or post-processing contamination of the food products had occurred. Another possible explanation is bacterial contamination at retail process, where improper handling and cross-contamination during transportation and storage are possible. The results that the prevalence of pathogens significantly decreased in 2013 compared to other years suggest the supervision of the food at the factory and circulation is effectual and should be strengthened to reduce food contamination continuously in China.

Seafood is gaining popularity worldwide because it is considered healthy and nutritious. However, infections by *VP* from seafood are frequently reported in worldwide (37) including in China (38), this is caused by consumption of raw or partially cooked seafood contaminated with *VP* (39, 40). In this study, we noticed that the average contamination rate of seafood was 16.84% for *VP* in the past four years, especially for shrimp, the average contamination rate was 23.12%. Furthermore, the contamination rate has been maintained at a relatively high level except in 2010. These facts suggested that the high prevalence of *VP* in seafood presents maybe a great threat to human health and effective microbial inactivation methods should be employed in seafood post-harvest processing to reduce seafood illness caused by *VP* in China. If possible, the effective intervention methods, for example, physical methods (41), chemical methods (42) and biological methods (43), for reducing *VP* used during seafood processing and consumption should be evaluated in the further studies. For physical methods, thermal processing, low-temperature freezing, high-pressure processing (HPP) and irradiation are reported to effectively inactivate or kill *VP* (41). Similar to the physical methods, chemical reagents and persistent broad-host-range Phages have been used for reducing the bacterial contamination in seafood (42-44). However, none of these absolutely dislodge *VP* from seafood, and further research is required that focuses on the screening and development of new methods.

There are two types of retail markets in China: supermarkets and farmer's markets. In this study, the result that a significant ( $P < 0.05$ ) difference in the selected pathogens contamination rate between the supermarkets and farmer's markets may suggest farmer's markets are more susceptible to cross-contamination than supermarkets, which may be caused by constant exposure to environmental factors such as dust, rodents, and insects (45,46). Moreover, farmer's markets are traditional open-air markets and not air-conditioned; these increase the risk of bacterial

contamination and proliferation. According to Chinese standards of RTE food, foodborne pathogenic bacteria cannot be detected. Therefore, efforts must be made by vendors and supervisors to improve the levels of hygienic conditions to reach that of supermarkets at least. For example, vendors should strengthen disinfection and sterilization of RTE food and marketing conditions, and supervisors should strengthen supervision and monitoring.

## Conclusion

*E. coli* O157: H7, *S. aureus*, *L. monocytogenes* and *Salmonella* spp. existed at a relatively low rate in retail foods. For VP, the contamination rate has been maintained at a relatively high level in Shandong Province in China. The potential of these foodborne pathogens in cooked meat and seafood should not be neglected. The supervision department should set rigorously national standard to ensure food safety. Meanwhile, special emphasis must be given to public education and mass awareness programs for strengthening food safety controls. The prevalence of microbial contamination in retail cooked meat and seafood can provide a foundation for future studies. Cooked meat and seafood obtained from farmer's markets are more susceptible to be contaminated compared to those from supermarkets. If possible, further research is necessary to understand the origin and transmission route of these pathogens in cooked meat and seafood, which can help reduce foodborne diseases.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Acknowledgments

This study was supported by grants from the Health and Family Planning Commission of

Shandong Province (2013WS0163, 2011HZ057) National Science and Technology Major Project of the Ministry of Science and Technology of China (2012ZX10004215) and National Science Foundation of China (NO. 81202260). The authors declare that there is no conflict of interest.

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