Int J Enteric Pathog. 2015 November; 3(4): e29009. doi: 10.17795/ijep29009

Published online 2015 November 2. **Research Article**

Distribution and Antimicrobial Resistance Profile of *Yersinia* Species Isolated From Chicken and Beef Meat

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Received 2015 April 15; **Revised** 2015 June 5; **Accepted** 2015 June 15

Abstract

Background: Foodborne diseases are widespread and growing public health problem in developed and developing countries. There are many microorganisms act as etiological agents for foodborne diseases such as *Campylobacter* spp., *Listeria*, *Staphylococcos*, *Salmonella*, *Bacillus*, *Yersinia* spp. High prevalence of gastrointestinal illness, including fatal cases attributable to yersiniosis, is also observed in many developing countries.

Objectives: The purpose of this study was to investigate the prevalence of *Yersinia enterocolitica* and other *Yersinia* species in meat and chicken samples in various seasons and to determine their antibiotic resistance profile.

Materials and Methods: To investigate the prevalence of *Yersinia* spp., a total of 450 samples, including chicken (n = 226) and beef meat (n = 224) were collected from supermarkets in Tehran. All samples were transported on ice to the laboratory and microbiological analysis was carried out within 2 hours after the collection. Susceptibility testing of bacterial strains was according to CLSI guideline at 28 °C by the disk diffusion assay.

Results: From a total of 450 samples, (226 chickens and 224 beef meats), 70 (15.5%) samples were positive for *Yersinia* spp. Of these isolates, (80%) 56 species were identified as *Y. enterocolitica*, 8 (11%) as *Y. frederiksenii*, 5 (7%) as *Y. intermedia* and 1 (1.4%) as *Y. kristensenii*. The highest rate of resistance was seen against cephalotin (98%), and ampicillin (52%). However, gentamicin and chloramphenicol were the most active antibiotics against the target cultures. Considering the season of isolation, *Yersinia* spp. were frequently isolated in autumn (52%), followed by spring (29%).

Conclusions: *Y. enterocolitica* was the most spp. distributed among other species. Many factors, such as isolation assay, season, and geographical location play critical role in reports of increase or decrease in the prevalence of the *Yersinia* spp. all over the world. Our findings demonstrate that the isolation ratio of *Y. enterocolitica* and other species is higher in colder regions. Most of the isolates were resistant to first generation cephalosporins (cephalothin). The most active antimicrobial agents were choloramphenicol, aminoglycozide and sulfunamdes. Regarding to the high sensitivity of *Yersinia* spp. to gentamicin and chloramphenicol, these antibiotics would be the choice for the treatment of *Yersinia* infections.

Keywords: Antimicrobial Susceptibility, Chickens, Beef Meat, Iran, *Yersinia*

1. Background

Foodborne diseases are widespread and growing public health problem in developed and developing countries. There are many microorganisms act as etiological agents for foodborne diseases such as *Campylobacter* spp., *Listeria, Staphylococcos*, *Salmonella*, *Bacillus*, *Yersinia* spp. Of these, food contamination with *Yersinia* species is the frequently reported (1). High prevalence of gastrointestinal illness, including fatal cases attributable to yersiniosis, is also observed in many developing countries (2). The high prevalence of foodborne illnesses acts as a major challenge for the development of food industry and has been one of the major concerns worldwide. Consumption of contaminated poultry and meat can be the main source of contamination (3). *Y. enterocolitica* is known as a psychotropic waterborne and foodborne enteropathogen. Also, *Y. enterocolitica* can grow on wide range of temperatures; therefore, food staff contaminated with the bacterium is an important health concern. *Yersinia* spp. was isolated from various sources like, foods, environment, water and clinical specimens (4). The global increase in chicken and beef meat consumption

is encouraged by its high protein content and its accessible price has drawn the attention to the necessity of rapid detection of the foodborne pathogens in the chicken and beef meat. Considering the high prevalence of *Yersinia* spp. infection in human community particularly in developing countries, the present study was carried out to screen raw chicken and beef meat samples collected from retails markets for the presence of *Yersinia* spp. pathogens.

2. Objectives

The aim of this study was to evaluate the prevalence of *Y. enterocolitica* and other *Yersinia* From raw meat and chicken in Tehran, Iran, in different seasons and to determine the antimicrobial susceptibility pattern of *Y. enterocolitica* and other *Yersinia* species obtained from meat and chicken in Tehran, Iran.

3. Materials and Methods

3.1. Sampling

To investigate the prevalence of *Yersinia* spp, a total of 450 samples, including chicken (n = 226) and beef meat (n = 224) were collected from Tehran, between April 2013 and April 2014. All samples were transported on ice to the laboratory of the Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran and microbiological analysis was carried out within 2 hours after the collection.

3.2. Isolation and Identification of Yersinia spp.

In this study, 25 g of meat was homogenized and added to 225 mL of phosphate-buffered saline and incubated at 4˚C. After 2 and 3 weeks, the cold-enriched samples were subjected to alkali treatment by adding 0.5 mL of 0.5% KOH into 4.5 mL of cold-enriched samples. The cold-enriched and alkali-treated cultures were streaked onto cefsulodin-irgasan-novobiocin (CIN) agar and incubated at 25˚C for 18 - 24 hours. Presumptive *Yersinia* colonies were identified by biochemical tests using the API 20E (bioMerieux, Inc.US).

3.3. Antimicrobial Susceptibility Testing

Susceptibilities of 70 strains (48 *Y. enterocolitica*, 7 *Y. frederiksenii*, 4 *Y. intermedia* and 1 *Y. kristensenii*) were determined against 10 antimicrobial drugs using the agar disk-diffusion method according to clinical and laboratory standards institute (CLSI 2013) (5). A disk diffusion assay according to the standard protocols was used to determine the susceptibility of *Yersinia* isolates to nalidixic acid (30 Ig), ciprofloxacin (5 Ig), tetracycline (15 Ig), streptomycin (30 Ig), gentamicin (10 Ig), chloramphenicol (30 Ig), ampicillin (30 Ig), trimethoprim (15 Ig), cephalexin (30 Ig) and cephotaxim (30 Ig). All the antibiotic discs were purchased from Mast Co., United Kingdom.

3.4. Statistical Analysis

Statistical analysis was performed using SPSS software version 11.5 (SPSS, Inc. Chicago, Illinois, USA). The chi-square test and Fisher's exact two-tailed test were used to analyze the data. A P value < 0.05 was used for statistical significance.

4. Results

From a total of 450 samples, 226 chickens and 224 beef meats, 70 (15.5%) isolates were detected as *Yersinia* spp based on biochemical and microbiological tests. Of these isolates, 56 species (80%) were identified as *Y. enterocolitica*, 8 (11%) as *Y. frederiksenii*, 5 (7%) as *Y. intermedia* and 1 (1.4%) as *Y. kristensenii*. Of *Yersinia* strains isolated from chicken, 35 (15%), 8 (3.5%), 4 (1.7%) and 1 (0.4%) were identified as *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii*, respectively. In case of isolates from beef meats, the more prevalent isolates were *Y. enterocolitica* (9.3%) and *Y. intermedia* (0.4%) (Table 1). Antibiotic susceptibility testing against 10 antimicrobial agents was done for 60 isolates out of 70. The highest rate of resistance (98%) was seen against cephalotin and (52%) against ampicillin, while gentamicin and chloramphenicol were the most active (98%) antibiotics against studied isolates (Table 2). There was no statistically significant difference in antibiotic susceptibility status of isolates among chickens and meats; however meat isolates were more susceptible to tetracycline (Table 3). Considering the season of isolation, *Yersinia* spp. were frequently isolated in autumn (52%), followed by spring (29%) (Table 4). In chicken, samples collected in April and December were more contaminated while for meats February and June were the months with highest contamination.

Table 1. Distribution of *Yersinia* spp. in Chicken and Beef Meat

Type of Samples	Number	Bacteria			
		Y. enterocolitica	*Y. frederiksenii*	*Y. intermedia*	*Y. kristensenii*
Chicken	226	35	8	4	1
Meat	224	21	0	1	0
Total	450	56	8	5	1

Table 2. Number and Percentages of Antimicrobial Pattern of *Yersinia* Strains Isolated From Chicken and Beef Meat[a,b]

Antibiotics	*Y. enterocolitica*, 48			*Y. frederiksenii*, 7			*Y. intermedia*, 4			*Y. kristensenii*, 1		
	S	I	R	S	I	R	S	I	R	S	I	R
Nalidixic Acid	37 (77)	0	11 (23)	5 (71)	0	2 (29)	4 (100)	0	0	0	0	1 (100)
Ciprofloxacin	45 (94)	3 (6)	0	7 (100)	0	0	4 (100)	0	0	1 (100)	0	0
Tetracycline	36 (75)	6 (12.5)	6 (12.5)	7 (100)	0	0	3 (75)	0	1 (25)	1 (100)	0	0
Streptomycin	28 (58)	15 (32)	5 (10)	6 (86)	1 (14)	0	4 (100)	0	0	0	1 (100)	0
Gentamicin	47 (98)	1 (2)	0	7 (100)	0	0	4 (100)	0	0	1 (100)	0	0
Chloramphenicol	47 (98)	1 (2)	0	7 (100)	0	0	4 (100)	0	0	1 (100)	0	0
Ampicillin	17 (35)	4 (8)	27 (56)	3 (43)	0	4 (57)	4 (100)	0	0	1 (100)	0	0
Trimethoprim	45 (94)	0	3 (6)	7 (100)	0	0	4 (100)	0	0	1 (100)	0	0
Cephalotin	1 (2)	0	47 (98)	0	0	7 (100)	0	0	4 (100)	0	0	1 (100)
Cephotaxim	40 (83)	8 (17)	0	7 (100)	0	0	4 (100)	0	0	1 (100)	0	0

[a] I, Intermediate; R, Resistant; S, Sensitive.
[b] The values are peresnted as No. (%).

Table 3. Antimicrobial Susceptibility Pattern Based on Type of Samples

Antibiotics	Type of Samples		P Value
	Meat	Chicken	
Nalidixic Acid			0.51
S/I	16	30	
R	3	11	
Ciprofloxacin			1
S/I	19	41	
R	0	0	
Tetracycline			0.08
S/I	19	34	
R	0	7	
Streptomycin			1
S/I	18	37	
R	1	4	
Gentamicin			1
S/I	19	41	
R	0	0	
Chloramphenicol			1
S/I	19	41	
R	0	0	
Ampicillin			0.78
S/I	10	19	
R	9	22	
Trimethoprim			1
S/I	18	39	
R	1	2	
Cephalotin			1
S/I	0	1	
R	19	40	
Cephotaxim			1
S/I	19	41	
R	0	0	

Table 4. Distribution of *Yesinia* spp. in Different Months of Year[a]

Month	Sample General		Total
	Meat	Chicken	
April	2.20 (10)	10.20 (50)	12.40 (30)
May	1.18 (5)	5.18 (28)	6.36 (17)
June	4.17 (23)	2.17 (12)	6.34 (18)
July	0.17 (0)	0.18(0)	0.35(0)
August	0.18 (0)	1.18 (5)	1.36 (3)
September	1.18 (5)	1.18 (5)	2.36 (6)
October	1.18 (5)	1.18 (5)	2.36 (6)
November	4.28 (14)	10.28 (36)	14.56 (25)
December	1.18 (5)	9.18 (50)	10.36 (27)
January	3.18 (16)	1.18 (5)	4.36 (11)
February	2.6 (33)	1.7 (14)	3.13 (23)
March	3.28 (11)	7.28 (25)	10.56 (18)
Total	22.224	48.226	70.450

[a]The values are presented as No. (%).

5. Discussion

Yersinia spp. is a Gram-negative rod from the Enterobacteriaceae family. *Y. enterocolitica* is a common Gram-negative foodborne enteric pathogen found in water, dairy products, and meats. It is one of the most common causes of foodborne gastroenteritis in the entire world and could become a significant health risk for consumers (6, 7). These bacteria are highly resistant to unfavorable conditions during food processing, such as low pH, salinity, or disinfectants. In addition, there are considerable problems with detecting pathogenic *Y. enterocolitica* in food due to the limited sensitivity of the laboratory methods (8). Frequency of *Yesinia* spp. in various sources such as meat, ground beef, pork, environment, water and human was reported from 9% to 99.5% percent in different regions (9-12). Our data showed that 15.5% of all studied samples were contaminated with *Yersinia* spp. and is in constant with other previous local studies in which the rate of contamination have been reported from 13.3% to 16% (13, 14). Prevalence of *Yersinia* spp in Iran is lower than other developing countries (from 32% - 38%) (15, 16). This may be contributed to isolation method, season, and geographical location.

Our results revealed that *Y. enterocolitica* was the most predominant *Yersinia* species recovered from chicken (15%) and meat (9%) which is slightly higher than reports by Siriken (16) in which they found that 8% samples of poultry and 4% samples of beef meat were positive for *Y. enterocolitica*. The results of our study are in agreement with most of the studies showed that chickens are more vulnerable to contamination with *Yesinia* spp. However, our result about isolation of *Y. enterocolitica* was different from studies conducted in Argentina (38.65%), Turkey (27.9%) and Brazil (25%) (15-17). Frequency of *Y. frederiksenii* is very diverse from 1.6% to 9% in different countries. In the present study, the prevalence of *Y. frederiksenii* (11.6%) was similar to other local studies (11%) (14).

According to our results, most of the isolates were resistant to cephalothine (98%) and ampicillin (56%), these results are similar to reports of Soltan-Dallal et al. (18), although in their report most of isolates were sensitive to chloramphenicol and gentamicin. Since the antibiotic profile of isolates in both chicken and meat was similar, it shows that they may share the same sources.

As documented by the results of numerous studies, *Yersinia enterocolitica* is an important etiological factor in food poisoning (1). Results revealed that a high proportion of chicken and beef meat in Tehran, Iran, is contaminated with *Yersinia*. The high rate of contamination of meats, particularly chicken, with enteropathogenic bacteria, remains a significant public health concern. Most of the isolates were antimicrobial-resistant; therefore, there is a possible risk to humans to such microorganisms especially from consumption of these products (18). Isolation method, season, and geographical regions are several factors that can play a significant role in reports of increase or decrease in the prevalence of the *Yersinia* spp.

Y. enterocolitica had the most prevalence among other species. The majority of isolates were resistant to first generation cephalosporins (cephalothine). The most active antimicrobial agents were choloramphenicol and aminoglycozide. Regarding to high Sensitivity of *Yersinia* spp. to gentamicin and chloramphenicol, these antibiotics would be efficient in the treatment of *Yersinia* spp.

Acknowledgments

The authors would like to thank Farahnoosh Doustdar for her assistance in preparation of the manuscript.

Footnotes

Funding/Support: The study was co-funded by Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran and by authors.

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