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Full Length Research Paper

Incidence and antibiotic susceptibilities of *YERSINIA ENTEROCOLITICA* and other *YERSINIA* species recovered from meat and chicken in Tehran, Iran

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The objective of this study is to investigate the prevalence and determine the antimicrobial susceptibility pattern of *YERSINIA* species in beef meat and chicken meat samples in different seasons. In this study 379 pieces of beef and chicken meats were examined for the presence of *YERSINIA* species between April 2007 and March 2008. 25 g of homogenized food samples was pre-enriched in PBS then it was cultured on cefsulodin-irgasan-novobiocin (CIN) agar. Susceptibility testing of bacterial strains was performed at 28˚C by the agar diffusion method. *YERSINIA* spp. was isolated from 60 of 379 (15.8%) beef meat and chicken meat samples. *Y. ENTROCOLITICA* was found in 48 of 60 (80%) positive samples. The rate of other 3 *YERSINIA* spp, *Y. FEREDERIKSENII*, *Y. INTERMEDIA*, *Y. KRISTENSENII*, were 7(11%), 4(6%) and1 (0.01%) out of 60 isolates, respectively. 98% of isolates were susceptible to choloramphenicol and gentamicin. The most antibiotic resistance belongs to cephalothine (98%). Our results showed that isolation ratio of *Y. ENTEROCOLITICA* and the other species is higher in cold climates. The majority of isolates were resistant to cephalothine. The most active pharmacologic agents were chloramphenicol, gentamicin and trimetoprim.

Key words: *Yersinia* enterocolitica, antimicrobial susceptibility**,** beef meat, chicken.

INTRODUCTION

Yersinia enterocolitica, a Gram-negative, urease positive and facultative anaerobic species, is highly heterogeneous microorganism and it can be divided into several bioserotypes and serotypes (Bottone, 1997; Robins-Browne, 1997). *Yersinia* contamination represents a significant problem in food supplies, since this bacterium needs long period to grow (Fredriksson-Ahomaa and Hannu Korkeala, 2003). *Y. enterocolitica* is known as a psychotropic waterborne and foodborne enteropathogen. This microorganism can grow to large numbers at refrigeration temperatures, so meat, chicken, milk, cheese contaminated with that organism could become a significant health risk for consumers (Black and Jackson, 1978; Stern and Pierson, 1979; Soltan-Dallal et al., 2004; Hudson et al., 2008). This microorganism is primarily a gastrointestinal tract pathogen with a strong propensity for extra intestinal spread under defined host conditions (Soltan-Dallal and Moezardalan, 2004a). It causes a broad range of diseases from acute bowel disease to

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Abbreviation: CIN, Cefsulodin-irgasan-novobiocin.

Table 1. Percentage number of contaminated samples.

Food type	Total number of samples	Number of contaminated samples	
		n	%
Fresh meat	189	19	31.7
Chicken meat	190	41	68.3
Total	379	60	100

extra intestinal manifestation such as reactive arthritis , uveitis and sepsis (Jacobs et al., 1989; Chandler and Parisi, 1994). Systemic and extraintestinal infections and enterocolitis in immune-compromised patients require antibiotic therapy, and the agents used most commonly include chloramphenicol, gentamicin, tetracycline, cotrimoxazole and ciprofloxacin (Butler, 1990; Hoog, 1987). Although there is data concerning the incidence of *Y. enterocolitica* and related species in foods are reported in some countries (Fredriksson-Ahomaa and Hannu, 2003; Siriken, 2002; Dominique et al., 1981; Soltan and Moezardalan, 2004b), but compared with other bacterial enteropathogenes, there are a few studies about the antimicrobial susceptibility of *Yersinia* spp, which are isolated from staff foods and human. The aims of this study are to investigate the prevalence of *Y. enterocolitica* and other *Yersinia* species in meat and chicken samples in different seasons and to determine the antimicrobial susceptibility pattern of *Yersinia entrocolitica* and other *Yersinia species* isolated from meat and chicken in Tehran, Iran**.**

METHODS AND MATERIALS

Sample collection

189 pieces of beef meat and 190 pieces chicken meat were purchased from 28 different local butcher's shops and supermarkets in Tehran, Iran, and were examined for the presence of *Yersinia* species between April 2007 and April 2008**.**

Cold enrichment, Bacterial isolation and identification

25 g of each sample was added to plastic bag containing 10 ml phosphate buffered saline (pH 7.2) and homogenized by Stomacher (400 circulator Seward-England) and incubated at 4°C for one, two, three and four weeks. At weekly intervals, a suspension aliquot was cultured linearly on Cefsulodin Irgasan Novobiocin Agar (CIN) (Merck-Germany) with Yersinia selective supplement. Cultured plates were incubated at 26°C, between 24 - 48 h. The colonies were considered macroscopically and microscopically after incubation. Suspected colonies were examined to motility test by SIM medium both at 25 and 37°C. In addition, biochemical test including urease activity, KIA and Simon citrate were used for the bacterial identification (Johnson, 1998).

Antimicrobial susceptibility

Susceptibility testing of bacterial strains was achieved at 28˚C on Mueller-Hinton agar (Merck-Germany) by the agar diffusion method. The sensitivity spectrum of each of the isolates to ten different antibiotics was determined according to CLSI (2005). (Clinical and Laboratory Standard Institute) protocol and zone size interpretative chart explained by High Media Company. Antibacterial agents included ampicillin (10 mcg), trimethoprim (5 mcg), chloramphenicol (30 mcg), tetracycline (30 mcg), streptomycin (10 mcg), cephalotin (30 mcg), ciprofloxacin (5 mcg), cephotaxime (30 mcg), nalidixic acid (30 mcg), and gentamicin (10 mcg). Antibiotic disks were provided by High Media Company.

Data analysis

Statistical analysis of results was performed with SPSS/PC 12 software (SPSS Chicago, IL). A P value<0.05 was used for statistical significance.

RESULTS

Out of 375 pieces including 189 beef meat and 190 chicken meat samples, 16% (n=60) *Yersinia* species were isolated (Table 1). 70% (n=42) of isolates were achieved from chicken meat and 30% (n=18) from beef meat. The prevalence of *Y. enterocolitica* with the most incidence was 80% (n=48), that were found in chicken meat and beef meat with rate of 62.5% (n=30) and 37.5% (n=18) respectively. The occurrence of *Y. enterocolitica* was slightly higher in chicken than meat. The frequency of the other *Yersinia* spp. including *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii* were 11.6% (n=7), 0.06% (n=4), and 0.01% (n=1). Except for *Y. frederiksenii* (n=1), none of *Y. intermedia* and *Y. kristensen* were not found in meat (Table 2).

As we stated above, all of samples were cultured at the end of week for four weeks. The highest isolation rate was 33, 75 and 42% in 4 ˚C/3-week enrichment for *Y. entrocolitica, Y. intermedia* and *Y. frederiksenii* respectively. The only isolated *Y. kristensenii* accrued in 2-week enrichment. The rates of Yersinia isolated in various seasons were 20, 16 and 13% in November, April and March respectively. The prevalence of *Yersinia entrocolitica* and other species in various month was mentioned in Table 3.

All isolates were subjected to antimicrobial resistance testing by the disc diffusion method. An overview of the antibiotic susceptibility of the strains is shown in Table 4. Our results indicated that among *Y. entrocolitica*, the sensitive rate for gentamicin and chloramphenicol was 97% (n=46) and for trimethoprim and ciprofloxacin was

Table 2. Percentage distribution of *Yersinia* spp in tested samples.

Food	Number of contaminated samples			
	Yersinia			
	Y. ENTEROCOLITICA	*Y. INTERMEDIA*	*Y. FREDERIKSENII*	*Y. KRISTENSENII*
	n (%)	n (%)	n (%)	n (%)
Fresh meat	18(30)	1(1.7)	0	0
Chicken meat	30(50)	3(5)	7(11.7)	1(1.7)
Total	48(80)	4(6.7)	7(11.7)	1(1.7)

Table 3. Frequency distribution of *Yersinia Spp* in different months of year.

month of sampling	Type of isolate				Total
	Y. KRISTENSENII	*Y.INTERMEDIA*	*Y.FREDERIKSENII*	*Y. ENTEROCOLITICA*	
	n:1	n:4	n:7	n:48	
April	0(%)	0	1	9	10
May	0	0	1	4	5
June	0	0	0	5	5
August	0	0	0	1	1
September	0	0	0	2	2
October	0	0	0	2	2
November	0	1	1	10	12
December	0	2	3	3	8
January	0	1	1	2	4
February	0	0	0	3	3
March	1	0	0	7	8
	1	4	7	48	60

Table 4. Antibiotic resistance patterns of isolated *Yersinia* spp.

Antibiotic	*Y. ENTROCOLITICA*			*Y.FREDERIKSENII*			*Y. INTERMEDIA*			*Y. KRISTENSENII*		
	n=48			n=7			n=4			n=1		
	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin (10 mcg)	17	4	27	3	0	4	4	0	0	1	0	0
Trimethoprim (5 mcg)	45	0	3	7	0	0	4	0	0	1	0	0
chloramphenicol (30 mcg)	47	1	0	7	0	0	4	0	0	1	0	0
Tetracycline (30 mcg)	36	6	6	7	0	0	3	0	1	1	0	0
Streptomycin (10 mcg)	28	15	5	6	1	3	4	0	0	0	1	0
Cephalotin (30 mcg)	1	0	47	0	0	7	0	0	4	0	0	1
Ciprofloxacin (5 mcg)	45	3	0	7	0	0	4	0	0	1	0	0
Cephotaxime (30 mcg)	40	8	0	7	0	0	4	0	0	1	0	0
nalidixic acid (30 mcg)	37	0	11	5	0	2	4	0	0	0	0	1
Gentamicin (10 mcg)	47	1	0	7	0	0	4	0	0	1	0	0

93.7% (n=45). Among ß-lactam antibiotics including ampicillin, cephalotin and cephotaxime the most resistant rate belongs to cephalotin (97%/n=59), followed by ampicillin (n=31), whereas none of *Yersinia* spp. was resistant to cephotaxime. All of *Y. intermedia*, *Y. kristensen* and Y*. frederiksenii* isolates were sensitive to trimethoprim, chloramphenicol, ciprofloxacin, cefotaxim and gentamicin.

In total, 15/8% (n=60) of this isolates exhibited a resistant phenotype, and most of these strains showed resistance to more than one antimicrobial test. 34 (56%) isolates were resistant to two or more antibiotic test

The highest co-resistance rate was seen for the ampicillin, cephalotin with rate of 28% (n=17), followed by 11% (n=7) for ampicilin, nalidixic acid and cephalotin. Four isolates (6%) were resistant to five antibiotics including ampicillin, nalidixic acid, cephalotin, tetracycline and streptomycin.

DISCUSSION

Yersinia spp. was reported from 9 to 99.5 percent in different sources such as meat, grandbeef, pork, environment, water and human (Kapperud, 1977; Ostroff, 1995; Soltan, 2001; Okwori et al., 2005). In our study, we analyzed two kinds of meat, chicken meat and beef meat, which are broadly consumed in Iran. Our results indicated *Y. entrocolitica* has a high incidence among of other *Yersinia* spp in meat and chicken. Similar findings had been achieved in Mexico (Ramirez and Vazquez-Salinas; 2000). The prevalence of *Y. frederiksenii* (11.6%) in present study was similar to results of Capita (Capita et al., 2002). Ibraham examined 50 beef samples for *Yersinia* spp. in Turkey and the isolation rate was 20% including of 9 (18%) *Y. enterocolitica* and 2 (4%) *Y. frederiksenii* (Ibraham and Mac Rae, 1991). Also in another study in Spain, *Y. enterocolitica* and *Y. frederiksenii* were detected in 22 (55%) and 6 (15%) samples, respectively (Capita et al., 2002). The various results between these different studies and our study probably can be due to several factors such as isolation methods, season of isolation and geographical location regarding to various temperatures and number of analyzed samples. These factors play an important role in isolation of the *Yersinia spp*. For instance, in this study the most of *Yersinia* spp. isolated in cold months such as November, April and December. It is known that the isolation rate of *Y. enterocolitica* is higher in cold climates (Warnken and Nunes, 1980). According present finding the highest rate of *Yersinia* spp, exception of *Y. kristensenii*, was isolated in 4 °C/3-week enrichment (Jiang and Kang, 2000).

Although *Y. entrocolitica* has been known as a most principal cause of human infections, however there are some reports about coursing gastroenteritis in human by other species such as *Y. intermedia*, *Y. kristensen* and Y*. frederiksenii* (Brenner and Bercovier, 1980; Hamama and Marrakchi, 1992). Strains of these pathogenic biotypes contain marker associated with virulence and these are located on the chromosome (ail) and on the virulence plasmid (PYV) (Goverde et al., 1993; Khorramizadeh et al., 2007). In USA the Center for Diseases Control and Prevention (CDC, 1982), conducts investigations of outbreaks of yersiniosis to control them and to learn more about how to prevent these infections. According with others authors, it can be assumed that further clinical studies are needed to assess the epidemiological importance, the occurrence and the possible etiological relevance of *Y. enterocolitica* (Ramirez and Vazquez-Salinas, 2000; Hoffmann et al., 2002).

Therefore it seems the investigation of susceptibility pattern and antibiotic resistance can be necessary for treatment of strains clinically. Antimicrobial resistance in foodborne pathogens and therapeutical intervention has always been an important issue in public health (Soltan et al., 2010). Using of antimicrobial agent in veterinary as a growth promotion, treatment or prophylactic can develop the antibiotic resistance in food animals. It can be a reason for antibiotic resistance transfer to humans via the food chain (Mayrhofer and Paulsen, 2004; Ezekiel et al., 2011). According to our results, the majority of isolates were resistant to cephalothine that may be due to presence of Enzyme B. The first report about B lactamase production by *Y. entrocolitica* was in 1973 (Cornelis et al., 1973). Soon afterwards, the presence of two types of chromosomal β-lactamase was described in some clinical isolates of *Y. enterocolitica*, a non-inducible broad-spectrum β-lactamase, enzyme A, and an inducible cephalosporinase, enzyme B (Cornelis and Abraham, 1975). The presence of two types of chromosomal B-lactamase was described in some clinical isolates of *Y. entrocolitica*, Enzyme B as Inducible cephalosporinase is one of them (Jeannette et al., 2000; Pham and Bell, 2000) The antibiotic susceptibility of *Y. enterocolitica* to gentamicin and chloramphenicol, ciprofloxacin and streptomycin are similar to reports of Okwori et al. (2005), who documented sensitivity of *Y. enterocolitica* strains of animal origin to ciprofloxacin and floxavid. The results indicated most active pharmacologic agents were chloramphenicol, gentamicin and trimethoprim. Regarding to high sensitivity of *Yersinia* spp. to gentamicin and chloramphenicol, these agents should be effective in the treatment of *Yersinia* spp when clinically indicated. It is suggested from results that the presence of *Yersinia* in beef meat and chicken meat represent a health risk for consumers. The education of people who involve in production, processing and final preparation of animal products is required to avoid cross-contamination.

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