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# Prevalence, Biotyping, and Antimicrobial Resistance of Yersinia enterocolitica Isolated from Traditional Iranian Cheeses - Evaluation of Yersinia enterocolitica in Traditional Cheeses

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RESEARCH ARTICLE

## Abstract

The present study aimed to investigate the contamination rate of various traditional Iranian cheese samples with *Yersinia enterocolitica*. In total, 200 cheese samples were collected from the northeast of Iran, and 10 types of traditional cheese were evaluated, including Lighvan, Kurdish, lactic, Tape-Salam, Onsory, Turkmen (type one and two), Sistani, Baluchi, and Kormange. The samples were analyzed using pre-enrichment Peptone-Sorbitol-Bile (PSB) broth, *Yersinia* selective agar (Cefsulodin-Irgasan-Novobiocin (CIN)) following polymerase chain reaction (PCR). Antimicrobial tests were carried out using 13 antibiotics on all the positive samples. From the cheese samples collected from Khorasan Razavi province, Kurdish cheese had the highest contamination rate (9/20; 45%), while the lowest contamination rate was observed in Lighvan and Onsory cheese. Also, the most commonly identified biotype was biotype 1A (23/38; 61%). *Y. entrocolitica* was mostly susceptible to ciprofloxacin, tetracycline, gentamicin, chloramphenicol, cefotaxime, and ceftazidime, while resistant to ampicillin and amoxicillin.

Keywords: Yersinia enterocolitica; Biotype; Antimicrobial Resistance; Traditional Cheese.

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## INTRODUCTION

Yersinia genus is a member of the Enterobacteriaceae family, which consists of 18 species, while Y. enterocolitica, Y. pseudotuberculosis and Y. pestis are pathogens in humans (Kiani, Bakhshi, Soltan-Dallal, & Najar-Peerayeh, 2019; Rusak et al., 2018). Yersinia enterocolitica is a foodborne pathogen that is present in various foods, especially dairy products. This bacterium causes yersiniosis infection, acute gastroenteritis, enterocolitis, hepatosplenic abscesses, septicemia, and Y. enterocolitica is a psychrotrophic microorganism, which is able to survive refrigeration, remaining in frozen foods and even in freezing conditions (Petsios, Fredriksson-Ahomaa, Sakkas, & Papadopoulou, 2016). This bacterium could be found in animals, food, water, and the environment, and pigs (pork and pork products) have been reported to be the main reservoirs for the transfer of pathogenic *Y. enterocolitica* (Bonardi et al., 2016). Evidence suggests that the foods that are contaminated with *Y. enterocolitica* could be the main cause of

yersiniosis development and main cause of *Yersinia* transmission to humans (Jamali *et al.*, 2015). Furthermore, the prevalence of this infection has been reported in food sources, such as meat, chicken, and dairy products (Sirghani, Zeinali, & Jamshidi, 2018; Jamali *et al.*, 2015; Soltan-Dallal, Tabarraie, & MoezArdalan, 2004).

According to previous studies, *Y. enterocolitica* has been detected frequently in cattles, which may be transmitted to its raw milk and non-pasteurized cheese (Jamali *et al.*, 2015; Ackers *et al.*, 2000; Milad Tavassoli, Jamshidi, Movafagh, & Afshari, 2019). In Iran, the consumption of dairy products (especially traditional cheese) is highly common in villages, while there is scarce data regarding the prevalence of *Y. enterocolitica* pathogens in Iranian dairy products (Hanifian & Khani, 2012a; Rahimi, Sepehri, Safarpoor Dehkordi, Shaygan, & Momtaz, 2014).

Limited studies in this regard have investigated the incidence of yersiniosis in southeastern Asia (Ananchaipattana, Hosotani, Kawasaki, Pongswat, *et al.*, 2012). Epidemiological information on yersiniosis is scarce in northeastern Iran, however, findings of *Y. enterocolitica* contamination in dairy products in northwest of Iran have been reported (Hanifian & Khani, 2012b).

*Y. enterocolitica* is a highly heterogeneous group of bacteria, consisting of six biotypes and more than 57 0serotypes. Biotypes 1B, two, three, four, and five are pathogenic, and biotype 1A is non-pathogenic (M. Tavassoli *et al.*, 2018; Bonardi et al., 2016). According to the literature, biotype 1A has been isolated from patients with diarrhea (Peng *et al.*, 2018). The most common pathogenic bio-serotypes in humans include 1B/0:8, 2/0:5,27, 2/0:9, 3/0:3, and 4/0:3 (Thoerner *et al.*, 2003). Biotypes 1B, two, three, four, and five carry the *Yersinia* virulence plasmid (pYV), while biotype 1A lacks the pYV plasmid (Tennant, Grant, & Robins-Browne, 2003). Despite the lack of pYV in biotype 1A of *Y. enterocolitica*, this biotype is an opportunistic pathogen, which has been isolated from patients with gastrointestinal diseases (Özdemir & Arslan, 2015).

*Y. enterocolitica* antimicrobial resistance is considered to be a major public health concern (M. Tavassoli *et al.*, 2018; Yang *et al.*, 2016). Excessive use of antibiotics has increased the antimicrobial resistance of *Y. enterocolitica* (Fàbrega & Vila, 2012). Previous studies have indicated that the *Y. enterocolitica* isolates in Iran have high sensitivity to antimicrobial agents (Bhaduri, Wesley, Richards, Draughon, & Wallace, 2009). *Y. enterocolitica* is often resistant to penicillin, ampicillin, cephalosporin, and amoxicillin/clavulanic acid antibiotics (Fàbrega & Vila, 2012).

Since the prevalence of *Y. enterocolitica* has not been investigated in northeast of Iran, we selected Khorasan Razavi and Golestan provinces as the largest provinces in this region for the monitoring of *Y. enterocolitica* contamination in traditional cheese samples. It is believed that these products are highly consumed in the northeast of Iran. Therefore, evaluation of the contamination rate of these local dairy products could provide beneficial data on the prevalence of *Y. enterocolitica* infection. The present study aimed to investigate the contamination rate, biotypes, and antimicrobial resistance of *Y. enterocolitica* in various types of traditional cheese in the northeast of Iran.

## **MATERIALS AND METHODS**

#### Sampling

This study was conducted during February-July 2018 on 200 traditional cheese samples, including Lighvan, Kurdish, lactic, Tape-Salam, Onsory, Turkmen (type one and two), Sistani, Baluchi, and Kormange, which were collected from the northeast of Iran (Khorasan Razavi and Golestan provinces). The samples were obtained from the manufacturers in different areas of Khorasan Razavi and Golestan provinces (10 areas), and 20 samples were collected from each area (every two weeks in three replicates) (Table 1). All the samples were transferred to the laboratory, preserved at the temperature of +4°C, and processed within 24 hours after collection (three days after production).

Types of Cheese	Number	No. of I 1A	Biotypes 1B	s 5	Region of Collection	Source of Milk for Cheese Production	City
Lighvan	20	1	2	0	Khorasan Razavi	Sheep Milk	Mashhad
Kurdish	20	6	3	0	Khorasan Razavi	Raw Cow Milk	Mashhad
Lactic	20	4	2	1	Khorasan Razavi	Cow Milk	Mashhad
Onsory	20	4	1	1	Khorasan Razavi	Cow Milk	Mashhad
Tape-Salam	20	2	1	0	Khorasan Razavi	Cow Milk	Mashhad
Turkmen	20	4	0	0	Golestan	Cow Milk	Gonbad
Turkmen	20	0	2	0	Golestan	Sheep Milk	Agh Ghala
Sistani	20	1	0	0	Golestan	Cow Milk	Kalaleh
Baluchi	20	1	1	0	Golestan	Cow Milk	Azadshahr
Kormange	20	0	1	0	Golestan	Goat Milk	Kordkuie

**Table 1.** Cheese samples collected from various regions and the frequency of various biotypes identified by biochemical tests in the northeast of Iran

#### Y. enterocolitica Isolation and Identification

Detection of *Y. enterocolitica* was performed using the described in the ISO No. 10273-2003 with minor modifications. Initially, 10 grams of each cheese sample was transferred into a stomacher bag containing 90 milliliters of peptone-sorbitol-bile (PSB) broth (Sigma-Aldrich, Germany) and homogenized for one minute. Following that, the isolates were incubated at the temperature of 25°C for 48 hours in a shaker incubator. Afterwards, 0.5 milliliter of the samples was mixed with 4.5 milliliters of 0.5% potassium hydroxide (KOH) and cultured onto Cefsulodin-Irgasan Novobiocin (CIN) agar plates (Merck, Darmstadt, Germany).

For each of the positive samples, 1-3 small colonies with deep red centers and translucent rims (bull's eye) in the CIN agar plate were selected. At the next stage, the isolates were stored in Brain-Heart-Infusion (BHI) broth containing 20% glycerol at the temperature of -20°C for further identification. Urease activity, indole production, and catalase and oxidase tests were performed on the samples grown in CIN agar.

### Y. enterocolitica Biotyping

The biotyping of the *Y. enterocolitica* isolates was performed using the method proposed by Wauters, Janssens, Steigerwalt, & Brenner (1988). The biotyping process involved the analysis of lipase activity, aesculin hydrolysis, indole production, and sugar fermentation (salicin, trehalose, sorbose, ornithine decarboxylase, inositol, and xylose).

#### DNA Extraction of Y. enterocolitica

DNA extraction was performed on 38 *Y. enterocolitica* isolates using the DNA isolation kit (QIAGEN GmbH-Germany) in accordance with the instructions of the manufacturer.

#### PCR Detection of Y. enterocolitica

Polymerase chain reaction (PCR) was determined following the method of Arnold, Neubauer, Nikolaou, Roesler, & Hensel (2004) with slight modification, the total volume of 25 microliters, including 12.5 microliters of 2× master mix (Merck, Germany) and one microliter (10 picomol) using PCR targeting *Y. enterocolitica*-specific 16S rRNA gene of forward (5'-AATACCGCATAACGTCTTCG-3') and reverse primers (5'-CTTCTTCTGCGAGTAACGTC-3') (Denazist Asia, Mashhad, Iran), as well as 4.5 microliters of the DNA template. Moreover, deionized water (CinnaGen, Tehran, Iran) was added with the final volume of six microliters, and the PCR reactions were performed in a thermal cycler (Bio-Rad Laboratories). In the thermal cycler program, initial denaturation was performed at the temperature of 94°C for 45 seconds, annealing was carried out at the temperature of 62°C for 45 seconds, extension was performed at the temperature of 72°C for 45 seconds, and final extension was carried out at the temperature of 72°C for seven minutes. In order to achieve the optimal annealing temperature, a gradient PCR was performed first, which temperature of 62°C was used to set in the PCR cycles. The PCR products (330 bp) were visualized via agarose gel electrophoresis (1.5% agarose gel) and *Y. enterocolitica* (PTCC 1151) was used as the positive control.

#### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed using the disk-diffusion method proposed by the Clinical and Laboratory Standards Institute (CLSI, 2006) at the temperature of  $26 \circ C$  on the Mueller–Hinton agar (Merck, Germany; CLSI, 2006). In total, 13 antimicrobial agents were tested, including ciprofloxacin (5 µg), ceftazidime (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), ampicillin (10 µg), amoxicillin and clavulanic acid (2:1; 30 µg), cefotaxime (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), and meropenem (10 µg). The growth inhibition zones were measured using a caliper to the nearest 0.01 millimeter.

#### **Statistical Analysis**

Data analysis was performed in SPSS version 16.0, and Differences in the prevalence of Y. enterocolitica in different cheese types and areas were analyzed by chi-square test.

## **RESULTS AND DISCUSSIONS**

#### Prevalence of Y. enterocolitica Isolated from the Cheese Samples

Among 200 cheese samples, *Y. enterocolitica* was isolated from 38 samples (19%). In Khorasan Razavi province, the contamination rate with *Y. enterocolitica* in Kurdish cheese was 9/20, while it was 7/20 in lactic cheese, 6/20

in Tape-Salam cheese, 3/20 in Lighvan cheese, and 3/20 in Onsory cheese. In Golestan province, the contamination rate of the cheese samples was determined to be 4/20 in Turkmen type one cheese, 2/20 in Turkmen type two cheese, 2/20 in Baluchi cheese, 1/20 in Sistani cheese, and 1/20 in Kormange cheese (Table 1).

## Biotype of *Y. enterocolitica*

The most commonly isolated biotype from the studied cheese types was biotype 1A (23/38), followed by biotype 1B (13/38) and biotype 5 (2/38). In the cheese samples collected from Khorasan Razavi province, biotype 1A was isolated from Lighvan cheese (1/20). Furthermore, biotype 1A was isolated from Turkmen type one cheese (4/20), while it was not considered significant in the other samples (Table 1).

Biotype 1B was more isolated in Khorasan Razavi cheeses than Kurdish cheese and biotype 1B was isolated from Turkmen type two cheese at the rate of 2/20 (10%), while it was not considered significant in the other cheese types. Biotype 5 was also observed in the cheese samples collected from Khorasan Razavi province, while it was not detected in the samples collected from Golestan province (Table 1).

## Antimicrobial Resistance

The antimicrobial resistance profiles of *Y. enterocolitica* in the present study are shown in (Table 2). According to the findings, all the *Y. enterocolitica* isolated from the cheese samples were sensitive to ciprofloxacin, tetracycline, gentamicin, chloramphenicol, cefotaxime and ceftazidime. The highest susceptibility rate was observed in ciprofloxacin and tetracycline (100%), followed by cefotaxime (35/38; 92.10%), gentamicin (33/38; 86.84%), and chloramphenicol (28/38; 73.68%). In addition, resistance to ampicillin, amoxicillin (100%), and kanamycin was common in *Y. enterocolitica*.

	•			•	
Antimicrobial Agents		Number of Yersinia enterocolitica (n = 38; %)			
Tasted Ranges (mm)	-	Ra	Ι	S	
Ampicillin	0 <sup>b</sup> -1 <sup>c</sup>	38 (100.00)	0 (0.00)	0 (0.00)	
Amoxicillin and Clavulanic Acid (2:1)	0-0	38 (100.00)	0 (0.00)	0 (0.00)	
Kanamycin	0-2	33 (86.84)	5 (13.15)	0 (0.00)	
Tetracycline	10-16	0 (0.00)	4 (10.52)	34 (89.47)	
Chloramphenicol	6-15	0 (0.00)	10 (26.31)	28 (73.68)	
Ciprofloxacin	10-18	0 (0.00)	0 (0.00)	38 (100.00)	
Ceftazidime	7-16	0 (0.00)	8 (21.05)	30 (78.94)	
Gentamicin	8-16	0 (0.00)	5 (13.15)	33 (86.84)	
Cefotaxime	10-18	0 (0.00)	3 (7.86)	35 (92.10)	
Nalidixic Acid	2-18	3 (7.86)	12 (31.56)	23 (60.52)	
Meropenem	3-15	8 (21.05)	9 (23.68)	21 (55.26)	
Trimethoprim/Sulfamethoxazole	6-15	6 (15.78)	23 (60.52)	9 (23.68)	
Streptomycin	5-16	7(18.42)	24 (63.15)	7 (18.42)	

Table 2. Antimicrobial resistance profiles of Y. enterocolitica isolated from cheese samples.

<sup>a</sup>R: resistant; I: intermediate; S: susceptible; <sup>b</sup>Min; <sup>c</sup>Max

There are diverse ethnic backgrounds in the northeast of Iran, and cheese production is common due to the high consumption of this food product in this region. However, data is scarce regarding the prevalence of *Y. enterocolitica* in various food products (Hanifian & Khani, 2012b). *Y. enterocolitica* is a psychotropic bacterium that easily grows in the refrigerator; if cheese production processes are not appropriate, contamination with this microorganism is highly likely (Ananchaipattana, Hosotani, Kawasaki, Pongsawat, *et al.*, 2012).

## Prevalence of Y. enterocolitica

According to the results of the present study, 19% (38/200) of the cheese samples were contaminated with *Y. enterocolitica*. Lighvan cheese had the lowest contamination rate, which could be due to the production of lactic acid bacteria in this cheese type, thereby preventing the growth of *Y. enterocolitica* (Garabal, Rodríguez-Alonso, & Centeno, 2008). Sheep milk is used in the preparation of Lighvan cheese, and the cheese is produced with no additive starter, which maintains the proper conditions for the growth of these pathogens (Hanifian & Khani, 2012a). Kurdish cheese is commonly used in Iran and is manufactured from raw cow milk during fermentation in goat skin (Hashemi, Shahidi, Mortazavi, Milani, & Eshaghi, 2014). Contamination of raw milk and the equipment in this process are considered to be the main causes of *Y. enterocolitica* transmission (Hanifian & Khani, 2012a). According

to a conducted study in the United States, the outbreak of yersiniosis was observed in 22 cases, most of which were the consumers of pasteurized milk (Longenberger *et al.*, 2013).

Kurdish cheese has the proper pH for *Y. enterocolitica* growth due to non-thermal treatment and lack of starter culture. Lactic cheese is obtained from cow milk and has relatively similar components to those of Kurdish cheese (e.g., less lactic acid bacteria) (Hanifian & Khani, 2012b; Morgan, Bonnin, Mallereau, & Perrin, 2001; Soltan-Dallal *et al.*, 2004). Tape-Salam and Onsory cheeses are traditionally made from cow milk, and this milk is considered to be an important source of *Y. enterocolitica* transmission (Hanifian & Khani, 2012b).

In the study conducted by Jamali *et al.* (2015), *Y. enterocolitica* was isolated more frequently from cow milk, and while sheep milk was reported to be less contaminated with these pathogens (Jamali *et al.*, 2015). Turkmen type one cheese is made from cow milk and was observed to be more contaminated compared to Turkmen type two, which is manufactured from sheep milk. In the Sistani and Baluchi traditional cheese types, minor contamination with *Y. enterocolitica* pathogens was reported as well. Kormange cheese is manufactured from goat milk and in present study was reported to have a low contamination rate (5%).

Consistent with the results of the present study, the findings of Jamali et al. regarding goat milk indicated only one case of contamination with *Y. enterocolitica* (Jamali *et al.*, 2015). Results of previous studies in this regard have also denoted that cow milk has higher contamination compared to other milk types. However, regarding the results of this study, it should be noted that due to the lack of data, there is not enough evidence to show that cheeses made from cow's milk are more contaminated than other milk types. In the research by Bursova et al., no significant difference was reported in the growth of *Y. enterocolitica* between cow and goat milk (Bursová, Necidová, Haruštiaková, & Janštová, 2017).

Another study in this regard was performed by Hanifian and Khani in the northwest of Iran on 200 cheese samples, and 21 *Y. enterocolitica* (10.5%) were isolated (Hanifian & Khani, 2012b), while in the present study, the contamination rate with *Y. enterocolitica* was higher (38/200; 19%). This could be due to the differences in the geographical regions or processing chain of cheese preparation, which affect the isolation rate (Kiani *et al.*, 2019). Several studies have reported the contamination rate of cheese samples with *Y. enterocolitica*; for instance, the contamination rate has been reported to be 2% and 4% in Egypt, 5% and 2.5% in Turkey, 4.5% in China, and 10.5% in Iran (Ahmed, Tahoun, Abou Elez, Abd El-Hamid, & Abd Ellatif, 2019; Atta, 2009; Hanifian & Khani, 2012b; Özdemir & Arslan, 2015; Y. W. Ye, Ling, Han, & Wu, 2014; Yucel & Ulusoy, 2006). On the other hand, another research conducted in Poland demonstrated that Polish cheese samples were not contaminated with *Y. enterocolitica* pathogens (Zadernowska & Chajęcka-Wierzchowska, 2017).

During the production of traditional cheese, no specific processes are performed to eliminate *Y. enterocolitica* along with the preservation of the samples in refrigerated conditions (Bursová *et al.*, 2017; Hanifian & Khani, 2012b). Notably, *Y. enterocolitica* are psychrotrophic bacteria, which grow in a wide range of temperatures (-2-42°C), therefore, it may also grow at refrigerated temperatures (Bursová *et al.*, 2017; McAuley, McMillan, Moore, Fegan, & Fox, 2014). In the present study, the cheese samples were collected three days after production and stored in a refrigerator, where *Y. enterocolitica* could grow. In line with our findings, Bursova *et al.* stated that *Y. enterocolitica* is able to grow at the temperature of 4°C within three days (Bursová *et al.*, 2017).

#### Biotype of Y. enterocolitica

Biotype 1A is often considered to be non-pathogenic due to the absence of pYV plasmids (Bancerz-Kisiel, Pieczywek, Łada, & Szweda, 2018; Milad Tavassoli *et al.*, 2019), while some studies reported that this biotype may produce symptoms similar to pathogenic biotypes (Bancerz-Kisiel *et al.*, 2018; Tennant *et al.*, 2003). Most of the strains that were isolated in the present study belonged to biotype 1A. In line with our findings, the studies conducted in Argentina, Chile, Egypt, Malaysia, China, the Czech Republic, and Iran have demonstrated that most of the *Y. enterocolitica* strains isolated from food are biotype 1A (Ahmed *et al.*, 2019; Jamali *et al.*, 2015; Kiani *et al.*, 2019; Mastrodonato, Favier, Lucero Estrada, Vidal, & Escudero, 2018; Tan, Ooi, & Thong, 2014; Verbikova, Borilova, Babak, & Moravkova, 2018; Q. Ye, Wu, Hu, Zhang, & Huang, 2016). *Y. enterocolitica* infection has been reported in the other food products in the studied area as well. For instance, the findings in Khorasan Razavi province indicated that the prevalence of *Y. enterocolitica* in chicken meat was 25% and all isolates belonged to biotype 1A (Sirghani, Zeinali, & Jamshidi, 2018). Since biotype 1A strains are isolated from the environment and may cause gastroenteritis in human subjects, special attention should be paid to this biotype and cheese production chain hygiene.

Biotype 1B of *Y. enterocolitica* is often associated with human yersiniosis (Bottone, 1997). In the current research, the isolated biotype 1B was more frequent compared to studies in China, Iran and Malaysia (Kiani *et al.*, 2019; Tan *et al.*, 2014; Y. W. Ye *et al.*, 2014). On the other hand, the studies conducted in Argentina, Chile, the Czech Republic, and Brazil showed no isolated biotype 1B of *Y. enterocolitica* (Frazão, Andrade, Darini, & Falcão, 2017; Mastrodonato *et al.*, 2018; Verbikova *et al.*, 2018). According to our findings and another study carried out in Iran, the prevalence rate of biotype 1B isolates was higher compared to other countries such as China, Turkey, and Egypt (Ahmed, Tahoun, Abou Elez, Abd El-Hamid, & Abd Ellatif, 2019; Özdemir & Arslan, 2015; Y. W. Ye, Ling, Han, & Wu, 2014; Yucel & Ulusoy, 2006). In Iran, Jamali et al. reported that 15.8% of the isolates were biotype 1B and in the

study of Kiani *et al.*, biotype 1B was only isolated from clinical samples while none of the environmental samples were positive for this biotype (Jamali *et al.*, 2015; Kiani *et al.*, 2019).

### Antimicrobial Resistance

According to the findings of the current research, all the *Y. enterocolitica* isolates were resistant to ampicillin, amoxicillin, clavulanic acid, and kanamycin, which is consistent with the previous studies in this regard (Fois *et al.*, 2018; Fondrevez *et al.*, 2014; Peng *et al.*, 2018; Verbikova *et al.*, 2018). Furthermore, *Y. enterocolitica*, mostly biotype 1B, was resistant to kanamycin (84%) which is in line with the study conducted in the Czech Republic. However, a research in Brazil indicated that the isolates of *Y. enterocolitica* were susceptible to kanamycin (Verbikova *et al.*, 2018).

In the current research, the highest sensitivity of *Y. enterocolitica* (mostly biotype 1A) was observed against ciprofloxacin, cefotaxime, tetracycline, gentamicin, ceftazidime, and chloramphenicol, which is in line with the previous studies in this regard (Fois *et al.*, 2018; Martins *et al.*, 2018; Peng *et al.*, 2018; Verbikova *et al.*, 2018). In another study conducted in Iran, *Y. enterocolitica* was reported to be resistant to tetracycline, which is inconsistent with the findings of the current research (Jamali *et al.*, 2015). This discrepancy might be due to geographical differences as they play a key role in the resistance pattern of *Y. enterocolitica* (Tavassoli *et al.*, 2018).

According to the study in China, the resistance pattern of *Y. enterocolitica* was closely correlated with the source of origin, while the biotypes and serotypes were less affected (Peng *et al.*, 2018). Pork is considered to be the main source of *Y. enterocolitica*, and the consumption of pork is prohibited in Islamic countries, such as Iran. Nevertheless, the prevalence of this bacterium is reported to be high in other food products (Hanifian & Khani, 2012a, 2012b; Jamali *et al.*, 2015; Soltan-Dallal *et al.*, 2004).

## CONCLUSIONS

According to the results of the present study, the prevalence of *Y. enterocolitica* in the cheese types collected from Khorasan Razavi province was higher compared to the samples collected from Golestan province. Some of the confounding factors in the prevalence of *Y. enterocolitica* are the differences in the geographical location, source of cheese products, production chain, and public health conditions. Evaluation of various traditional cheese types in Iran in terms of *Y. enterocolitica* infection indicated that cheese consumption may play a key role in *Y. enterocolitica* transmission in the northeast of Iran. Therefore, it is recommended that further investigations be conducted on the emerging antibiotic resistance in order to identify the foods with significant risks and ensure the effectiveness of the treatments. According to our findings, the overall antimicrobial resistance of *Y. enterocolitica* was the same as the previous reports in Iran and other countries, with the exception of resistance against tetracycline and kanamycin. Therefore, further investigations and constructive strategies are required for the prevention and control of *Y. enterocolitica* in Iran.

**Author Contributions:** Please specify the individual contributions of every author if there are several. Ex.: A.B. Conceived and designed the analysis; C.D. Collected the data; E.F. Contributed data or analysis tools; G.H. Performed the analysis; I.J. Wrote the paper. You can add other contributions if necessary.

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### **Conflicts of Interest**

Authors must declare any conflicts of interest or state "The authors declare that they do not have any conflict of interest." A conflict of interest can occur when you have a financial, commercial, legal, or professional relationship with other organizations, or with the people working with them, that could influence your research. Authors must identify and declare any personal circumstances or interest that may influence their work.

## REFERENCES

- 1. Ackers, M.-L., Schoenfeld, S., Markman, J., Smith, M. G., Nicholson, M. A., DeWitt, W., Slutsker, L. (2000). An Outbreak of Yersinia enterocolitica O:8 Infections Associated with Pasteurized Milk. *The Journal of Infectious Diseases*, *181*(5), 1834-1837. doi:10.1086/315436
- Ahmed, H. A., Tahoun, A. B. M. B., Abou Elez, R. M. M., Abd El-Hamid, M. I., & Abd Ellatif, S. S. (2019). Prevalence of Yersinia enterocolitica in milk and dairy products and the effects of storage temperatures on survival and virulence gene expression. *International Dairy Journal*, 94, 16-21. doi:https://doi.org/10.1016/j.idairyj.2019.02.010
- 3. Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongsawat, S., Md.Latiful, B., Isobe, S., & Inatsu, Y. (2012). Prevalence of Foodborne Pathogens in Retailed Foods in Thailand. *Foodborne Pathogens and Disease, 9*(9), 835-840. doi:10.1089/fpd.2012.1169
- 4. Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongswat, S., Latiful, B. M., Isobe, S., & Inatsu, Y. (2012). Bacterial Contamination of Soybean Curd (Tofu) Sold in Thailand. *Food Science and Technology Research*, *18*(6), 843-848. doi:10.3136/fstr.18.843
- Arnold, T., Neubauer, H., Nikolaou, K., Roesler, U., & Hensel, A. (2004). Identification of Yersinia enterocolitica in Minced Meat: A Comparative Analysis of API 20E, Yersinia Identification Kit and a 16S rRNA-based PCR Method. *Journal of Veterinary Medicine, Series B*, 51(1), 23-27. doi:10.1046/j.1439-0450.2003.00718.x
- 6. Atta, S. F. A. A. E. A. a. M. A. E. H. B. (2009). Occurrence of Listeria and Yersinia species in milk and some milk products *Assiut Veterinary Medical Journal*, *55*, 45-60.
- 7. Bancerz-Kisiel, A., Pieczywek, M., Łada, P., & Szweda, W. (2018). The Most Important Virulence Markers of Yersinia enterocolitica and Their Role during Infection. *Genes*, *9*(5), 235.
- 8. Bhaduri, S., Wesley, I., Richards, H., Draughon, A., & Wallace, M. (2009). Clonality and Antibiotic Susceptibility of Yersinia enterocolitica Isolated from U.S. Market Weight Hogs. *Foodborne Pathogens and Disease, 6*(3), 351-356. doi:10.1089/fpd.2008.0197
- Bonardi, S., Bruini, I., D'Incau, M., Van Damme, I., Carniel, E., Brémont, S., . . . Brindani, F. (2016). Detection, seroprevalence and antimicrobial resistance of Yersinia enterocolitica and Yersinia pseudotuberculosis in pig tonsils in Northern Italy. *International Journal of Food Microbiology*, 235, 125-132. doi:https://doi.org/10.1016/j.ijfoodmicro.2016.07.033
- 10. Bottone, E. J. (1997). Yersinia enterocolitica: the charisma continues. *Clinical Microbiology Reviews*, *10*(2), 257-276. doi:10.1128/cmr.10.2.257
- 11. Bursová, Š., Necidová, L., Haruštiaková, D., & Janštová, B. (2017). Growth potential of Yersinia enterocolitica in pasteurised cow's and goat's milk stored at 8 °C and 24 °C. *Food Control, 73*, 1415-1419. doi:https://doi.org/10.1016/j.foodcont.2016.11.006
- 12. Fàbrega, A., & Vila, J. (2012). Yersinia enterocolitica: Pathogenesis, virulence and antimicrobial resistance. *Enfermedades Infecciosas y Microbiología Clínica, 30*(1), 24-32. doi:https://doi.org/10.1016/j.eimc.2011.07.017
- Fois, F., Piras, F., Torpdahl, M., Mazza, R., Ladu, D., Consolati, S. G., . . . De Santis, E. P. L. (2018). Prevalence, bioserotyping and antibiotic resistance of pathogenic Yersinia enterocolitica detected in pigs at slaughter in Sardinia. *International Journal of Food Microbiology, 283*, 1-6. doi:https://doi.org/10.1016/j.ijfoodmicro.2018.06.010
- Fondrevez, M., Minvielle, B., Labbé, A., Houdayer, C., Rose, N., Esnault, E., & Denis, M. (2014). Prevalence of pathogenic Yersinia enterocolitica in slaughter-aged pigs during a one-year survey, 2010–2011, France. *International Journal of Food Microbiology*, 174, 56-62. doi:https://doi.org/10.1016/j.ijfoodmicro.2013.12.027
- Frazão, M. R., Andrade, L. N., Darini, A. L. C., & Falcão, J. P. (2017). Antimicrobial resistance and plasmid replicons in Yersinia enterocolitica strains isolated in Brazil in 30 years. *The Brazilian Journal of Infectious Diseases*, 21(4), 477-480. doi:https://doi.org/10.1016/j.bjid.2017.04.006
- Garabal, J. I., Rodríguez-Alonso, P., & Centeno, J. A. (2008). Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). *LWT - Food Science and Technology*, 41(8), 1452-1458. doi:https://doi.org/10.1016/j.lwt.2007.09.004
- 17. Hanifian, S., & Khani, S. (2012a). Fate of Yersinia enterocolitica during manufacture, ripening and storage of Lighvan cheese. *International Journal of Food Microbiology*, *156*(2), 141-146. doi:https://doi.org/10.1016/j.ijfoodmicro.2012.03.015

- 18. Hanifian, S., & Khani, S. (2012b). Prevalence of virulent Yersinia enterocolitica in bulk raw milk and retail cheese in northern-west of Iran. *International Journal of Food Microbiology*, 155(1), 89-92. doi:https://doi.org/10.1016/j.ijfoodmicro.2012.01.012
- 19. Hashemi, S. M. B., Shahidi, F., Mortazavi, S. A., Milani, E., & Eshaghi, Z. (2014). Potentially Probiotic Lactobacillus Strains from Traditional Kurdish Cheese. *Probiotics and Antimicrobial Proteins*, 6(1), 22-31. doi:10.1007/s12602-014-9155-5
- 20. Jamali, H., Paydar, M., Radmehr, B., & Ismail, S. (2015). Prevalence, characterization, and antimicrobial resistance of Yersinia species and Yersinia enterocolitica isolated from raw milk in farm bulk tanks. *Journal of Dairy Science*, *98*(2), 798-803. doi:https://doi.org/10.3168/jds.2014-8853
- 21. Kiani, P., Bakhshi, B., Soltan-Dallal, M. M., & Najar-Peerayeh, S. (2019). Heterogeneity of Highly Susceptible Yersinia enterocolitica Isolates of Clinical and Environmental Origin: A 5-Year Survey from Iran (2011–2016). *Microbial Drug Resistance, 26*(1), 46-53. doi:10.1089/mdr.2018.0469
- Longenberger, A. H., Gronostaj, M. P., Yee, G. Y., Johnson, L. M., Lando, J. F., Voorhees, R. E., . . . Ostroff, S. M. (2013). Yersinia enterocolitica infections associated with improperly pasteurized milk products: southwest Pennsylvania, March–August, 2011. *Epidemiology and Infection, 142*(8), 1640-1650. doi:10.1017/S0950268813002616
- Martins, B. T. F., Botelho, C. V., Silva, D. A. L., Lanna, F. G. P. A., Grossi, J. L., Campos-Galvão, M. E. M., ... Nero, L. A. (2018). Yersinia enterocolitica in a Brazilian pork production chain: Tracking of contamination routes, virulence and antimicrobial resistance. *International Journal of Food Microbiology*, 276, 5-9. doi:https://doi.org/10.1016/j.ijfoodmicro.2018.03.028
- 24. Mastrodonato, A. C., Favier, G. I., Lucero Estrada, C. S. M., Vidal, R., & Escudero, M. E. (2018). Bioserotypes, virulence genes, antimicrobial susceptibility and genomic diversity of Yersinia enterocolitica isolates from Argentina and Chile. *Journal of Food Safety*, *38*(5), e12491. doi:doi:10.1111/jfs.12491
- 25. McAuley, C. M., McMillan, K., Moore, S. C., Fegan, N., & Fox, E. M. (2014). Prevalence and characterization of foodborne pathogens from Australian dairy farm environments. *Journal of Dairy Science*, *97*(12), 7402-7412. doi:https://doi.org/10.3168/jds.2014-8735
- 26. Morgan, F., Bonnin, V., Mallereau, M. P., & Perrin, G. (2001). Survival of Listeria monocytogenes during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. *International Journal of Food Microbiology*, *64*(1), 217-221. doi:https://doi.org/10.1016/S0168-1605(00)00452-9
- 27. Özdemir, F., & Arslan, S. (2015). Genotypic and phenotypic virulence characteristics and antimicrobial resistance of Yersinia spp. isolated from meat and milk products. *Journal of Food Science, 80*(6), M1306-M1313. doi:10.1111/1750-3841.12911
- 28. Peng, Z., Zou, M., Li, M., Liu, D., Guan, W., Hao, Q., . . . Li, F. (2018). Prevalence, antimicrobial resistance and phylogenetic characterization of Yersinia enterocolitica in retail poultry meat and swine feces in parts of China. *Food Control*, *93*, 121-128. doi:10.1016/j.foodcont.2018.05.048
- 29. Petsios, S., Fredriksson-Ahomaa, M., Sakkas, H., & Papadopoulou, C. (2016). Conventional and molecular methods used in the detection and subtyping of Yersinia enterocolitica in food. *International Journal of Food Microbiology*, *237*, 55-72. doi:https://doi.org/10.1016/j.ijfoodmicro.2016.08.015
- 30. Rahimi, E., Sepehri, S., Safarpoor Dehkordi, F., Shaygan, S., & Momtaz, H. (2014). Prevalence of Yersinia Species in Traditional and Commercial Dairy Products in Isfahan Province, Iran. *Jundishapur Journal of Microbiology*, *7*(4), e9249. doi:10.5812/jjm.9249
- 31. Rusak, L. A., de Castro Lisboa Pereira, R., Freitag, I. G., Hofer, C. B., Hofer, E., Asensi, M. D., & Vallim, D. C. (2018). Rapid detection of Yersinia enterocolitica serotype 0:3 using a duplex PCR assay. *Journal of Microbiological Methods*, 154, 107-111. doi:https://doi.org/10.1016/j.mimet.2018.10.014
- 32. Sirghani, K., Zeinali, T., & Jamshidi, A. (2018). Detection of Yersinia enterocolitica in Retail Chicken Meat, Mashhad, Iran. *Journal of Pathogens, 2018*, 4. doi:10.1155/2018/1286216
- 33. Soltan-Dallal, M.-M., Tabarraie, A., & MoezArdalan, K. (2004). Comparison of four methods for isolation of Yersinia enterocolitica from raw and pasteurized milk from northern Iran. *International Journal of Food Microbiology*, *94*(1), 87-91. doi:https://doi.org/10.1016/j.ijfoodmicro.2003.10.017
- 34. Stamm, I., Hailer, M., Depner, B., Kopp, P. A., & Rau, J. (2013). <em>Yersinia enterocolitica</em> in diagnostic fecal samples of European dogs and cats: Identification by FT-IR and MALDI-TOF MS. *Journal of Clinical Microbiology*. doi:10.1128/jcm.02506-12

- 35. Tan, L. K., Ooi, P. T., & Thong, K. L. (2014). Prevalence of Yersinia enterocolitica from food and pigs in selected states of Malaysia. *Food Control*, *35*(1), 94-100. doi:https://doi.org/10.1016/j.foodcont.2013.06.053
- 36. Tavassoli, M., Afshari, A., Drăgănescu, D., Arsene, A. L., Burykina, T. I., & Rezaee, R. (2018). Antimicrobial resistance of yersinia enterocolitica in different foods. A review. *Farmacia*, *66*(3), 399-407.
- 37. Tavassoli, M., Jamshidi, A., Movafagh, F., & Afshari, A. (2019). Virulence Characteristics of Yersinia enterocolitica Isolated from Dairy Products in the Northeast of Iran. *zums-jhehp*, *5*(2), 72-78. doi:10.29252/jhehp.5.2.5
- 38. Tennant, S. M., Grant, T. H., & Robins-Browne, R. M. (2003). Pathogenicity of Yersinia enterocolitica biotype 1A. *FEMS Immunology & Medical Microbiology*, *38*(2), 127-137. doi:10.1016/S0928-8244(03)00180-9
- Thoerner, P., Bin Kingombe, C. I., Bögli-Stuber, K., Bissig-Choisat, B., Wassenaar, T. M., Frey, J., & Jemmi, T. (2003). PCR Detection of Virulence Genes in <em>Yersinia enterocolitica</em> and <em>Yersinia pseudotuberculosis</em> and Investigation of Virulence Gene Distribution. *Applied and Environmental Microbiology*, 69(3), 1810-1816. doi:10.1128/aem.69.3.1810-1816.2003
- 40. Verbikova, V., Borilova, G., Babak, V., & Moravkova, M. (2018). Prevalence, characterization and antimicrobial susceptibility of Yersinia enterocolitica and other Yersinia species found in fruits and vegetables from the European Union. *Food Control, 85*, 161-167. doi:https://doi.org/10.1016/j.foodcont.2017.08.038
- 41. Wauters, g., janssens, m., steigerwalt, a. g., & Brenner, d. j. (1988). Yersinia mollaretii sp. nov. and Yersinia bercovieri sp. nov., Formerly Called Yersinia enterocolitica Biogroups 3A and 3B. *International Journal of Systematic and Evolutionary Microbiology*, *38*(4), 424-429. doi:doi:10.1099/00207713-38-4-424
- 42. Yang, X., Huang, J., Wu, Q., Zhang, J., Liu, S., Guo, W., . . . Yu, S. (2016). Prevalence, antimicrobial resistance and genetic diversity of Salmonella isolated from retail ready-to-eat foods in China. *Food Control, 60*, 50-56. doi:https://doi.org/10.1016/j.foodcont.2015.07.019
- 43. Ye, Q., Wu, Q., Hu, H., Zhang, J., & Huang, H. (2016). Prevalence and characterization of Yersinia enterocolitica isolated from retail foods in China. *Food Control,* 61, 20-27. doi:https://doi.org/10.1016/j.foodcont.2015.09.016
- 44. Ye, Y. W., Ling, N., Han, Y. J., & Wu, Q. P. (2014). Detection and prevalence of pathogenic Yersinia enterocolitica in refrigerated and frozen dairy products by duplex PCR and dot hybridization targeting the virF and ail genes. *Journal of Dairy Science*, *97*(11), 6785-6791. doi:https://doi.org/10.3168/jds.2014-8382
- 45. Yucel, N., & Ulusoy, H. (2006). A Turkey survey of hygiene indicator bacteria and Yersinia enterocolitica in raw milk and cheese samples. *Food Control*, *17*(5), 383-388. doi:https://doi.org/10.1016/j.foodcont.2005.01.005
- 46. Zadernowska, A., & Chajęcka-Wierzchowska, W. (2017). Prevalence, biofilm formation and virulence markers of Salmonella sp. and Yersinia enterocolitica in food of animal origin in Poland. *LWT*, *75*, 552-556. doi:https://doi.org/10.1016/j.lwt.2016.10.007