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RESEARCH ARTICLE

Incidence and Pathogenicity of *Yersinia enterocolitica* Isolates from Foods in Turkey [1]

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Summary

During a year period, at 150 from each food group, a total of 750 samples including ice cream, raw milk, feta cheese, chicken drumsticks and minced meat collected from markets located in the northeast region of Turkey (Kars, Ardahan and Iğdır) were analyzed for determining of *Yersinia spp.* incidence. Fifty seven of samples (7.6%) were evaluated as positive for *Yersinia spp.* and 18 (2.4% in total) of them, isolated from 6 feta cheese, 4 ice cream, 2 chicken drumsticks, 4 minced meat and 2 raw milk samples, were identified as pathogenic *Y. enterocolitica.* All the 18 pathogenic strains were tested for their antimicrobial susceptibility and some of the isolates were found to be resistant to ticarcillin (n=6), netilmicin (n=5), tetracycline (n=1) streptomycin (n=17), gentamicin (n=12), kanamycin (n=17), furazolidone (n=6), clindamycin (n=18) and cephazolin (n=18) while all of them were susceptible to sulphamethoxazole/trimethoprim, ciprofloxacin, chloramphenicol and imipenem. According to findings, cold enrichment at 4°C for 14 days seems to be more effective for isolation of *Yersinia spp.* than enrichment at 25°C for 24 h.

Keywords: *Yersinia enterocolitica, Pathogenicity, Animal Originated Foods*

Türkiye'de Gıdalardan İzole Edilen *Yersinia entocolitica*'nın Yaygınlığı ve Patojenitesi

Özet

Kars, Ardahan ve Iğdır'da yer alan marketlerden, bir yıl boyunca, her bir gıdadan 150'şer adet olmak üzere, toplam 750 örnek (çiğ süt, dondurma, taze beyaz peynir, tavuk budu ve kıyma) toplanarak *Yersinia spp.* varlığı araştırılmıştır. Örneklerin 57'sinden (%7,6) *Yersinia spp.* izole edilmiş ve bunlar içerisinde 6'sı beyaz peynir, 4'ü dondurma, 2'si tavuk butu, 4'ü kıyma ve 2'si çiğ süt olmak üzere toplam 18 gıdadan (% 2,4) elde edilen izolatlar, patojenik *Yersinia enterocolitica* olarak identifiye edilmiştir. 57 gıda örneğinden %31,57 oranında patojenik *Y. enterocolitica* identifiye edilmiştir. Antibiyotik duyarlılığı yönünden incelenen 18 suşun ticarcillin (n=6), netilmicin (n=5), tetracycline (n=1) streptomycin (n=17), gentamicin (n=12), kanamycin (n=17), furazolidone (n=6), clindamycin (n=18) ve cephazolin'e (n=18) dirençli olduğu görülürken, tümünün sulphamethoxazole/trimethoprim, ciprofloxacin, chloramphenicol ve imipenem'e duyarlı olduğu belirlenmiştir. Elde edilen bulgular, soğuk zenginleştirme (4°C'de 14 gün) yönteminin, 25°C'de 24 saatlik zenginleştirme prosedürüne göre *Yersinia spp.* izolasyonunda daha başarılı olduğuna işaret etmiştir.

Anahtar sözcükler: *Yersinia enterocolitica, Patojenite, Hayvansal gıdalar*

INTRODUCTION

The genus *Yersinia* is a member of the family *Enterobacteriaceae* and includes pathogenic and several non-pathogenic strains [1,2]. The genus *Yersinia* is composed of 11 species, of which three (*Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica*) have clearly been shown to cause human disease. The remaining

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eight species considered as nonpathogen (*Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. bercovieri*, *Y. mollaretii, Y. rohdei, Y. ruckeri* and *Y. aldovae*) have not been studied extensively due to the absence of classical *Yersinia* virulence markers [3]. Currently, *Y. enterocolitica* is represented by six biovars (1A, 1B and 2-5) and more than 50 serovars. The virulence of the pathogenic biovars namely 1B and 2-5 is attributed to the presence of a 70-kb pYV (plasmid for *Yersinia virulence*) and certain chromosomal genes [4]. *Y. enterocolitica* is an important entero-pathogen can cause acute enteritis (especially in children), enterocolitis, mesenteric lymphadenitis, and terminal ileitis. Colonization of the intestinal tract is the primary event of the successful enteric pathogen [5].

The pathogenic bacterium *Y. enterocolitica* has become increasingly important as a food contaminant. Of special significant in food hygiene is the ability of *Y. enterocolitica* to grow in refrigerated foods [6]. The psychrotrophic bacterium *Y. enterocolitica* which is able to grow at temperatures close to 0°C is characterized by temperature-dependent adaptations [7]. Yersiniosis is a typical foodborne disease. Yersinia has been frequently isolated from a variety of foods like untreated milk, chocolate milk, dairy cream and ice cream, vegetables like carrots, tomatoes, lettuce, celery and mushrooms, raw hare, beef and lamb. It has also been isolated from drinking water [8].

Difficulties associated with the isolation of pathogenic *Y. enterocolitica* stem from the small number of pathogenic strains in the samples and the large number of organisms in the background flora, especially in food and environmental samples. However, in order to get epidemiological information, *Y. enterocolitica* isolates are needed. Thus, at least one culture methods has to be used in parallel to PCR method. Selective enrichment is needed, especially when food samples are studied. However, no single procedure is currently available which will recover all bioserotypes [9]. However, the detection, isolation and enumeration of *Y. enterocolitica* remain problematic. The development of isolation procedures which clearly differentiate pathogenic from non-pathogenic variants has been difficult [6,9].

Y. enterocolitica and related species have been isolated from many types of food [8,10-16]. The majority of these food isolates differ in biochemical and serological characteristics from typical clinical strains and are usually mentioned 'non-pathogenic' or 'environmental' Yersinia strains [6,17]. It is important to determine the pathogenic significance of the isolates. *Y. enterocolitica* is thought to be a significant food-borne pathogen although the incidence of the pathogenic isolates in foods is low [6,17-19].

In the northeast region of Turkey, cheese, cream, butter and ice cream are made traditionally using raw milk. Although the consumption rate of unpasteurized dairy products is gradually decreasing in Turkey, little no more information about the incidence of pathogenic *Yersinia spp.* has been documented. In this study, it was aimed to evaluate the presence of pathogenic *Y. enterocolitica* in some foods marketed in a part of the northeast area of Turkey and determine of resistance of isolates to some antibiotics.

MATERIALS and METHODS

Samples

A total of 750 samples including 150 ice cream, 150 raw milk, 150 fresh (feta) cheese, 150 chicken drumsticks and 150 minced meat were collected from markets in three cities (Kars, Ardahan and Iğdır) located at the Northeast of Turkey. Food samples were randomly selected and delivered to the laboratory in an ice box at 4°C within 2 h from collection and tested immediately upon arrival.

Isolation and Identification of Yersinia spp.

A 25 g of sample was aseptically added to 100 ml 0.01 M Phosphate Buffered Saline (PBS, pH 7.6) in a sterile stomacher plastic bag and homogenized for 2 min. The homogenates were incubated at 25°C for 10 min. Different enrichment procedures were applied. One of the methods was adding a 20 ml from homogenate to 80 ml TSB (Trypticase soya broth- Oxoid CM 0129B) and enriching at 25°C for 24 h. Another procedure was adding a 20 ml from homogenate 80 ml PBS (Phosphate Buffered Saline) and enriching at 4°C for 14 days (cold enrichment). The samples were treated with KOH (0.5% KOH in 0.5% saline) to suppress background flora after enrichment. Subculturing on selective CIN agar plates (Yersinia Selective Agar Base -Oxoid CM 0653B) was applied according to the method of the FDA [20]. One to five susceptible colonies of typical “bull’s eye” appearance on the CIN agar plates, if available, were streaked onto Tryptone Soya Agar (Oxoid CM 0131B) plates to create a pure culture. All the isolates from pure cultures were examined for Gram staining, utilization of Simmon’s citrate, Kligler’s Iron agar reaction and urease activity [20].

Confirmation and Biogrouping of Yersinia enterocolitica

All the isolates which were negative for utilization of citrate, positive for urease activity and giving an alkaline slant/acid butt without gas or H_2S on KIA were submitted to further testing. In order to identification

and biogrouping of isolates as *Y. enterocolitica;* activities of oxidase, lysine decarboxylase, ornithine decarboxylase, β-D-glucosidase, lipase and pyrazinamidase, utilization of rhamnose, sucrose, xylose and trehalose, and salicin were evaluated. Further analyses were also conducted applying Esculin hydrolysis, Indole and Voges Proskauer tests to isolates. The reference strain *Y. enterocolitica* O: 3 (serotype 920) used in this study were purchased from culture collection of Refik Saydam Hygiene Center Ankara, Turkey. *Y. enterocolitica* isolates were biotyped according to the revised biogroup scheme of Wauters et al.[21], Schiemann and Wauters [22] and FDA [20].

Testing for Pathogenicity Markers

Y. enterocolitica strains were tested for virulence by Temp-Dependent autoagglutination (25°C-35°C) in Methyl Red-Voges Proskauer broth (Oxoid CM 0043B), occur of small red colonies on CR-MOX agar and Congo red binding/crystal violet binding assays [20,23,24].

Antimicrobial Susceptibility

Determination of antimicrobial susceptibility of *Y. enterocolitica* strains to antibiotics which are used to treat of Yersiniosis was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) [25] using Mueller Hinton agar (Oxoid CM 0337B) and commercially available antimicrobial test discs *(Table 2).* Results were recorded by measuring the inhibition zones and scored as sensitive, intermediate susceptibility and resistant according to the NCCLS [25] recommendations.

RESULTS

Presence of Yersinia spp. in Food Samples

In this study, two different enrichment procedures were applied to each sample as overnight and cold enrichment. All of 18 strains were recovered after cold enrichment for 14 days, but no strain was isolated from the samples enriched at 25°C for 24 h in TSB. Out of the 750 analyzed food samples, 57 samples (7.6%) were evaluated as positive for *Yersinia spp.* and 18 (2.4% in total) of them, isolated from 6 feta cheese, 4 ice cream, 2 chicken drumsticks, 4 minced meat and 2 raw milk samples, were evaluated as pathogenic *Y. enterocolitica.* All the 18 pathogenic strains were tested for their antimicrobial resistance. A total of 31.57% of the 57 food samples including *Yersinia spp.* were contaminated by pathogenic *Y. enterocolitica.* Biotypes 1B, biotype 2, 3 and 4 were identified. Biotype distribution and test applied were documented in *Table 1.*

Antimicrobial Susceptibility Test Results

No strain was resistant to sulphamethoxazole/ trimethoprim, ciprofloxacin, chloramphenicol and imipenem. Six isolates were resistant to ticarcillin, 5 isolates netilmicin, and 1 isolate tetracycline whereas 17 isolates were resistant to streptomycin, 12 isolates gentamicin, 17 isolates kanamycin, and 6 isolates furazolidone. All of strains were resistant to clindamycin and cephazolin *(Table 2).*

Table 1. *Biogrouping results of Y. enterocolitica isolated from different food samples*
Tablo 1. *Değişik gıda örneklerinden elde edilen Y. enterocolitica izolatlarının biogrupları*

Isolates	Feta Cheese						Icecream				Chicken Drumsticks		Minced Meat				Milk	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Indole	+	+	+	+	+	-	-	+	-	+	+	+	-	-	+	+	+	+
Voges Proskauer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-D-Glucosidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lipase	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CR-MOX *	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Autoagglutination	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyrazinamidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Biotyping	**1B**	**1B**	**1B**	**1B**	**2**	**3**	**3**	**2**	**4**	**2**	**2**	**2**	**4**	**4**	**2**	**2**	**2**	**2**

** Small red colonies on CR-MOX agar*

Table 2. *Antimicrobial susceptibility test results of pathogenic Y. enterocolitica isolated from food samples*
Tablo 2. *Patojenik Y. enterocolitica izolatlarının antimikrobiyel duyarlılık test sonuçları*

Antimicrobial Agent	Numbers of Isolates (%)		
	Susceptible	Intermediate	Resistant
Amikacin (30 mcg) (Oxoid CTO 107B)	7 (38.8)	3 (16.6)	8 (44.4)
Amoxycillin/Clavulanic acid (30 mcg) (Oxoid CTO 223B)	6 (33.3)	7 (38.8)	5 (27.7)
Ampicillin (10 mcg) (Oxoid CTO 003B)	- (0)	8 (44.4)	10 (55.5)
Cefoperazone (75 mcg) (Oxoid CTO 249B)	1 (5.5)	6 (33.3)	11 (61.1)
Cefotaxime (30 mcg) (Oxoid CTO 166B)	13 (72.2)	5 (27.7)	- (0)
Cephazolin (30 mcg) (Oxoid CTO 011B)	- (0)	- (0)	18 (100)
Ciprofloxacin (5 mcg) (Oxoid CTO 425B)	18 (100)	- (0)	- (0)
Chloramphenicol (30 mcg) (Oxoid CTO 013B)	18 (100)	- (0)	- (0)
Clindamycin (2 mcg) (Oxoid CTO 064B)	- (0)	- (0)	18 (100)
Furazolidone (15 mcg) (Oxoid CTO 448B)	4 (22.2)	- (0)	14 (77.7)
Gentamicin (10 mcg) (Oxoid CTO 024B)	6 (33.3)	- (0)	12 (66.6)
Imipenem (10 mcg) (Oxoid CTO 455B)	18 (100)	- (0)	- (0)
Kanamycin (5 mcg) (Oxoid CTO 025B)	1 (5.5)	- (0)	17 (94.4)
Netilmicin (30 mcg) (Oxoid CTO 225B)	10 (55.5)	3 (16.6)	5 (27.7)
Streptomycin (10 mcg) (Oxoid CTO 047B)	1 (5.5)	- (0)	17 (94.4)
Sulphamethoxazole/trimethoprim (25 mcg) (Oxoid CTO 052B)	18 (100)	- (0)	- (0)
Tetracycline (30 mcg) (Oxoid CTO 041B)	15 (83.3)	2 (11.1)	1 (5.5)
Ticarcillin (75 mcg) (Oxoid CTO 167B)	7 (38.8)	5 (27.7)	6 (33.3)
Ticarcillin/Clavulanic acid (85 mcg) (Oxoid CTO 449B)	17 (94.4)	1 (5.5)	- (0)
Trimethoprim (5 mcg)	11 (61.1)	- (0)	7 (38.8)

All the 18 pathogenic strains were tested for their antimicrobial susceptibility and some of the isolates were found to be resistant to ticarcillin (n=6), netilmicin (n=5), tetracycline (n=1) streptomycin (n=17), gentamicin (n=12), kanamycin (n=17), furazolidone (n=6), clindamycin (n=18) and cephazolin (n=18) while all of them were susceptible to sulphamethoxazole/trimethoprim, ciprofloxacin, chloramphenicol and imipenem.

DISCUSSION

In this study, 750 food samples were analyzed. Pathogenic *Y. enterocolitica* was isolated from 18 samples (2.4%) of all the samples. Two different enrichment procedures were applied to each sample as overnight and cold enrichment. All of 18 strains were recovered after cold enrichment for 14 days, but no strain was isolated from the samples enriched at 25°C for 24 h in TSB. A possible explanation for the very low recovery rate after cold enrichment might be the low number of pathogenic *Y. enterocolitica* strains contaminated in food samples or high background flora on the selective agar. In overnight enrichment at room temperature, endogenous microflora overgrew, suppressing the growth of *Y. enterocolitica.* The psychrotrophic nature of *Y. enterocolitica* is unusual among other *Enterobacteriaceae*; consequently, enrichment in different solutions at 4°C for prolonged periods has been used for isolation of *Yersinia spp.*[9]. However, the time needed for this method is a disadvantage for routine analysis.

The isolation rate of *Y. enterocolitica* observed in this study was close or considerably lower than that of studies which had reported previously *(Table 3)*. This can be explained by differences in isolation and identification methods, false analysis results which might be occurred depend on methods used, different seasons those samples obtained, and diversity of kind, hygienic condition and also competing microflora of samples. In this study, cheese and ice cream samples appeared to be more noticeable samples among others.

Variety of research findings related to antimicrobial susceptibility of *Y. enterocolitica* has been published [33,35,36]. Antibiotic susceptibility data for *Y. enterocolitica*

Table 3. *Isolation of Y. enterocolitica from foods (literature data)*
Tablo 3. *Gıdalardan Y. enterocolitica izolasyon oranları (literatür bilgisi)*

Foods	Country	Incidence (%)	Reference
Raw milk	USA	6.1	26
Raw milk	Turkey	20	16
Raw milk	Iran	1.6	27
Raw milk	Normandy	36	28
Cheese	Argentina	0	29
Cheese	Turkey	35.7	15
Cheese	Morocco	4	30
Ice cream	India	40.3	14
Ice cream	India	0	31
Chicken meat	Argentina	4.3	32
Chicken meat	Spain	50	10
Chicken meat	Austria	44.9	33
Minced meat	German	0.5	34
Meat	Spain	0	13

has been somewhat inconsistent and has validated between concordance and nonconcordance among different serogroups and biotypes [2]. Aarestrup et al.[37] reported that some of their *Y. enterocolitica* strains were intermediate resistant against ampicillin although it had been previously noted as naturally resistant. Indeed, eight years before that study, Kwaga and Iversen [38] showed that all or most of the strains were resistant against ampicillin, clindamycin, cephazolin and amoxicillin-clavulanic acid while 100% of them were susceptible to sulfamethoxazole-trimethoprim, imipenem and ticarcillin-clavulanic acid. We also found 8 (44.4%) isolates evaluated as intermediate resistant against ampicillin in addition to 10 (55.5%) resistant strains. Those results are important for their role warning about ampicillin resistance. In another study, Lyons et al.[39] reported that 100% of strains were resistant against tetracycline and trimethoprim. In this study no strain was resistant against trimethoprim and tetracycline. In another study, Pham et al.[40] assessed the antibiotic susceptibility profile of 100 clinical isolates of *Y. enterocolitica.* According to their results, all the 100 isolates were uniformly susceptible to chloramphenicol, ciprofloxacin, gentamicin, tetracycline and trimethoprim. Our findings representing susceptibility of all the strains investigated in this study were parallel to those of Pham et al.[40]. In an early study, all *Y. enterocolitica* isolates were subjected to resistance against tetracycline, gentamicin, kanamycin, trimethoprim and chloramphenicol [33]. In this study, antimicrobial susceptibility results of *Y. enterocolitica* isolates were generally similar to that of previous ones. However, it is difficult to predict warning signals about gaining resistance ability of that Yersinia strain in time, investigating just 18 isolates.

Consequently, our results showed that raw and ready to eat animal originated foods tested in this study were contaminated with pathogenic biotype *Y. enterocolitica* even though in low percentage and thereby represented a risk to the consumers in regard to yersiniosis.

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