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Isolation of pathogenic *Yersinia enterocolitica* strains from different sources in Izmir region, Turkey

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Abstract *Yersinia enterocolitica* is a foodborne pathogen that is very rarely encountered in Turkey. In this work, several human, porcine, and environmental samples collected from Izmir region in Turkey were examined for the presence of *Y. enterocolitica* using different cultivation and enrichment methods. A total of nine pathogenic *Y. enterocolitica* strains were isolated; five strains from pig stool and manure samples and four strains from waste water samples. On the other hand, no *Y. enterocolitica* was isolated from human diarrheal stool samples (n=102) and from 12 gulf, canal, municipal pool, and well water samples. Biochemical and serological characterization of the nine *Y. enterocolitica* strains revealed that they belonged to three different bioserotypes: 4/O:3, 2/O:9, and 2/O:5,27. All the strains were deemed pathogenic based on virulence factor-specific PCR analysis. Detection of pathogenic *Y. enterocolitica* strains from the pig and waste water samples from the Izmir region indicates that *Y. enterocolitica* is a potential risk for public health.

Introduction

The genus *Yersinia* of the family *Enterobacteriaceae* includes three well-established human pathogens (*Yersinia pestis*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*) and several nonpathogenic species (EFSA 2007). The human and animal pathogenic *Yersinia* strains carry the 70–75-kb *Yersinia* virulence plasmid. Since the 1960s, *Y. enterocolitica* has been identified as a frequent and important cause of enteric yersiniosis in developed countries and is the most prevalent *Yersinia* species comprising ca. 91.0 % of all the confirmed cases (EFSA 2012). *Y. enterocolitica* is the third most commonly reported zoonotic pathogen in the European Union (EFSA 2007). As consequences of yersiniosis are severe and might include prolonged acute infections, pseudoappendicitis, and long-term sequelae such as reactive arthritis, the economic and public health effects of yersiniosis are of greater magnitude than the actual number of cases would suggest (Nesbakken 2013).

Y. enterocolitica and related species are grouped in over 70 serotypes that are determined mainly by the variability of the lipopolysaccharide (LPS) O-antigen structures. LPS is an important component of the outer membrane of Gram-negative bacteria. LPS is composed of two main components, which are the lipid A-core and the O-specific polysaccharide (O-PS, also known as O-antigen).The O-PS can be either homo- or heteropolymeric, i.e., a polymer of a single sugar or a polymer of oligosaccharide repeats (O-units). The O-antigen is the immunogenic part of LPS and therefore contributes in serotyping (Beczala et al. 2012). Pathogenic *Y. enterocolitica* strains most commonly belong to serotypes O:3, O:8, O:5,27, and O:9, and to biotypes 1B, 2, 3, 4, and 5. Biotype 1A *Y. enterocolitica* strains are generally considered nonpathogenic. They are widespread in nature both in aquatic and animal reservoirs. Some 1A strains of *Y. enterocolitica* have been reported to cause clinical illness in humans (Drummond et al. 2012). In addition, *Y. enterocolitica* biotype 1A strains fall into two phylogenetic lineages with different pathogenic potential, for example, the ability to resist human (Sihvonen et al. 2012). Pathogenic strains of *Y. enterocolitica* are transmitted by the

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fecal-oral route. Pigs are considered the principal reservoir for *Y. enterocolitica* pathogenic to humans, although other animal species, such as cattle, sheep, poultry, fish, deer, small rodents, cats, and dogs, may also carry pathogenic biotypes (Denis et al. 2011). Pigs are considered healthy carriers of the human pathogenic strains such as bioserotypes 4/O:3, 2/O:5,27, 2/O 9, and 1B/O:8. Bacteria are detected in the oral cavity, tongue, and tonsils of the pigs as well as from the intestine and feces. *Y. enterocolitica* is also widely distributed in nature, such as aquatic and animal reservoirs (Bottone 1997) and is therefore a common contaminant of water supplies. *Y. enterocolitica* has also been isolated from natural waters including lakes, oceans, and streams (Percival and Williams 2014). Water is a significant reservoir of *Y. enterocolitica* as shallow wells, rivers, and lakes are exposed to fecal contamination from wild or domestic animals in the surrounding areas (Nesbakken 2013).

In Turkey, the occurrence of *Y. enterocolitica* has not been systematically studied and presence of the bacterium is not routinely assessed from human, food, or environmental sources. The only food-related report of *Y. enterocolitica* in Turkey was in raw milk and cheese samples (Yucel and Ulusoy 2006). In the first human case of yersiniosis in Turkey, *Y. enterocolitica* was isolated from a type I diabetes patient in Istanbul (Mert et al. 2011).

Compared with Europe, the USA, and China, pig farming, and pork meat production is almost nonexistent in Turkey. According to the Turkish Food and Agricultural Policy Research Institute, there are only three pig farms in Turkey; two of them in Antalya and one close to Izmir. Accordingly, the likelihood of yersiniosis due to consumption of contaminated pork in Turkey is minimal. Due to the lack of studies on the incidence of *Y. enterocolitica* from various sources in Turkey, the risk of yersiniosis in the country remains unknown. The purpose of this project was to isolate, identify, and characterize *Y. enterocolitica* strains from various sources in the Izmir region, Turkey.

Materials and methods

Bacterial strains

The *Y. enterocolitica* strains used as positive controls in the microbiological tests, MALDI-TOF®MS analysis and PCR in the laboratory at Ege University in Turkey were the serotype O:3 strain 6471/76 (Skurnik 1984), serotype O:5,27 strain JDE657 (Schiemann et al. 1981), serotype O:8 strain 8081 (Portnoy and Falkow 1981), and serotype O:9 strain Ruokola/71 (Skurnik 1984). The strains were obtained from the bacterial strain collection of the Yersinia Research Laboratory at University of Helsinki.

Isolation of *Y. enterocolitica* from human stool samples

During the period from October 2012 to January 2013, a total of 102 diarrheal stool samples from 62 pediatric and 40 adult patients admitted to Ege University hospital were collected for this study. All the patients had abdominal pain and had diarrheal symptoms, i.e., three or more loose liquid or watery stools which contained leukocytes and/or erythrocytes within a 24-h period. The stool samples, in addition to isolation of *Y. enterocolitica*, were screened routinely for the presence of *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., and diarrheagenic *Escherichia coli* strains. The samples were transferred immediately to the Microbiology Research and Genetics Laboratory at Ege University, Biology Department. *Y. enterocolitica* was isolated according to the ISO10273 method: 1 ml stool sample was transferred to 9 ml phosphate-buffered saline (PBS, pH 7.45) supplemented with 1 % glucitol and 0.15 % bile salts (PSB) and incubated at 25 °C for 2 days. Following the pre-enrichment step, a 10-μl aliquot was spread onto Cefsulodin Irgasan Novobiocin Agar (CIN) (Oxoid, UK) plate. A 1-ml aliquot was mixed with 9 ml of irgasan-ticarcillin-potassium chlorate (ITC) broth (Oxoid, UK) and incubated at 25 °C for 48 h. A 10-μl aliquot was then spread onto *Salmonella-Shigella*-sodium deoxycholate-calcium chloride (SSDC) agar (Oxoid, UK) (Denis et al. 2011).

The Department of Food and Environmental Hygiene (DFEH, University of Helsinki) method (Laukkanen et al. 2010) was also used for isolation of *Y. enterocolitica* strains. The DFEH method includes two different enrichment buffers: a cold enrichment in PBS supplemented with 0.5 % peptone, 1 % mannitol, and 0.15 % bile salts (PMB/PSMB) and PBS supplemented with 1 % mannitol, 1 % glucitol, and 0.15 % bile salts (PSMB). Two milliliters of the stool sample was added to 18 ml PMB/PSMB (1:9, *V/V*), and the suspension was incubated at +4 °C for 2 weeks. A 1-ml sample was then inoculated into 9 ml of ITC broth (Oxoid, UK) that was incubated at 25 °C for 2–3 days. From each enrichment culture, 100-μl aliquots were spread onto CIN agar (Oxoid, UK) plates, and the plates were incubated at 25 °C for 24–48 h (Atobla et al. 2012; Laukkanen et al. 2010). For alkaline treatment, 0.5 ml samples from the enrichment cultures were mixed with 4.5 ml of 0.25 mM KOH and 0.5 % NaCl (Aulisio et al. 1980); 100 μl of the mixture was then plated immediately onto CIN agar (Oxoid, UK).

Isolation of *Y. enterocolitica* from pig stool and pig manure

The presence of *Y. enterocolitica* in a pig farm located in Kesik village was investigated with special permission from the pig farm owner. Two pig stool and two pig manure samples were collected into sterile plastic bags and transported in an ice box to the laboratory and processed within 24 h. The samples were

analyzed by two methods: (i) direct streaking on selective agar plates in duplicate and pre-enrichment with the DFEH method and ISO10273 method as described above, with a modified pre-enrichment incubation time up to 3 weeks. Presumptive *Y. enterocolitica* pure cultures were analyzed with biochemical tests as described below.

Isolation of *Y. enterocolitica* from water sources

Gulf, canal, municipal pool, and well water samples were collected in fall 2012 from the north and south part of Izmir (n=12). All water samples were aseptically collected in sterile 500 ml containers and transported in an ice box immediately to the laboratory. The 500-ml samples were concentrated by filtering the water through a 0.45-μm nitrocellulose membrane (S-Pak, Sigma Aldrich) using a vacuum pump. The filters were rotated at 150 rpm for 1 h at room temperature in 10 ml sterile PBS. Four different enrichment methods were used to detect *Y. enterocolitica* form the concentrated samples. One milliliter of concentrated water sample was added to 9 ml of (i) PSB at 25 °C for 2 days, (ii) yeast extract-rosebengal broth (YER) at 4 °C for 9 days, (iii) PSMB/PMB at 4 °C, 7 days, and (iv) tryptic soy broth (TSB) at 22 °C for 1 day (Atobla et al. 2012; Fredriksson-Ahomaa and Korkeala 2003; Laukkanen et al. 2009). A loopful of each enriched sample was spread onto CIN agar (Oxoid, UK) and incubated at 25 °C for 24–48 h. The enriched samples were alkaline-treated and plated on CIN agar (Oxoid, UK) as described above. Suspected *Y. enterocolitica* isolates were characterized as described below.

Isolation of *Y. enterocolitica* from waste water treatment facility

The presence of *Y. enterocolitica* in waste water from waste water treatment facilities located in Aliaga, Menemen, and Cigli was investigated with official permission from the Izmir Metropolitan Municipality. Two untreated incoming 500-ml sewage samples from each facility were collected in sterile containers and transported in an ice box to the laboratory. The samples were processed by the modified pre-enrichment method as described (Cheyne 2008). The 500-ml samples were concentrated by filtering the water through a 0.45-μm nitrocellulose membrane (S-Pak, Sigma Aldrich) using a vacuum pump. The filters were then rotated at 100 rpm for 24 h at 12 °C in 10-ml modified tryptic soy broth (mTSB; per liter, 30 g TSB, 2.5 g yeast extract, 2 g bile salt, pH adjusted to 7.6 with 2 M NaOH). Irgasan (Sigma Aldrich; 4 mg/ml in methanol) was added to each enrichment culture to a final concentration of 4 μg/ml. After the enrichment period, 50 μl samples were plated on CIN agar (Oxoid, UK) plates with and without alkaline treatment.

Identification of *Y. enterocolitica*

At least five colonies from each CIN agar plate showing the bull's eye colony morphology were selected and tested by biochemical assays, i.e., Gram-staining, citrate, lactose fermentation, and Kligler agar tests. All Gram-negative, citrate-negative, and lactose fermentation-negative colonies which in the Kligler tube also presented an alkaline (red) slant without gas or H_2S were further identified by MALDI-TOF®MS (bioMérieux, France).

Biotyping and pathogenicity related assays of *Y. enterocolitica* strains

The strains identified as *Y. enterocolitica* by MALDI-TOF®MS were biotyped using lipase, esculin hydrolysis, salicin utilization, indole, D-xylose utilization, trehalose utilization, pyrazinamidase, Voges-Proskauer, and DNAse tests (EFSA 2007). The pathogenicity related assays, including autoagglutination in MR-VP broth and Congo red-binding test, were carried out as described by Johnson (1998).

PCR methods

Bacterial DNA was isolated using a genomic DNA isolation kit (Invitrogen) according to the manufacturer's instructions. The primers used are listed in Table 1, and PCR reaction conditions were as described by Thoerner et al. (2003). A duplex PCR assay with primers targeting the *ail* (attachment and invasion locus) and the 16S rRNA genes for species identification of *Y. enterocolitica* was used to identify pathogenic *Y. enterocolitica* strains (Wannet et al. 2001). In addition to *ail*, the chromosomal virulence genes *ystA*, responsible for the production of heat-stable enterotoxin, *ystB*, encoding the enterotoxin present mainly in biotype 1A strains of *Y. enterocolitica* and the virulence plasmid genes *yadA*, encoding the adhesion and serum resistance factor, and *virF/lcrF*, encoding the transcriptional activator, were tested by PCR.

Serotyping of *Y. enterocolitica* strains

Serotyping of *Y. enterocolitica* strains was carried out using serotype-specific bacteriophages available in the Skurnik Laboratory as described (Baker and Farmer 1982). O-antigen genotyping was performed using O-antigen operon specific primers (Table 1).

LPS profile of *Y. enterocolitica* strains

LPS characterization of pathogenic *Y. enterocolitica* strains, deoxycholate-polyacrylamide-gel-electrophoresis (DOC-PAGE) and silver staining analysis were carried out as described (Skurnik et al. 1995).

Table 1 Primers used in this study

Description/PCR target	Primer	Sequence (5′→3′)	Size (bp)	Reference
ail, the attachment/invasion locus gene	A1 A2	ttaatgtgtacgctgggagtg agtattcatatgaagcgtc	425	Wannet et al. (2001)
16S rRNA gene	Y1 Y2	aataccgcataacgtcttcg cttcttctgcgagtaacgtc	330	Wannet et al. (2001)
ystA heat-stable enterotoxin	*yst*A	atcgacaccaataaccgctgag ccaatcactactgacttcggct	79	Thoerner et al. (2003)
ystB, enterotoxin mainly biotype 1A	*ystB*	gtacattaggccaagagacg gcaacatacctcacaacacc	146	Thoerner et al. (2003)
Yersinia enterocolitica yadA gene	*yad*A	cttcagatactggtgtcgctgt atgcctgactagagcgatatcc	849	Thoerner et al. (2003)
Y. enterocolitica and *Y. pseudotuberculosis* transcriptional activator of *yop* regulon	*vir*F/l*cr*F	ggcagaacagcagtcagacata ggtgagcatagagaatacgtcg	561	Thoerner et al. (2003)
Y. enterocolitica O:9/O-antigen operon	per-6 per-7	attgacttatattgcttcggtta aaggtacctacatgtcgtccat	272	Skurnik Laboratory
Y. enterocolitica O:3 / O-antigen operon	o3wb5959r o3wb5119f	attagcaatctcgggggaat gaattgcatctgcacagaac	840	Skurnik Laboratory
Y. enterocolitica O:5/O-antigen operon	O5-7699r O5-7011f	actctccgctgattgtcgtt taggcctgccaactcaagat	688	Skurnik Laboratory

Results

Nine of the 259 suspected *Y. enterocolitica*-like colonies on CIN agar were confirmed as *Y. enterocolitica* by culture, MALDI-TOF®MS, PCR, and biochemical tests (Table 2). No *Y. enterocolitica* strains were isolated from 102 stool samples of diarrheal patients or from the 12 water sources studied. However, 5 of the 69 suspected colonies from the pig stools and manure and 4 of 43 suspected colonies from six waste water samples were confirmed as *Y. enterocolitica*. *Y. enterocolitica* strains were isolated from four of the six waste water treatment facility samples, three strains from Aliaga and one from Menemen. Of the pig stool and manure samples, all samples except for one stool sample, were positive for *Y. enterocolitica* (Table 3).

The O-serotypes of the nine *Y. enterocolitica* isolates were determined by PCR specific to O-antigen operons of different O-serotypes, by serotype-specific bacteriophages and by DOC-PAGE analysis (Fig. 1). Four of the strains belonged to serotype O:3, four to O:9 and one to O:5,27. The DOC-PAGE LPS analysis of the strains corroborated the serotyping results showing the typical serotype-specific LPS with distinct O-polysaccharide (O-PS) profiles (Skurnik and Toivonen 2011). While strain A2-1 showed the serotype O:5,27-specific single length O-PS, the other strains had the typical smears of serotype O:3 and O:9 homopolymeric O-PS. The strains A2-2, A2-22, A2-25, and AS-8-15 had the shorter O:9-specific homopolymeric O-PS, and strains A1-1, AS-11-45, AS-12-35, and AS-11-47 had the longer O:3-specific homopolymeric O-PS. Based on the PCR analysis, all nine *Y. enterocolitica* strains were pathogenic as they were PCR positive for the virulence genes *ail, yadA, virF,* and *ystA* (Table 3).

In addition to *Y. enterocolitica* strains, the suspected Gram-negative, citrate-negative, and lactose fermentation-negative colonies on CIN agar were identified by MALDI-TOF®MS as *Serratia liquefaciens* ($n=6$), *Serratia odorifera* ($n=1$), *Pseudomonas fluorescens* ($n=3$), *Morganella morganii* ($n=1$), *Vibrio alginolyticus* ($n=2$), *Aeromonas hydrophila* ($n=2$), *Aeromonas hydrophilia/caviae* ($n=1$), *Stenotrophomonas maltophilia* ($n=2$), *Erwinia rhapontici* (n=1), and *Phobacterium damselae* ($n=1$).

Discussion

In this study, nine *Y. enterocolitica* strains were isolated from a pig farm and two waste water treatment facilities located close to Izmir in Turkey. This is the first report of detection of *Y. enterocolitica* from pig stool and pig manure in Turkey. Surprisingly, the isolated nine strains represented three different bioserotypes, i.e., 2/O:9, 2/O:5,27, and 4/O:3 that are the most common pathogenic bioserotypes of *Y. enterocolitica* isolated from humans in EU countries. All the isolates were found to be pathogenic based on virulence factor-specific PCR analysis.

Y. enterocolitica has been isolated worldwide from animals, raw food materials, environment, water sources, and also from human patients (Rahman et al. 2011). Extensive studies have been performed on the detection of *Y. enterocolitica* from slaughter pigs, pig tonsils, pig tongues, pig oral cavity, and fecal

Table 2 Summary of the suspected *Yersinia enterocolitica* colonies isolated from different sources and characterized in this work by culture, biochemical, and PCR methods

Material/isolation source	Sampling time/period	Samples (*n*)	Suspected colonies (*n*)	No. of positive (%)[a]	
				MALDI-TOF®MS	PCR (*ail/16S rRNA*)
Stool of diarrhea patients	Oct 2012–Jan 2013	102	132	–	–
Pig stool and pig manure	Feb 2013	4	69	5 (7.24)	5 (7.24)
Gulf, canal, municipal pool, and well water	Aug–Sept 2012	12	15	–	–
Waste water	Feb 2013	6	43	4 (9.3)	4(9.3)
Total		124	259	9 (3.48)	9 (3.48)

[a] All nine isolates were Gram, citrate, and lactose fermentation negative and presented in the Kligler tube an alkaline (red) slant without gas or H_2S

samples (Biasizzo et al. 2013; Bonardi et al. 2013; Vanantwerpen et al. 2014). *Y. enterocolitica* 4/O:3 is the most common bioserotype isolated from pigs (Fondrevez et al. 2014). Human yersiniosis has been associated with the consumption of pork and pork products in many investigations and case-control studies (Laukkanen et al. 2010). Pigs usually do not develop clinical signs of illness, but they carry *Y. enterocolitica* in their tongues and tonsils, and they excrete this bacterium in their feces. They also have raised antibody titers against *Y. enterocolitica*. Tan et al. (2014) examined 165 pigs from 9 farms in Malaysia and reported isolation rate of 1.8 % for pathogenic *Y. enterocolitica* 3/O:3 strains. In Italy, 24 out of 115 (20.9 %) *Y. enterocolitica* strains isolated from pigs were of bioserotype 4/O:3 and 4 (3.5 %) of 2/O:9 (Bonardi et al. 2013). In 2011, an outbreak involving 21 cases of *Y. enterocolitica* 2/O:9 were reported in Norway due to a salad mix from Italy as a possible source (MacDonald et al. 2011).

Although the number of pig samples investigated in this study was small, pathogenic *Y. enterocolitica* strains could be isolated from three out of four samples. In order to fully understand the *Y. enterocolitica* epidemiology in pigs reared in Turkey, more pig-derived samples should be analyzed. Being an Islamic country, pig farming is extremely rare in Turkey and in practice all the pig farms could be subjected to epidemiologic *Y. enterocolitica* screens in future. However, our data suggests that pork meat, mostly consumed by tourists in Turkey, is most likely contaminated by pathogenic *Y. enterocolitica* and is therefore a risk of foodborne yersiniosis.

On the other hand, no *Y. enterocolitica* were isolated from the patient stool samples. The patients were local people who do not consume pork or pork products and this may be the reason why yersiniosis is so rare in Islamic countries. In spite of this, a few yersiniosis cases have been reported in Turkey; a yersiniosis case of a type I diabetic patient with a liver abscess whose blood culture was positive for *Y. enterocolitica* in Istanbul (Mert et al. 2011). In addition, Ozden et al. (2012) reported one *Y. enterocolitica* strain from a 51-year-old man who was a peritoneal dialysis patient in Elazıg, in the eastern part of Turkey. Bulbuloglu et al. (2010) reported an adult case of *Y. pseudotuberculosis* colitis who presented with severe gastrointestinal bleeding in the province of Kahramanmaras, Turkey.

In our study, stool samples were also screened for the presence of *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., and diarrheagenic *E. coli* strains as a part of routine screening tests in hospital. Out of 102 diarrheal stool samples, *E. coli* O157:H7 was detected in one patient. Moreover, 1 patient out

Table 3 Summary of the bioserotypes, isolation sources, isolation methods, and virulence markers of the *Yersinia enterocolitica* strains isolated in this work

Y. enterocolitica strains	Isolation source	Isolation method	Bioserotype	Presence of virulence genes
A1-1	Pig manure A/Menemen	Direct inoculation from manure, CIN	4/O:3	*ail, yadA, virF, ystA*
A2-1	Pig manure B/Menemen	Direct inoculation from manure,CIN	2/O:5,27	*ail, yadA, virF, ystA*
A2-2	Pig manure B/Menemen	Direct inoculation from manure, CIN	2/O:9	*ail, yadA, virF, ystA*
A2-22	Pig stool A/Menemen	PSMB, ITC +4 °C, 3 weeks, CIN	2/O:9	*ail, yadA, virF, ystA*
A2-25	Pig stool A/Menemen	PSB, ITC +4 °C, 3 weeks, CIN	2/O:9	*ail, yadA, virF, ystA*
AS-8-15	Waste water/Menemen	mTSB, 24 h, 12 °C, 100 rpm, CIN	2/O:9	*ail, yadA, virF, ystA*
AS-12-35	Waste water Aliaga	mTSB, 24 h, 12 °C, 100 rpm, CIN	4/O:3	*ail, yadA, virF, ystA*
AS-11-45	Waste water/Aliaga	mTSB, 24 h, 12 °C, 100 rpm, CIN	4/O:3	*ail, yadA, virF, ystA*
AS-11-47	Waste water/Aliaga	mTSB, 24 h, 12 °C, 100 rpm, CIN	4/O:3	*ail, yadA, virF, ystA*

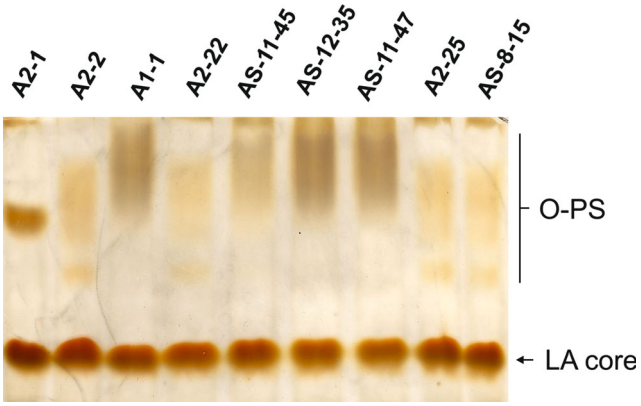

Fig. 1 DOC-PAGE analysis of silver-stained LPS of the *Yersinia enterocolitica* strains isolated in this work. The strains are indicated on *top of the lanes*. The locations of the O-polysaccharide (*O-PS*) and the lipid A core (*LA core*) bands are indicated at the *right*. Strain A2-1 is of serotype O:5,27 that has a single length O-PS, strains A2-2, A2-22, A2-25, and AS-8-15 are of serotype O:9 that have a shorter homopolymeric O-PS, and strains A1-1, AS-11-45, AS-12-35, and AS-11-47 are of serotype O:3 that have a longer homopolymeric O-PS

of 102 had co-infections with *Campylobacter jejuni* and enteropathogenic *E. coli*.

Water sources are exposed to fecal contamination from wild or domestic animals or by leakage from septic tanks to the surroundings. Therefore, water also can be considered as a reservoir of *Y. enterocolitica* (Nesbakken 2013). In 2009, Cheyne et al. examined 200 river samples from Ontario, Canada, but all the isolated *Y. enterocolitica* strains belonged to the nonpathogenic biotype 1/A (Cheyne et al. 2009). However, Falcao et al. (2004) documented isolation of virulent *Y. enterocolitica* 2/O:5,27 and 3/O:5,27 strains from water sources and sewage in Brazil. Another study was carried out by Waage et al. (1999) from environmental water and sewage in Norway, where *Y. enterocolitica* O:3 was detected by nested PCR. Also in Izmir, a microbiological quality survey was performed by Gonul and Karapinar (1991) from a drinking water supply. They reported 5 *Y. enterocolitica* strains out of 100 water samples collected from piped public supplies, wells with and without motor pumps, springs, and commercially bottled spring waters. However, there was no additional information on the bioserotypes of the *Y. enterocolitica* strains isolated. In the present study, we tried to isolate *Y. enterocolitica* from 12 water samples from gulfs and canals located north and south of Izmir, from wells, from municipal pools, and from waste water treatment facilities. Four pathogenic bioserotype 2/O:9 and 4/O:3 *Y. enterocolitica* strains were isolated from two waste water treatment facilities; three 4/O:3 strains from the Aliaga, and one 2/O:9 strain from the Menemen facility. Due to the enrichment culture approach to isolate strains, it is possible that the three Aliaga facility 4/O:3 strains and also the three pig farm 2/O:9 strains (Table 3) represent a single clone; however, this possibility was not studied here. While the Aliaga and Memenen treatment facilities receive human and domestic animal sewage, the origin of the *Y. enterocolitica* isolates remains elusive at present.

Isolation of *Y. enterocolitica* is very rarely reported in Turkey. This could be due to several reasons. Firstly *Y. enterocolitica* has not had any clinical significance earlier in Turkey and therefore this zoonotic pathogen is not routinely monitored in diarrheal patients. Secondly, in Islamic countries where pork and pork products are not consumed by the main population, yersiniosis cases are rare; however, presence of pathogenic *Y. enterocolitica* strains in pig farms may be a threat for public health also in Turkey. Finally, the isolation of *Y. enterocolitica* from stool and water samples is demanding as other members of *Enterobacteriaceae* present in stool samples could easily overgrow other slow-growing bacteria, such as *Y. enterocolitica*.

We conclude that pathogenic *Y. enterocolitica* represent a potential risk for public health in Turkey and this should be taken into account, perhaps by a routine monitoring of water supplies and diarrheal patients for *Y. enterocolitica*. Clearly, the sample numbers analyzed in this work are too small to understand the epidemiology of *Y. enterocolitica* in Turkey; however, this should serve as a starting point for more thorough epidemiological survey.

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